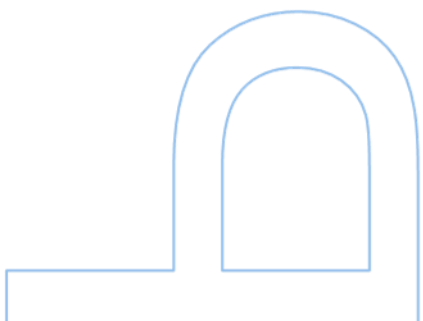
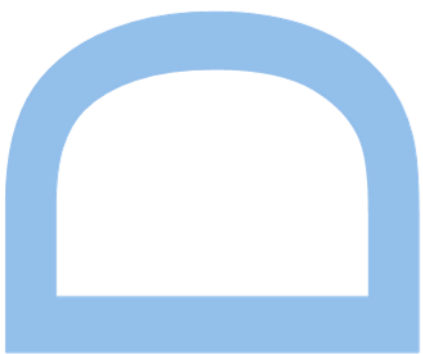
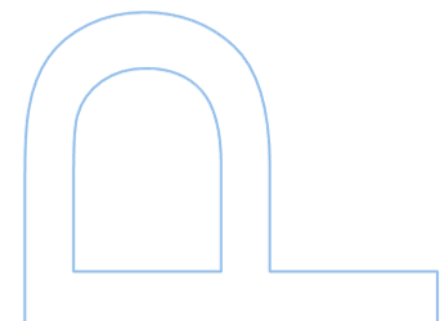
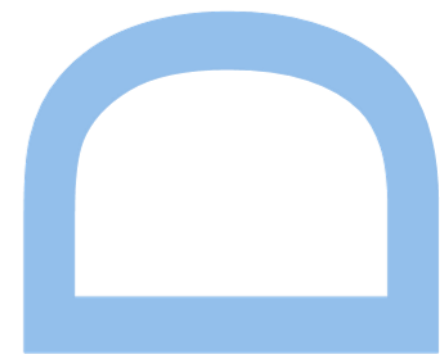
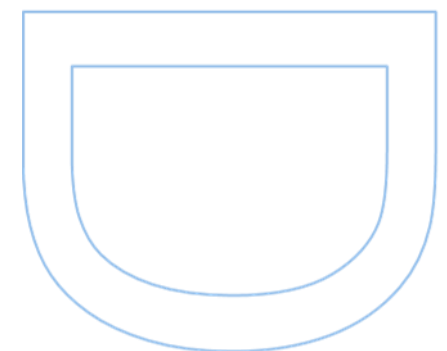




Development of Carbon-based Nanomaterials as Organocatalysts for Sustainable CO₂ Recycling

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Department of Geosciences, Environment and Spatial Plannings
Faculty of Sciences of the University of Porto
2025



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Thesis carried out as part of Doctoral Program in Environmental Sciences and Technology
Department of Geosciences, Environment and Spatial Plannings, 2025

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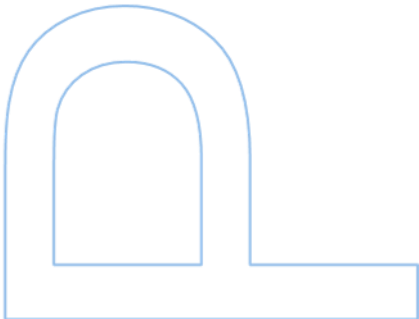
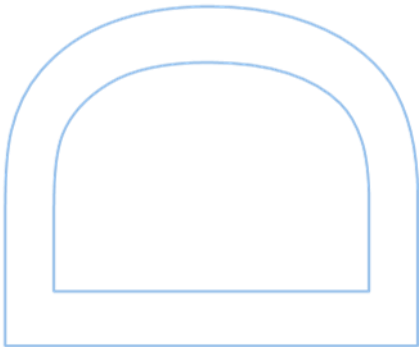
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Dedicated to Francisco and Maria Francisca

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Resumo

A dependência mundial de combustíveis fósseis para energia e matérias-primas resultou num aumento significativo dos níveis de CO₂ atmosférico, subindo cerca de 50 % desde a Revolução Industrial. Esse aumento nas emissões de CO₂ tem sido um grande contribuinte para as alterações climáticas, tornando a redução do CO₂ atmosférico uma das questões mais urgentes da atualidade.

Apesar de o CO₂ ser considerado uma fonte de carbono abundante, renovável, barata e não tóxica, o desenvolvimento de métodos eficientes em termos de custo e energia para a sua reciclagem contribuirá para reduzir as suas emissões totais e transformá-lo em produtos de valor acrescentado.

Catalisadores baseados em metais de transição permitem que a reciclagem do CO₂ ocorra à temperatura ambiente com altos rendimentos. No entanto, os organocatalisadores serão melhores opções, pois são considerados de baixo custo, não tóxicos e prontamente disponíveis. No entanto, os organocatalisadores ainda requerem altas temperaturas e pressões de CO₂. Portanto, o desenvolvimento de organocatalisadores eficientes é essencial para desenvolver um processo de reciclagem de CO₂ sustentável, não tóxico e económico.

Nanopartículas à base de carbono, como os *carbon dots* (CDs), podem ser bons candidatos para serem usados como catalisadores na reciclagem do CO₂ em produtos de valor acrescentado. Os CDs são solúveis em água, altamente estáveis, biocompatíveis, não tóxicos e fáceis de funcionalizar. Além disso, podem ser produzidos em massa a partir de fontes de carbono de baixo custo e renováveis, com abordagens sintéticas quase sem reagentes.

Para abordar isso, na primeira parte deste projeto de doutoramento, exploramos o potencial dos resíduos de borra de café (RBC) como uma fonte para sintetizar CDs. Os CDs derivados dos RBC, produzidos via carbonização sem solvente em uma única etapa, foram comparados aos CDs feitos de precursores convencionais como ácido cítrico e ureia. Os CDs derivados dos RBC, tinham tamanhos entre 2,1 e 3,9 nm, exibiram fluorescência azul com rendimentos quânticos moderados (2,9 % a 5,8 %) e, adicionalmente, mostraram-se promissores como sensores fluorescentes para detecção de iões Fe³⁺ em água. A avaliação do ciclo de vida confirmou a sustentabilidade ambiental da produção de CDs baseados em RBC em comparação com métodos convencionais.

No entanto, é sabido que os CDs podem ser sintetizados por várias abordagens de “*bottom up*”, que influenciam as suas características estruturais e ópticas. Para compreender bem as diferenças nas propriedades dos CDs preparados

por diferentes sínteses de “bottom up”, na segunda parte deste trabalho examinamos três métodos de síntese (hidrotérmico, assistido por micro-ondas e “*dry-heating*”) usando ácido cítrico e ureia para entender seu impacto nas propriedades dos CDs. Embora todos os métodos tenham produzido CDs com tamanhos semelhantes e emissões dependentes da excitação, os métodos de micro-ondas e “*dry-heating*” foram mais eficientes na dopagem com nitrogénio, resultando em rendimentos quânticos de fluorescência mais elevados (~27-29 %) em comparação com a síntese hidrotérmica (~2 %). A avaliação do ciclo de vida destacou a síntese por micro-ondas como a opção mais sustentável.

Outra propriedade essencial dos CDs é seu alto rendimento quântico de fluorescência (QYFL). Uma estratégia comumente usada para melhorar as suas propriedades de fluorescência envolve a dopagem com heteroátomos, particularmente o nitrogénio (dopagem com N), usando precursores contendo os heteroátomos desejados. Com base nos resultados anteriores, nesta terceira parte do trabalho exploramos CDs dopados com N sintetizados a partir de misturas de glicose e ureia via “*dry-heating*” de misturas sólidas com dois objetivos principais: primeiro, investigar a influência da estequiometria nas propriedades ópticas e na composição dos CDs e, segundo, explorar a formação de nanopartículas potencialmente responsivas a pH alcalino e a viabilidade dessa abordagem para obter CDs. Os resultados revelaram dois tipos de nanopartículas à base de carbono: CDs solúveis em água e CDs solúveis em pH básico. Estes últimos apresentaram rendimentos de síntese mais elevados, mas rendimentos quânticos e estabilidade térmica menores. Este estudo não encontrou correlação direta entre a estequiometria dos dopantes de N e os rendimentos quânticos, fornecendo-nos assim uma base para o desenvolvimento de CDs com fluorescência e rendimentos de síntese melhorados.

Na parte final deste projeto de doutoramento, investigamos o potencial dos CDs como dadores de pontes de hidrogénio (HBD) na reação de abertura de anel de epóxido, uma etapa chave na conversão de CO₂. Os CDs demonstraram uma atividade catalítica significativa, com uma constante de velocidade de $0,2361 \pm 0,008 \text{ h}^{-1}$ e uma taxa de conversão de 70,8 %, superando outros HBD moleculares alternativos. Estes resultados sugerem que os CDs podem ser HBD eficazes em processos de conversão de CO₂ organocatalisados, oferecendo uma abordagem promissora para a geração de produtos de valor acrescentado.

Keywords: Emissões de CO₂; organocatalisadores, carbon-dots; conversão de CO₂; catalisador, alterações climáticas; dador de pontes de hidrogénio; nucleófilo.

Abstract

The world's dependence on fossil fuels for energy and raw materials has resulted in a significant increase in atmospheric CO₂ levels, rising around 50 % since the Industrial Revolution. This increase in CO₂ emissions has been a major contributor to climate change, reducing atmospheric CO₂, one of the most pressing issues today. Despite CO₂ being considered an abundant, renewable, cheap, and non-toxic carbon source, the development of cost-/energy-efficient methods for CO₂ recycling will contribute to reducing its overall emissions and transform it into a valuable commodity.

Catalysts based on transition metals have allowed CO₂ recycling to occur at room temperature with high yields. However, organocatalysts should be preferred instead, as they are considered low-cost, non-toxic, and readily available. Nevertheless, organocatalysts still require high temperatures and CO₂ pressures. So, the development of efficient organocatalysts is essential for developing a cost-efficient, non-toxic, and sustainable CO₂ recycling process.

Carbon-based nanoparticles, namely carbon dots (CDs), can be a promising candidate to be used as a catalyst for CO₂ recycling into value-added products. CDs are water-soluble, highly stable, biocompatible, nontoxic, and are easy to be functionalized. Moreover, they can be mass-produced from low-cost and renewable carbon sources, with almost reagentless synthetic approaches

To address this, in the first part of this PhD project, we explored the potential of spent coffee grounds (SCGs) as a source for synthesizing carbon dots (CDs) using a nanotechnological approach. SCG-derived CDs, produced via one-pot, solvent-free carbonization, were compared to CDs made from conventional precursors like citric acid and urea. The SCG-derived CDs measured between 2.1 and 3.9 nm, exhibited blue fluorescence with moderate quantum yields (2.9 % to 5.8 %), and additionally showed promise as fluorescent probes for detecting Fe³⁺ ions in water. Life cycle assessments confirmed the environmental sustainability of SCG-based CD production compared to conventional methods.

However, it is known that CDs can be synthesized through various bottom-up approaches, which influence their structural and optical features. To well understand the differences in the CDs properties prepared by different bottom-up syntheses, in the second part of this work, we examined three synthesis methods (hydrothermal, microwave-assisted, and calcination) using citric acid and urea to understand their impact on CD properties. While all methods produced CDs with similar sizes and excitation-dependent emissions, microwave and calcination methods were more

efficient in nitrogen-doping, resulting in higher fluorescence quantum yields and synthesis efficiencies (~27-29 %) compared to hydrothermal synthesis (~2 %). Life cycle assessments highlighted microwave synthesis as the most sustainable option.

Another essential property of CDs is their high fluorescence quantum yields (QYFL). A common strategy to enhance their fluorescence properties involves heteroatom-doping, particularly nitrogen (N-doping), using precursors containing desired heteroatoms. Based on the previous results, in the third part of the work, we explored N-doped CDs synthesized from glucose and urea mixtures via dry heating of solid mixtures with two primary objectives: first, to investigate the influence of stoichiometry on the optical properties and composition of CDs, and second, to explore the formation of potential alkaline-responsive nanoparticles and the feasibility of this approach for achieving CDs. The results revealed two types of carbon-based nanoparticles: water-soluble and basic pH-soluble CDs. The latter had higher synthesis yields, but lower quantum yields and thermal stability. Our study found no direct correlation between N-dopants' stoichiometry and quantum yields, providing insights for developing CDs with enhanced fluorescence and synthesis yields.

In the final part of this PhD project, we investigated fluorescent CDs as catalytic hydrogen bond donors (HBD) in the epoxide ring-opening reaction, a key step in CO₂ conversion. CDs demonstrated a significant catalytic influence, with a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$ and a reactant conversion rate of 70.8 %, outperforming alternative molecular HBD. These findings suggest that CDs could be effective HBD in organocatalyzed CO₂ conversion processes, offering a promising approach for generating value-added products.

Keywords: CO₂ emissions; organocatalysts, carbon-dots; CO₂ conversion; catalyst, climate changes: hydrogen bond donor; nucleophile.

Publications and Presentations of the Author Containing Work Related to this Thesis

The work here reported was presented in scientific meetings and was published in peer-reviewed journals.

A. Published papers as first author

1. Crista, Diana M. A.; Esteves da Silva, Joaquim C. G.; Pinto da Silva, Luís. “Application of Fluorescent Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides”. *Materials* 16, 24 (2023):7620.

2. Crista, Diana M. A.; Algarra, Manuel; Martínez de Yuso, Maria Valle; Esteves da Silva, Joaquim C. G.; Pinto da Silva, Luís. “Investigation of the role of pH and the stoichiometry of the N-dopant in the luminescence, composition, and synthesis yield of carbon dots”. *Journal of Materials Chemistry B* 11, 5 (2023):1131-1143.

3. Crista, Diana M. A.; Esteves da Silva, Joaquim C. G.; Pinto da Silva, Luís. “Evaluation of different bottom-up routes for the fabrication of carbon dots”, *Nanomaterials* 10, 7 (2020):1-15.

4. Crista, Diana M. A.; El Mragui, Abderrahim; Algarra, Manuel; Esteves da Silva, Joaquim C. G.; Luque, Rafael; Pinto da Silva, Luís. “Turning Spent Coffee Grounds into Sustainable Precursors for the fabrication of carbon dots”. *Nanomaterials* 10, 6 (2020):1209.

B. Published papers not as first author

1. Vale, Nuno; Silva, Sara; Duarte, Diana; Crista, Diana M. A.; Pinto da Silva, Luís; Esteves da Silva, Joaquim C. G. “Normal breast epithelial MCF-10^a cells to evaluate the safety of carbon dots”. *RSC Medicinal Chemistry* 12 (2020): 245-253.

B. Oral Communications as Presenting author

1. Crista, Diana M. A. (2022), Evaluation of the role of different bottom-up synthesis procedures for carbon dots in their potential as candidates as drug carriers,

The 8th International Electronic Conference on Medical Chemistry (ECMC 2022), Online Conference, MDPI.

2. Crista, Diana M. A. (**2023**), Evaluation of the pH and stoichiometry of the N-dopant effect into carbon dots properties, PhotOnline 2023, SP2P.

3. Crista, Diana M. A. (**2023**), Effect of pH and stoichiometry on the properties of N-doped CDs, XXVII Encontro Luso-Galego de Química, Porto, Portugal.

4. Crista, Diana M. A. (**2023**), Investigation of the N-dopant stoichiometry into the composition, luminescence and catalytic properties of carbon dots, 2º Encontro Anual do Centro de Investigação em Química (CIQUP), Matosinhos, Portugal.

Structure of the Thesis

This thesis is divided into three major sections: the introduction, the presentation, the discussion of the results, and a conclusion with a future perspective section. Each section comprises chapters corresponding to papers published in specialized peer-reviewed international journals.

The introduction provides a comprehensive review of the climate changes and the crucial role of carbon dioxide followed by a description of the different technologies for CO₂ conversion. Then, a summary of the different catalysts used in CO₂ conversion with emphasis in metal-based catalysts and organocatalysts. The last part comprises a description of CDs properties, the methods used in their synthesis, and their application as catalysts. Then, a brief description of the project's main objectives is included.

The second section encompasses four chapters corresponding to the synthesis of carbon dots from waste materials (Chapter 2), the study of three different bottom-up strategies for CDs synthesis (Chapter 3), the influence of nitrogen doping into CDs synthesis (Chapter 4), and the use of CDs as catalysts for the ring opening reaction of epoxides (Chapter 5).

The third and last section present the main conclusion of the work performed and the future perspectives of this research. Besides, in the attachment section are present other contribution relevant to this work.

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List of Abbreviations

CCS	CARBON CAPTURE AND STORAGE
CCU	CARBON CAPTURE AND UTILIZATION
CCUS	CARBON CAPTURE, UTILIZATION, AND SEQUESTRATION
CDS	CARBON DOTS
CO ₂ RR	CARBON DIOXIDE REDUCTION REACTIONS
COFS	COVALENT ORGANIC FRAMEWORKS
DBD	DIELECTRIC BARRIER DISCHARGE
ER	ELECTROCATALYTIC REDUCTION
FT	FISCHER-TROPSCH
GA	GLIDING ARC
GC-MS	GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROSCOPY
GHGS	GREENHOUSE GASES
HBD	HYDROGEN BOND DONORS
HPLC	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
HR-TEM	HIGH-RESOLUTION TRANSMISSION ELECTRON MICROSCOPY
IPCC	INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE
LCA	LIFE CYCLE ASSESSMENT
MOFS	METAL ORGANIC FRAMEWORKS
MW	MICROWAVE
N-CDS	NITROGEN DOPED CARBON DOTS
QY	QUANTUM YIELD
RWGS	REVERSE WATER-GAS SHIFT
SACS	SINGLE ATOM CATALYSIS
SCGS	SPENT COFFEE GROUNDS
SWCNTS	SINGLE WALLED CARBON NANOTUBES
XPS	X-RAY PHOTOELECTRON SPECTROSCOPY

1. Introduction

1.1. Climate change and the crucial role of carbon dioxide

Climate change, driven predominantly by anthropogenic activities such as the combustion of fossil fuels and deforestation, manifests through alterations in global and regional climate patterns. The primary driver of climate change is the increase in greenhouse gas concentrations in the atmosphere, particularly carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), which trap outgoing heat radiation, resulting in a net warming effect on the Earth's surface [1,2].

Consequences of climate change are multifaceted and encompass a spectrum of environmental, societal, and economic impacts:

- Warming Trends: Global mean surface temperatures are rising, leading to shifts in temperature extremes and altered thermal regimes across terrestrial and aquatic ecosystems [3].

- Changes in Precipitation: Climate change influences precipitation patterns, resulting in alterations in rainfall intensity, frequency, and distribution. This leads to an increased incidence of extreme precipitation events, as well as changes in drought frequency and severity in certain regions [4].

- Sea Level Rise: Thermal expansion of seawater and the melting of polar ice caps and glaciers contribute to rising sea levels. This phenomenon poses risks to coastal regions, exacerbating coastal erosion, inundation, and saltwater intrusion into freshwater sources [5].

- Extreme Weather Events: Climate change amplifies the frequency, intensity, and duration of extreme weather events, including heatwaves, droughts, hurricanes, and storms. These events cause significant socio-economic disruptions, damage to infrastructure, and loss of life [6].

- Ocean Acidification: Increased CO₂ levels in the atmosphere lead to higher absorption of CO₂ by the oceans, resulting in ocean acidification. This phenomenon has detrimental effects on marine ecosystems, particularly coral reefs, shellfish, and other calcifying organisms [7].

- **Loss of Biodiversity:** Climate change poses significant threats to biodiversity through habitat loss, altered species distributions, disruptions to ecological interactions, and increased susceptibility to environmental stressors such as temperature extremes and habitat fragmentation [8].

- **Food and Water Security:** Changes in temperature and precipitation patterns affect agricultural productivity, crop yields, and water availability, posing risks to food and water security, particularly in vulnerable regions with limited adaptive capacity [9].

- **Health Impacts:** Climate change influences human health through various pathways, including heat-related illnesses, respiratory problems associated with air pollution, increased incidence of vector-borne diseases, and compromised food and water safety [10].

Addressing the challenges posed by climate change requires comprehensive mitigation efforts to reduce greenhouse gas emissions, as well as adaptation measures to build resilience and facilitate sustainable socio-economic development in a changing climate.

CO₂ is one of the greenhouse gases (GHGs) resulting from human activities, stands as a primary driver of climate change [11]. By 2022, global CO₂ emissions had surged to 36.8 Gt, with the predominant share stemming from fossil fuel combustion across various industrial sectors and transportation [12]. The continued utilization of oil and natural gas, alongside robust economic growth forecasts, suggests a probable uptick in anthropogenic CO₂ emissions in 2019 [13]. Data from the National Oceanic and Atmospheric Administration/Earth System Research Laboratory indicates a steady rise in global CO₂ emissions, culminating in a record-high atmospheric concentration of 425 ppm in 2023 (Figure 1) [14]. BP's Energy Outlook forecasts a continued escalation in CO₂ emissions from energy usage over the coming decades, with an estimated 10 % increase by 2040 under the evolving transition scenario [15]. In response to these alarming trends, the Intergovernmental Panel on Climate Change (IPCC) advocated for limiting global warming to 1.5 °C, rather than 2.0 °C, to mitigate the risks of catastrophic climate change [16].

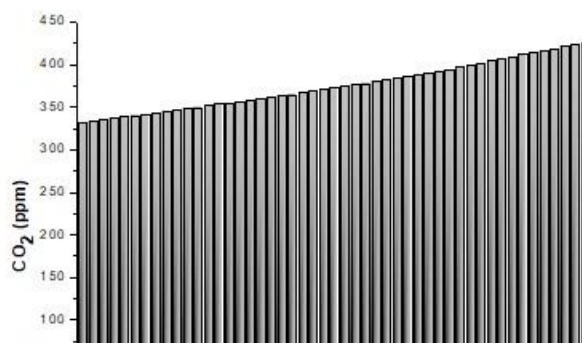


Figure 1. CO₂ emissions in the atmosphere over the past 50 years.

Despite its harmful consequences when mismanaged, this gas can also be regarded as an environmentally friendly, abundant, and low-cost C1 source. Also, CO₂ has several valuable applications and benefits. It is essential for photosynthesis, enabling plants to convert sunlight into energy, which supports plant growth and oxygen production. In agriculture, elevated CO₂ levels can enhance crop yields through CO₂ fertilization, particularly in controlled environments like greenhouses. Industrially, CO₂ is used as a refrigerant, in fire extinguishers, and for carbonating beverages. It also plays a role in enhanced oil recovery, helping extract more oil from reservoirs. Additionally, CO₂ can be used in producing polymers and other chemicals, and emerging technologies are exploring its potential in creating sustainable fuels and materials, thus transforming a GHG into valuable resources. [17].

As a result, developing methods to convert CO₂ into valuable products is crucial for various industries and for reducing climate-altering emissions. Several international agreements recognize the importance of carbon capture, utilization, and sequestration (CCUS)—a combination of carbon capture and utilization (CCU) and carbon capture and storage (CCS)—as essential strategies for sustainably cutting CO₂ emissions and mitigating climate change [14,18,19].

In the CCUS process, CO₂ is first captured from the exhaust gases of fossil fuel combustion and purified to produce a high-purity CO₂ stream. This stream can then be either sequestered or converted into valuable products, yielding environmental, economic, and social benefits. CO₂ can be utilized both physically and chemically across various fields, including chemical, biological, and food processes, which are at different stages of development and demonstration. Despite these efforts, we are significantly failing to meet our greenhouse gas reduction targets, evidenced by arctic

changes and ocean acidification [20,21]. Therefore, it is imperative to employ all available options, including CCS for gas and coal projects and CCU, to reduce energy and resource consumption and enhance innovation and competitiveness [22].

CO₂ storage, or CCS, involves capturing carbon dioxide emissions from sources like power plants and industrial processes and storing it underground to prevent it from entering the atmosphere. This process typically involves capturing CO₂ at the emission source, transporting it via pipelines or ships, and injecting it into deep geological formations such as depleted oil and gas reservoirs, saline aquifers, or unmineable coal seams. By sequestering CO₂ in these secure underground sites, CCS helps mitigate climate change by reducing greenhouse gas concentrations in the atmosphere. While it offers significant environmental benefits, CCS faces challenges related to high costs, potential leakage risks, and the need for long-term monitoring and public acceptance.

CCU involves using captured CO₂ in industrial applications rather than storing it permanently. Currently, CO₂ is utilized in the production of urea, enhanced oil recovery, carbonates, methanol, dimethyl ether, formaldehyde, and other chemicals, with a total market size of around 300 Mt/year. While the market size necessitates that most captured CO₂ be stored, CCU presents a highly attractive method for reducing CO₂ emissions by adding value to the captured CO₂, aligning with the circular economy concept. Some CO₂-derived products can replace more environmentally harmful compounds, and others can be used in new applications, creating fresh business opportunities [23].

The main obstacles to expanding CO₂ applications are the significant energy required to break the CO₂ molecule and the associated capture costs. These issues can be mitigated by enhancing the efficiency of existing technologies or developing new ones. Over the past two decades, research, development, and innovation in CCU have grown exponentially [24].

To meet climate mitigation goals and achieve net-zero targets by 2050, the global CO₂ uptake needs to increase significantly, reaching at least 51.7 Gt of captured CO₂ per year [25]. This substantial rise will greatly expand the potential CO₂ market. The most promising uses for CO₂-derived products include its application as fuel and solid materials primarily for construction, followed by biochar, enhanced oil recovery, and chemical products like methanol and other C₂+ compounds. Although CO₂-based products made from captured CO₂ and green hydrogen are analogous to their market counterparts, they are currently more expensive due to the early stage of technology

development and the cost of clean energy required. Their marketability can be enhanced by their clear environmental benefits and potential superior performance. However, overcoming technological barriers is essential to achieve this stage [24].

Mature technologies produce products like urea, methanol, polyurethane, and polycarbonates, while others such as ethylene carbonate, propylene carbonate, formaldehyde, and acrylic acid are still under development. Furthermore, new sustainable processes for converting CO₂ into methanol, methane, and other chemicals using renewable energy are at various stages of development. E-fuels, produced through these CCU processes, offer significant sustainability advantages by potentially replacing conventional fossil fuels and utilizing existing transport infrastructure. This is particularly beneficial for decarbonizing difficult sectors like heavy transportation, including maritime and aviation. Chemicals derived from captured CO₂, such as methanol, methane, formic acid, and formaldehyde, are also appealing within a circular economy framework. Additionally, algae, which absorb CO₂ from the air or concentrated sources, are emerging as a sustainable source of biomass, food, fuel, and other products [26]. Biodiesel from microalgae [27], though still in its early development stages, has the potential to significantly replace fossil fuels in the future if economic feasibility is achieved.

When developing new processes or products from CO₂, it is crucial to ensure they are sustainable and marketable by meeting these criteria: (1) they should reduce global CO₂ emissions, (2) they must use less and cleaner energy and materials than the processes/products they are replacing, (3) they must be safer, (4) they should facilitate better environmental conditions, and (5) they must be economically viable [24].

1.2. Technologies for CO₂ conversion

CO₂ is a stable molecule, requiring advanced technologies for its conversion into valuable products. Direct and indirect conversion of CO₂ are approaches aimed at transforming CO₂ into useful products to mitigate its environmental impact. Indirect conversion involves using CO₂ as a feedstock to produce intermediate products, which are then further processed into final products. On the other hand, direct conversion involves processes like electrochemical reduction, where CO₂ is directly converted into fuels and chemicals. Each approach offers distinct advantages and challenges, contributing to the development of sustainable technologies for CO₂ utilization and

reducing its atmospheric concentration. The existing conversion methods include CO₂ carbonation, as well as thermochemical, electrochemical, bioelectrochemical, photocatalytic, and plasma-catalytic conversion processes [24].

1.2.1. CO₂ Carbonation

Stable solid carbonates, primarily used in the construction industry, are created by reacting CO₂ with metal cations found in natural minerals or industrial solid wastes. This technology is based on the same principles as in situ CO₂ storage, where CO₂ injected into geological formations is permanently stored as carbonates. The primary industrial application of CO₂ carbonation involves using CO₂ for concrete curing. This process enhances the strength of the cured concrete by forming calcium carbonates [28]. Other methods for creating CO₂-derived aggregates and construction materials, are still under development.

1.2.2. Thermochemical Conversion

Heat and pressure are used to thermochemically convert CO₂ into various value-added products, including methanol, methane, urea, and syngas. Because of the harsh operating conditions, catalytic materials are often employed to enable these reactions under milder conditions.

Urea is the primary commercial product from this technology. In 2021 [23], 130 Mt of urea were produced, with demand expected to rise due to population growth. The energy-intensive process involves the Bazarov reaction, where CO₂ and ammonia are converted into ammonium carbamate at high temperature and pressure [29], followed by dehydration to form urea. This step uses a copper(II) sulfate complex catalyst to speed up the endothermic reaction [29].

Other CO₂-derived products include methane, produced via the Sabatier process (power-to-gas) [30]. This highly exothermic reaction involves reacting CO₂ with H₂ at high temperatures. Commercial methanol production similarly relies on the thermochemical reaction between CO₂ and H₂ [31]. Additionally, the reverse water-gas shift (RWGS) reaction can convert CO₂ and H₂ into CO and water, which can then be processed via the Fischer-Tropsch (FT) reaction to produce synthetic fuels like kerosene, gasoline, and diesel [32,33].

Key challenges in producing sustainable fuels and chemicals from CO₂ thermochemical conversion include developing efficient catalytic materials that can withstand high operating conditions and producing low-cost, low-carbon hydrogen.

1.2.3. Electrochemical Conversion

Electrochemical conversion involves the nonspontaneous reduction of CO₂ into products with lower oxidation states using electricity as the energy source. This process can produce a variety of products, including methanol, carbon monoxide (CO), carboxylic acids, alcohols, and alkenes [34,35].

Electrocatalytic reduction (ER) can be performed at both low and high temperatures, resulting in different products. High-temperature electrolysis typically occurs in the gaseous phase within solid oxide electrolyzers, producing methane, CO, and syngas. In contrast, low-temperature electrolysis is more versatile but less energy efficient, capable of generating C₂ products such as ethanol, ethylene, and formic acid [36].

Recent studies suggest using formic acid as a hydrogen carrier instead of ammonia, despite its lower volumetric capacity (1.77 kW·h/L versus 3.2 kW·h/L for ammonia), because it offers a higher capacity than commercial hydrogen tanks (1.4 kW·h/L at 70 MPa hydrogen pressure) and has lower toxicity and flammability under ambient conditions. Additionally, with suitable catalysts, the energy required to release hydrogen from formic acid is lower than from ammonia, making it a potential candidate for fuel cell applications [37]. However, using formic acid for this purpose results in CO₂ emissions that must be captured, adding extra costs and complexity compared to directly using hydrogen in fuel cells.

Electrochemical conversion pathways have the potential to revolutionize industrial decarbonization by providing sustainable methods for CO₂ conversion and chemical production. However, cost remains a significant challenge compared to conventional thermochemical conversion. Achieving cost competitiveness will depend on the availability and price of renewable electricity and the maturity of CO₂ capture technologies [24].

1.2.4. Bioelectrochemical Conversion

Natural organisms such as plants, microalgae, and cyanobacteria use CO₂ in photosynthesis to produce sugars and other organic materials. Similarly, biochemical conversion uses enzymes or microbial catalysts to transform CO₂ and water into various products, including methane, methanol, CO, and biomass [38]. Promising species for this process include spirulina microalgae [39], cyanobacteria, and carbonic anhydrase [40,41]. The efficiency of this process depends on the organism's growth rate, contamination tolerance, and control of operating conditions.

This process is highly sustainable as it uses biocatalysts rather than metal-based catalysts. However, direct conversion of CO₂ into value-added products via bioelectrochemical processes is still in its early stages. This is due to the high costs associated with controlling the bioreaction environment, the need for precise reaction conditions, and the challenges of providing a continuous nutrient supply for large-scale microbial cultures [42].

An alternative two-step process for methane production is closer to commercialization. This process starts with hydrogen production, followed by converting H₂ and CO₂ into methane in bioreactors using anaerobic methanogens.

1.2.5. Photocatalytic Conversion

This technology employs light irradiation and photocatalysts to reduce CO₂ into methane, syngas, and other hydrocarbons. It is considered one of the most sustainable CO₂ conversion methods compared to electrochemical and thermochemical processes because it does not require additional energy input. However, it faces challenges such as low yield and selectivity, which depend on the choice of catalysts [43].

In the photocatalysis process, light radiation hits the surface of the photocatalyst, a light-sensitive material, generating numerous electron (e⁻) and hole (h⁺) pairs. These pairs participate in oxidation or reduction reactions, producing the final products. Various photocatalysts have been used for CO₂ reduction, utilizing different light irradiation types, including visible light and, to a lesser extent, UV light. TiO₂ is the most widely studied catalyst for CO₂ photocatalytic conversion due to its excellent stability, nontoxicity, and low-cost [44]. However, it suffers from low visible light absorption and rapid electron-hole recombination, prompting the search for novel strategies to fine-tune the photocatalyst [45,46].

1.2.6. Plasma-catalytic Conversion

This technology utilizes highly reactive weakly ionized plasma, generated by applying a light electrical field to a gas. This creates a reactive environment that enables chemical reactions under mild conditions and throughout the entire reactor volume, rather than solely on the catalyst surface [47–49]. A major advantage of this technology is that excited electrons can activate CO₂ molecules to produce new products without heating the entire gas. The reaction mechanisms vary with the type of plasma used, such as dielectric barrier discharge (DBD), microwave (MW), and gliding arc (GA) [50,51].

However, this approach reduces process selectivity, complicating the targeted production of specific products. This issue has been addressed by coupling plasma with solid catalysts. Despite achieving adequate conversion levels at room temperature and pressure, the technology faces challenges with energy efficiency and scalability for industrial applications [49].

1.3. CO₂ Conversion into ethanol and methanol

With the introduction of "emission peaking" and "carbon neutralization" initiatives, the conversion and utilization of CO₂ have gained significant attention [52–56]. Photocatalytic conversion of CO₂ into valuable fuels is a promising approach as it addresses climate issues related to greenhouse gas emissions and simultaneously stores renewable solar energy as chemical energy [57–60]. The products of CO₂ photocatalytic conversion include CO [61–63], CH₄ [64,65], CH₃OH (methanol) [66,67], C₂H₅OH (ethanol) [68–70], HCOOH [71], among others. Converting CO₂ into methanol and ethanol is vital for environmental sustainability, energy security, and economic development. This process helps mitigate climate change by reducing atmospheric CO₂ levels, turning a GHG into valuable resources. Methanol and ethanol serve as alternative fuels, reducing reliance on fossil fuels and enhancing renewable energy use. Economically, this conversion creates value-added products and new revenue streams, while also providing essential chemical feedstocks for various industrial applications [72].

Catalysts are crucial for the efficiency of photocatalytic CO₂ conversion to ethanol. Numerous photocatalysts have been developed, including TiO₂ [73], Bi₂MoO₆ [74], g-C₃N₄ [75], Cu/TiO₂ [76], and AuCu/g-C₃N₄ [77]. These catalysts can be broadly

categorized into semiconductors, plasmonic metal-based catalysts, and other types based on their properties and mechanisms [72].

These catalysts facilitate the absorption of light and the subsequent chemical reactions that convert CO₂ and water into ethanol. The efficiency of these reactions depends on the catalysts' ability to enhance light absorption, improve charge separation, and reduce recombination rates [72].

Despite progress, challenges remain in enhancing the selectivity and efficiency of photocatalysts, as well as scaling up the process for industrial applications. Future research aims to develop more efficient catalysts, understand reaction mechanisms better, and integrate these processes with renewable energy sources to make them economically viable [78].

Also, methanol is a versatile chemical used to produce formaldehyde, acetic acid, and other chemicals. It also serves as a fuel and can be blended with gasoline to reduce emissions. The ability to produce methanol from CO₂ not only helps in reducing greenhouse gases but also provides a renewable pathway to generate important industrial chemicals and fuels [41].

The hydrogenation of CO₂ to methanol is a well-researched method involving the use of thermal catalysts. This process typically requires a combination of CO₂ and H₂ over catalysts like Cu/ZnO/Al₂O₃ under high temperatures and pressures. This method is advantageous due to its high selectivity and yield of methanol, making it a promising approach for large-scale application [79].

Electrochemical and photochemical methods are also used for CO₂ conversion into methanol. These methods use electricity (from renewable sources) or light to drive the conversion of CO₂ to methanol. Electrochemical conversion involves using electrodes and catalysts to reduce CO₂ in an aqueous solution, while photochemical methods use light-absorbing materials to drive the reaction. These methods are still under development but offer the potential for more sustainable and efficient conversion processes compared to traditional thermal methods [41].

In conclusion, converting CO₂ into ethanol and methanol represents a crucial strategy for addressing climate change and advancing sustainable energy technologies. Continued research and development are needed to overcome existing challenges and to implement these technologies on a commercial scale effectively.

1.4. Catalysts for CO₂ conversion

1.4.1. Metal-based catalysts

Metal-based catalysts play crucial roles in a wide range of chemical reactions due to their ability to increase reaction rates and selectivity. These catalysts help reduce CO₂ under various reaction conditions, including thermal, electrochemical, and photochemical processes. Here's an overview of the different types of metal-based catalysts used for CO₂ conversion:

1.4.2. Transition Metal Catalysts

1.4.2.1. Cobalt-Based Catalysts

Cobalt is extensively studied for its role in CO₂ reduction reactions (CO₂RR). Cobalt-based catalysts, including cobalt single atoms, multi-metal alloys, complexes, and metal-organic frameworks (MOFs), exhibit good stability and efficiency. These catalysts can reduce CO₂ at lower overpotentials, which is crucial for practical applications [80].

1.4.2.2. Nickel-Based Catalysts

Nickel is effective in electrochemical CO₂ reduction, often used in combination with other metals like copper (NiCu) or iron (NiFe). These combinations enhance catalytic activity and selectivity towards hydrocarbons and alcohols. Nickel catalysts are valued for their relatively low cost and robust performance [81].

1.4.3. Precious Metal Catalysts

1.4.3.1. Gold and Silver Catalysts

Gold and silver are renowned for their high catalytic efficiency. Gold nanoparticles, in particular, show excellent activity in reducing CO₂ to CO and subsequently to methanol. Silver, while less active than gold, is cost-effective and selective towards CO production, which can be further converted into other products [80,82].

1.4.3.2. Platinum and Palladium Catalysts

Platinum and palladium are highly effective for hydrogenation reactions, essential for converting CO₂ to methanol and ethanol. They facilitate CO₂ adsorption and activation, leading to high reaction rates and selectivity. Despite their high cost, their performance makes them critical for research and development [81].

1.4.3.3. Single-Atom Catalysts

Single-atom catalysts (SACs) involve individual metal atoms dispersed on support materials. These catalysts offer high atom efficiency and unique electronic properties that enhance catalytic performance. Metals like cobalt, nickel, and iron are used as SACs, showing promising results in activity, selectivity, and stability for CO₂ reduction [82].

1.4.4. Nanostructured and Hybrid Catalysts

1.4.4.1. Metal-Organic Frameworks (MOFs) and Covalent Organic Frameworks (COFs)

These materials provide high surface areas and tunable pore structures, beneficial for CO₂ adsorption and activation. Incorporating metals like cobalt, nickel, or copper into MOFs or COFs significantly enhances their catalytic properties. These frameworks offer a versatile platform for designing efficient CO₂ conversion catalysts [80].

1.4.4.2. Plasmonic Metal-Based Catalysts

Plasmonic metals such as gold and silver can utilize light energy to drive CO₂ reduction, making them suitable for photocatalytic applications. Combining these metals with semiconductors can improve their efficiency and selectivity for converting CO₂ into ethanol and methanol [82].

1.5. Organocatalysts

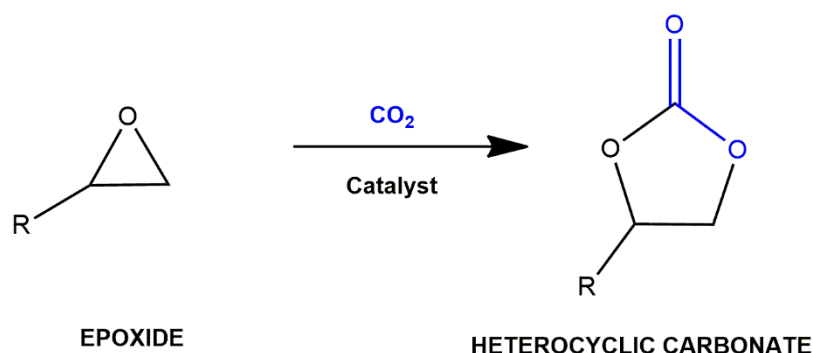
In nature, CO₂ is captured, converted, and reduced to carbohydrates through a series of cascade reactions facilitated by specific metalloenzymes found in photosynthetic organisms [83]. Similarly, the field of CO₂ conversion catalysis is largely dominated by transition metal-based catalysts, which can be either heterogeneous or homogeneous in nature [84–87]. While using a catalytic system generally suggests an improvement in sustainability, there are notable differences between metal-based and organo-catalysts. It has been demonstrated that the activation and/or stabilization of substrates or intermediates through metal complexes or immobilized metal species via coordination motifs is significantly more efficient than through organo-based catalytic mediators. However, organocatalysts are typically low-cost, non-toxic molecules characterized by good stability and resistance to moisture and air. Thus, from a reaction design perspective, they can be considered safer reaction components. Additionally, organocatalysts can sometimes be readily derived from renewable feedstocks, enabling the development of technically and economically viable procedures starting from biomass and CO₂, which can eventually lead to "carbon-neutral" processes for producing sustainable chemicals, fuels, and materials [88].

It is known that organocatalysts can sometimes result in higher energy consumption during reactions and work-up time, necessitating higher catalyst loadings, higher operating temperatures, and prolonged reaction times. Nevertheless, in certain cases (e.g., when using ionic liquids as reaction solvents and/or catalysts), reagents and products can be recovered through simple distillation, devising safer and more atom-economical processes while avoiding waste-generating work-up procedures. Catalyst heterogenization has been extensively utilized to extend the lifetime of organo-catalysts, simplifying reaction monitoring and work-up procedures and providing a potential pathway towards developing continuous flow reactions. Organocatalysis is particularly advantageous for developing metal-free products or processes, as including chemicals in consumer products requires minimal metal residues to avoid

toxicity issues. As these requirements become increasingly stringent, alternative processes that bypass the use of metal catalysts could produce targeted chemicals in a safer and potentially greener manner. In this context, organo-based catalysts (i.e., organocatalysts) are of growing interest and importance in the area of CO₂ catalysis and conversion. Nevertheless, organocatalytic systems also present some limitations, such as requiring higher temperatures and CO₂ pressures [89]. To overcome these issues, researchers have been focused on the development of two-component organocatalytic systems, which are composed of a nucleophile and a hydrogen bond donor (HBD), in which the limitations of both transition metal-based catalysts and organocatalysts are bridged.

1.5.1. Organocatalyzed coupling of carbon dioxide with epoxides for the synthesis of cyclic carbonates

One of the most promising methods for utilizing CO₂ is its conversion into cyclic carbonates via its reaction with epoxides (Scheme 1) [90].



Scheme 1. Cycloaddition of CO₂ to epoxides.

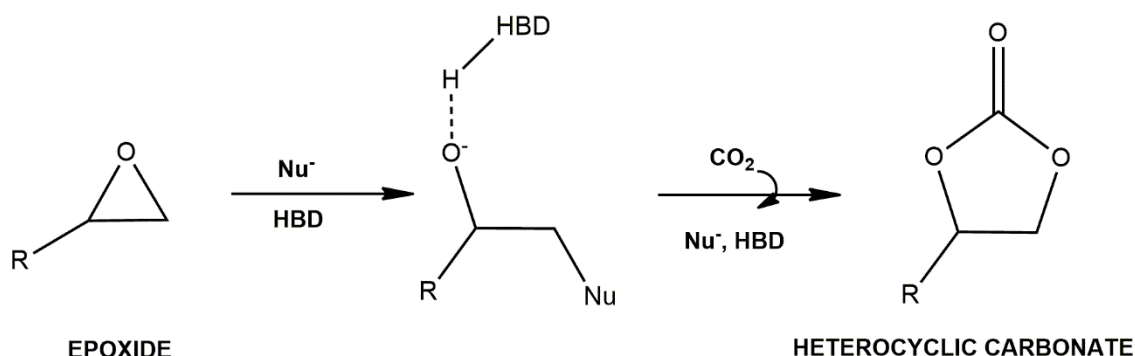
Cyclic carbonates are organic compounds that exhibit desirable properties such as low vapor pressure, high boiling point, low toxicity, and biodegradability. Due to these characteristics, cyclic carbonates are widely used as aprotic high-boiling polar solvents, electrolytes for batteries, precursors for polymeric materials, fuel additives, plastic materials, and intermediates in the synthesis of fine chemicals, including dialkyl carbonates, glycols, carbamates, and pyrimidines [91–93].

Traditionally, cyclic carbonates have been synthesized industrially using phosgene. However, this method produces equimolar amounts of carbonates and chlorinated salts, along with large quantities of chlorinated solvents. Although phosgene is a versatile building block in producing plastics and pesticides, it is also hazardous, toxic, corrosive, and challenging to handle safely. Exposure to phosgene can lead to severe health issues. Therefore, replacing phosgene with more sustainable and environmentally friendly alternatives in large-scale industrial synthesis is highly desirable.

One such alternative is the synthesis of cyclic carbonates from CO₂ and epoxides, which offers a low-toxicity route [94].

The simplest and most cost-effective catalysts for the cycloaddition of CO₂ to epoxides are halide salts, particularly organic halides such as imidazolium salts, quaternary ammonium salts, and phosphonium salts, which demonstrate the best catalytic performance [95,96]. These compounds contain a halide nucleophilic species that catalyzes the reaction by first opening the epoxide ring, followed by the nucleophilic attack of the resulting alkoxide on CO₂, and finally, ring closure to form the cyclic carbonate product [96,97]. Among organic halides, phosphonium salts like PPNCI (bis(triphenylphosphine) iminium chloride) show high catalytic activity due to the delocalization of the positive charge and the bulky nature of the cation, making the halide more readily available as a nucleophile [98–100]. However, phosphonium salts are not ideal from a green chemistry perspective because their synthesis involves toxic organophosphines [101,102]. Generally, these organocatalysts are less active than catalytic systems that include metal centers acting as Lewis acids, which enhance the reaction by activating the epoxide toward nucleophilic attack by the halide [97,103,104]. State-of-the-art metal-based homogeneous and heterogeneous catalysts enable the reaction of CO₂ with epoxides under milder conditions (i.e., $T \leq 45\text{ }^{\circ}\text{C}$ and $p\text{CO}_2 \leq 10\text{ bar}$) compared to organic halides alone [97,103,105]. However, the synthesis of these metal-based catalysts is costly and complex, and the toxicity of some metals used raises concerns for large-scale applications [106].

HBD have emerged as a greener alternative to metal centers as Lewis acid species and have been increasingly studied in recent years. HBD can activate the epoxide by forming a hydrogen bond with its oxygen atom [96,107]. In this type of system, with the goal of facilitating the epoxide's ring opening by nucleophilic attack, the HBD interact with the O-donor of the epoxide via hydrogen bonding in a process involving a suitable nucleophile (Scheme 2).



Scheme 2. Reaction scheme for CO₂ conversion in the presence of a nucleophile (Nu[−]) and a hydrogen bond donor (HBD).

Furthermore, the HBD are expected to stabilize the oxyanion resulting from the ring-opening reaction via hydrogen bonding. These synergistic effects should make the ring-opening reaction of the epoxide more efficient, allowing the production of heterocyclic carbonates from CO₂ at lower temperatures and CO₂ pressures. It should be noted that in this type of catalytic scheme, the ring opening of the epoxide through nucleophile attack typically represents the rate-limiting step of the process. So, adding an HBD is made with the expectancy of decreasing the activation barrier for this step, thus increasing efficiency. Considering this, there is a growing interest in the development and identification of new HBD with the potential to be used in CO₂ conversion reactions [108].

Various HBD compounds have been explored, including organic acids [109,110], alcohols [111,112], phenolic derivatives [113,114], silanediols [115], and water [99,111]. Bio-based compounds, often rich in HBD groups, have been utilized for this purpose, such as amino acids, chitosan, cellulose, and lignin [116]. HBD are generally used in binary catalytic systems with an organic halide, most commonly Bu₄NX or PPNX (X = Cl, Br, I) [99,112,113]. Compared to organic HBD, water is preferable in the context of green chemistry due to its non-toxicity, low cost, abundance, renewability, and ease of separation [99].

An appealing alternative is to design a single-component bifunctional catalyst that includes both the nucleophile and an HBD group (e.g., −OH, −COOH, or −NH−) within the same compound, ideally in close proximity to enhance their cooperation [117–119]. Such metal-free bifunctional catalysts have been developed for both homogeneous and heterogeneous catalysts [96,107,120]. Homogeneous catalysts

generally exhibit higher activity but face challenges in catalyst separation and recycling [121]. This issue is inherently resolved with heterogeneous catalysts, although they often suffer from lower catalytic activity due to reduced accessibility of the active sites [94,122–125]. Therefore, designing metal-free bifunctional catalysts that combine the benefits of both homogeneous and heterogeneous systems while avoiding their limitations is a desirable but challenging goal [126].

1.6. Carbon-Dots

Fluorescent carbon nanoparticles, also known as CDs, have emerged as a promising alternative to traditional semiconductor quantum dots, constituting a novel category of carbon nanomaterials.[127] CDs were accidentally discovered in 2004 by Xu et al. when they isolated carbon nanoparticles as a by-product during the synthesis of single-walled carbon nanotubes [128]. CDs are typically defined as a fascinating type of carbon nanomaterials with a core that can be either amorphous or nanocrystalline and have sizes typically below 10 nm. Their structure consists of sp² and sp³ carbon atoms with various polymer chains or functional groups, such as -OH, -COOH, and -CHO, attached to their surfaces. These groups enhance CDs' water solubility and provide binding sites for further functionalization with other molecules [129–131].

1.6.1. Properties and applications of CDs

CDs have attracted significant attention from the research community due to their quite remarkable features, such as high photoluminescence [132,132–134], low toxicity [135], good water solubility [127,132,132], biocompatibility [128,136], and good physical–chemical and photochemical stability [127,137,138]. Therefore, it is not surprising that CDs have gained relevance in various fields, such as sensing [139–141], bioimaging [142], photocatalysis [134], drug delivery [143], solar cells [144], and photodynamic therapy [145].

Synthetic approaches for CDs are generally classified into two categories: top-down and bottom-up.[127] The former approach is based on the fragmentation of large graphitic materials into smaller carbon-based materials [146]. However, top-down methodologies typically necessitate rigorous reaction conditions, costly materials and equipment, and long processing durations. Conversely, bottom-up methodologies are arguably the most prevalent synthesis techniques [147–149]. These methods typically involve the pyrolysis of smaller organic molecules, either in powder form (via

calcination) [150] or in solution using hydrothermal [148,151] or microwave-based techniques [138,152,153]. Bottom-up approaches offer advantages such as scalability for mass production, eco-friendliness, and cost-effectiveness [149].

While top-down approaches are constrained to utilizing carbon "bulk" samples as precursors (e.g., graphite powders, graphene, and black carbon) [154], bottom-up methodologies can employ a diverse array of molecular organic precursors, ranging from simpler chemicals like carbohydrates and acids to more heterogeneous sources such as rice, coffee beans, and waste materials [147,155–159]. The structure, surface functionalization, and photoluminescence of carbon dots (CDs) are heavily influenced by the selection of precursors and synthesis methodologies. The adaptability of bottom-up strategies enables many possibilities for tailoring the properties of CDs and pioneering novel applications.

Another attractive property of CDs is their ability to be synthesized from a broad range of precursors, eliminating the need for costly, toxic materials or complex equipment. In essence, their typical synthesis pathway involves straightforward thermal or microwave-assisted treatment of organic molecules, commonly using glucose or citric acid as a carbon source. However, synthesis using only carbon precursors usually leads to CDs with low photoluminescence. Due to that, researchers typically add also precursors containing appropriated heteroatoms such as N, S, and P, to achieve heteroatom-doping of carbon nanoparticles, which generally enhances the fluorescence quantum yield (QY_{FL}) of the CDs [160–163]. Among these, N-doping is the most used heteroatom doping to enhance the QY_{FL} of the CDs. N-doping is achieved by the addition of nitrogen-containing small organic molecules as a nitrogen source of the carbon precursor in which urea and ethylenediamine are the most common options.

1.6.2. CDs as catalysts

Carbon dots (CDs) have been proposed for use as photocatalysts, electrocatalysts, and photoelectrocatalysts due to their diverse structures, properties, and applications [164]. Utilizing solar energy, a renewable and clean energy source, has made photocatalysis a prominent application for CDs. They are used as photocatalysts for degrading pollutants (such as organic dyes [165,166] and metal ions [167–169]), water splitting [170–172], CO₂ reduction [172,173], and chemical reactions during solar energy conversion [174]. This is possible due to their broad light

absorption range (from UV to NIR), low cost, excellent photostability, and efficient charge separation and transfer capabilities.

CDs with nitrogen atoms positioned at the edge sites of aromatic domains facilitate efficient interfacial electron transfer, enhancing photocatalytic hydrogen generation from water [175]. Typically, CDs are hybridized with other nanomaterials (such as Fe₂O₃ or g-C₃N₄) to serve as light absorbers and/or electron acceptors in heterojunctions, thereby improving photocatalytic performance [168,171].

1.7. Objectives

The world depends on fossil fuels for energy and as raw materials [176,177]. The combustion of fossil fuels releases CO₂ into the atmosphere, causing its levels to rise by a third since the Industrial Revolution began [178]. This increase in atmospheric CO₂ has contributed to the rise in Earth's surface temperature, making CO₂ a major driver of climate change. Consequently, reducing atmospheric CO₂ levels is one of the most pressing issues of our time.

Despite this, CO₂ can be seen as an abundant, renewable, cheap, and non-toxic carbon source [83,178–180]. Its catalytic coupling with epoxides to form heterocyclic carbonates presents a promising approach for recycling CO₂ into valuable organic compounds. These heterocycles are essential in the synthesis of drugs, polymers, and other fine chemicals [181–183]. Therefore, developing cost- and energy-efficient methods for CO₂ recycling could significantly reduce emissions and transform CO₂ into a valuable commodity.

Transition metal-based catalysts have enabled CO₂ recycling at room temperature with high yields [83,84,179,180,184]. However, organocatalysts are preferable due to their lower cost, non-toxicity, and availability [83,180,185]. Nonetheless, current organocatalysts still require high temperatures and CO₂ pressures [83,180]. Thus, advancing efficient organocatalysts is crucial for developing a cost-effective, non-toxic, and sustainable CO₂ recycling process.

The objective of this PhD project is to develop a catalyst based on carbon-based nanoparticles, specifically CDs, capable of recycling CO₂ and epoxides into valuable cyclic carbonates under mild conditions with significant yields. CDs are water-soluble, highly stable, biocompatible, non-toxic, and easily functionalized. Additionally, they can be mass-produced from low-cost and renewable carbon sources using nearly reagentless synthetic approaches [132,140,186–189].

These CDs will be doped with nitrogen heteroatoms introducing pyridinic nitrogen to the surface of CDs, which facilitates the activation of both the epoxide and CO₂ [190–192].

Essentially, this project has the distinct objectives:

- I. Evaluation of different synthetic methodologies for CDs production and its effect on their characteristics and properties.
- II. CDs synthesis from waste materials sources;
- III. Evaluate the catalytic activity of the synthesized CDs towards CO₂ recycling.

2. Carbon dots production from organic waste materials

2.1. Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots

Article 1

Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots

Diana M.A.Crista, Abderrahim El Mragui, Manuel Algarra, Joaquim C.G. Esteves da Silva, Rafael Luque and Luís Pinto da Silva, *Nanomaterials*, **2020**, 10, 1209.

2.1.1. Contextualization

Spent coffee grounds (SCGs) are recognized for their abundance in various organic compounds such as carbohydrates, lipids, phenolic compounds, and proteins. Thus, we explored their potential as a viable source for synthesizing CDs employing a nanotechnological approach. We conducted a comparative analysis between CDs derived from SCGs and conventional precursors like citric acid and urea.

The SCG-derived CDs were synthesized through one-pot, solvent-free carbonization of solid samples, yielding nanoscale particles measuring between 2.1 and 3.9 nm. These nanoparticles exhibited blue fluorescence with moderate quantum yields ranging from 2.9% to 5.8% and displayed an excitation-dependent emission characteristic typical of carbon dots. The SCG-derived CDs demonstrated promise as environmentally significant fluorescent probes for detecting Fe³⁺ ions in water. Notably, life cycle assessment studies affirmed the environmental sustainability of CD production from SCG samples compared to conventional precursors, whether assessed by weight or function-based functional units.

2.1.2. Contributions to this work

All synthesis of CDs, as well as their optical characterization and life cycle assessment (LCA) studies, was performed by me (Diana Crista) with the assistance of Dr. Abderrahim El Mragui under the supervision of my supervisors. XPS analyses were performed by the team of Dr. Manuel Algarra at the University of Malaga. AFM and Zeta Potential measurements and discussion were carried out with my assistance.



Article

Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots

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Abstract: Spent coffee grounds (SCGs) are known for containing many organic compounds of interest, including carbohydrates, lipids, phenolic compounds and proteins. Therefore, we investigated them as a potential source to obtain carbon dots (CDs) via a nanotechnology approach. Herein, a comparison was performed between CDs produced by SCGs and classic precursors (e.g., citric acid and urea). The SCG-based CDs were obtained via the one-pot and solvent-free carbonization of solid samples, generating nanosized particles (2.1–3.9 nm). These nanoparticles exhibited a blue fluorescence with moderate quantum yields (2.9–5.8%) and an excitation-dependent emission characteristic of carbon dots. SCG-based CDs showed potential as environmentally relevant fluorescent probes for Fe³⁺ in water. More importantly, life cycle assessment studies validated the production of CDs from SCG samples as a more environmentally sustainable route, as compared to those using classic reported precursors, when considering either a weight- or a function-based functional unit.

Keywords: carbon dots; nanotechnology; fluorescence; spent coffee grounds; life cycle assessment; sensing

1. Introduction

Carbon dots (CDs) are fluorescent carbon-based nanomaterials with a near-spherical shape and are typically sized below 10 nm [1,2]. CDs possess remarkable properties, including high photoluminescence, biocompatibility, high photostability and chemical stability, good water solubility and simplicity of functionalization [3–7]. Thus, it is not surprising that CDs have emerged as a suitable replacement for more traditional fluorophores, being used in several fields, such as the fabrication of light emission devices, bioimaging, sensing, drug delivery and biomedicine [2,8–11].

Another very attractive feature of CDs is that they can be fabricated from a large variety of precursors without expensive and/or toxic materials or sophisticated equipment [12–15]. In fact, their general synthetic route is based on the simple thermal or microwave-assisted treatment of organic molecules as a carbon source (generally citric acid), together with other organic molecules as a nitrogen source (e.g., urea as a common nitrogen source), both in aqueous solution or in powder form [16–18].

These approaches make CDs a sustainable and non-toxic alternative to metal-based quantum dots. However, most available organic molecules are still expensive, and their use and/or synthesis can pose significant challenges to the environment and to human health [19]. It has become quite desirable to use biomass waste as alternative precursors in the synthesis of CDs [20], given that biomass waste material is ubiquitous, nontoxic, cheap and renewable [21]. Furthermore, the use of such biomass would allow the valorization of waste material of other industries, a desirable outcome toward the establishment of a circular economy.

One of the biomass waste materials of increasing importance is spent coffee grounds (SCGs), which are the residues obtained from treatment of coffee powder with hot water or steam during the brewing process [22]. In fact, coffee consumption has increased significantly in recent years, with a worldwide consumption of over 9.5 million tons in 2017. Among the different ways of consuming coffee, one of the most popular ways is by using disposable espresso capsules, being a method adopted in homes, offices and cafeterias [23]. This significant increase in coffee consumption has consequently led to an increase in waste products, including used coffee capsules containing coffee grounds. In fact, their increasing numbers has forced companies to create various recycling programs. For example, Nespresso created a worldwide collection system consisting of 14,000 collection points and over 88,000 United Parcel Service (UPS) drop off points in the USA. Residues from espresso coffee consumption are also considered to be the main waste generated by cafeterias widespread all over the world. In simple terms, used coffee capsules are composed by the capsule itself and by SCGs [22], with most of a used capsule's weight corresponding to SCGs. Thus, SCGs are becoming a quite problematic waste product. Interestingly, SCGs have been found to contain many organic compounds of interest, such as carbohydrates, lipids, phenolic compounds and proteins [24]. Thus, SCG waste has significant potential for valorization.

Given this, it is not surprising that several researchers have already used SCGs as precursors for the fabrication of CDs. Chang and co-workers pioneered the use of SCGs in the fabrication of CDs, by performing the one-pot thermal carbonization of the samples and obtaining nanoparticles with a size of 5 ± 2 nm and a fluorescent quantum yield of 3.8% [25]. These CDs were then used in cell imaging and surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) of angiotensin I and insulin. CDs with similar properties (a size of 4.4 nm and a quantum yield of 5.5%) were also tested for cells and fish imaging [26]. SCG-based CDs were also applied for the sensing of heavy metals, [27] after synthesis via the hydrothermal treatment of SCGs in the presence of hydrazine and subsequent functionalization with poly(ethylene imine), from which resulted nanoparticles with average sizes between 1.88 ± 0.72 nm and 2.67 ± 0.81 nm. Finally, Li and co-workers have prepared CDs from the treatment of coffee bean shells with an aqueous sodium hydroxide solution, followed by precipitation with hydrochloric acid. This resulted in the formation of CDs with diameters ranging from 1 to 5 nm, which were later used for banana storage (based on their antioxidation ability), and the imaging of cellular nuclei and in vivo bioimaging [28].

These results demonstrated that SCGs can indeed be used in the fabrication of CDs with relevant practical applications (bioimaging and sensing), which indicated that SCGs could provide a more environmentally sustainable route for the synthesis of CDs. However, these studies failed to assess some significant aspects. Namely, they did not investigate the environmental impacts associated with the synthesis of CDs from SCGs. They also did not compare the properties of the SCG-based CDs with those of CDs obtained from standard precursors (such as citric acid and urea). Thus, while the claim was made, there is not enough information to determine if the use of SCGs as precursors can indeed lead to a more environmentally sustainable fabrication of CDs, when in comparison with the use of standard precursors. This can be particularly problematic if we consider that, while the use of standard precursors might lead to a less sustainable synthesis route (when considering only the amount of CDs), they also might lead to improved properties that could justify a more resource-intensive synthesis.

It should be pointed out that most studies found in the literature regarding CDs are focused on the development of new individual nanoparticles with novel applications at the laboratory scale. However, and despite the hundreds to thousands of such papers, the translation of this vast research

to industrial/commercial applications is non-existent. This is due to the fact that, while each new study uses different synthesis protocols and/or precursors, leading to almost unrepeatable nanoparticles, there are only limited data on trends that may allow us to predict the structure/surface functionalization and photoluminescence of CDs. This makes the reproduction of existent results difficult and prevents the rational development of new CDs, leaving the research community to serendipitous discoveries, given that there has been an insufficient effort to develop a well-defined and reproducible scaffold structure for CDs, which researchers can use to improve and modify in a rational and incremental way toward new applications.

Herein, we have compared the fabrication of CDs prepared from different SCG samples and from standard precursors (citric acid and urea), [16,29,30] via a one-pot and solvent-free carbonization method [21]. The objective of this comparison is to determine if the use of SCGs as precursors really leads to a more environmentally sustainable synthesis route than the use of standard precursors, even when the functional properties of all CDs are considered (such as synthesis yield and fluorescent quantum yield). Our work also aims to determine if different SCGs can be used as a family of precursors that can generate reproducible and predictable CDs, with identical structure/surface functionalization and photoluminescence, and in this way lead to the formation of common and dependable CD scaffolds.

To this end, the resulting CDs were characterized by atomic force microscopy (AFM), dynamic light scattering (DLS) and X-ray photoelectron (XPS), UV-Vis and fluorescence spectroscopy. A comparative life cycle assessment (LCA) study was also performed for all CDs, to determine which is the most environmentally sustainable fabrication method for fluorescent CDs.

2. Materials and Methods

2.1. Materials

Three different SCG samples were obtained from three different used espresso capsules, each one corresponding to different Portuguese coffee brands: Chave D'Ouro[®] descafeinado (decaffeinated) blend, Nicola[®] Rossio (intense) blend, and Chave D'Ouro[®] Prestígio (Prestige) blend. Citric acid and urea were purchased from Sigma-Aldrich (St. Louis, MI, USA).

2.2. Fabrication of CDs

CDs were fabricated via a one-pot and solvent-free carbonization method, based on the pioneering work of Hsu and co-workers in the use of SCGs as CD precursors [25]. More specifically, the three SCG-based CDs were prepared from different coffee blends from Portuguese brands: Chave D'Ouro[®] decaffeinated blend, Nicola[®] Rossio (intense) blend, and Chave D'Ouro[®] Prestígio (Prestige) blend. The resulting CDs were then identified as: decaf-CDs from Chave D'Ouro[®] decaffeinated blend, rossio-CDs from Nicola[®] Rossio (intense) blend and prestige-CDs from Chave D'Ouro[®] Prestígio (Prestige) blend.

All CDs were fabricated according to the same procedure. Namely, 2.5 g of each SCG sample were placed in a beaker, which was heated at 200 °C for 4 h in a VWR DL 112 Prime oven (2500 W power consumption). The synthesized CDs were subsequently dissolved in water, while discarding unsuspended and insoluble aggregates. Purification was initially done by centrifuging the aqueous solutions at 6000 rpm for 30 min in order to eliminate suspended impurities. The CDs were further purified by dialysis (Float-A-Lyzer[®] G2 Dialysis Device, MWCO: 500 Da), which ran continuously for three days. After that, the samples were freeze-dried, and the resulting powder was stored for further use. The CDs synthesized from citric acid and urea (CA@U-CDs) were prepared with the same procedure, by using 2.5 g of citric acid + urea (3:1) as precursor powder instead of SCGs.

2.3. Characterization

Zeta potential measurements were determined by using a particle analyzer Anton Paar LitesizerTM 500. X-ray photoelectron spectroscopy (XPS) data were obtained with a Fi Kratos Axis Ultra HAS-VISION.

AFM analysis was carried out using a Veeco Metrology Multimode/Nanoscope IVA by tapping. A silica plate was used to deposit the sample for analysis and an AFM RTEP cantilever was used. UV–Vis spectra were obtained with a UV-3100PC spectrophotometer, by using quartz cells. Fluorescence spectra were obtained in standard 10 mm fluorescence quartz cells and collected in a Horiba Jovin Yvon Fluoromax-4 spectrofluorimeter. Spectra were obtained with a 1 nm interval and 5 nm slit widths. The assessment of photostability was made by subjecting CDs to irradiation by a low-pressure lamp (40 W, model Vilber, VL-340.BL) emitting UV radiation at 365 nm.

2.4. Calculation of Fluorescence Quantum Yield

The relative fluorescence quantum yield (QY_{FL}) was calculated with a standard procedure, [30,31] which is based on the comparison of the integrated luminescence intensities and absorbance values of the relevant sample (different CDs) with that of a reference fluorophore, with the following equation:

$$QY_{FL}^{Sample} = QY_{FL}^{Quinine\ Sulphate} \times \frac{Grad_{Sample}}{Grad_{Quinine\ Sulphate}} \times \frac{\eta_{Sample}^2}{\eta_{Quinine\ Sulphate}^2} \quad (1)$$

In Equation (1), *Grad* is the gradient from the plot of integrated fluorescence intensity versus absorbance and η is the refractive index. Quinine sulphate was chosen as a reference fluorophore of known quantum yield ($QY_{FL} = 0.54$) [32]. Fluorescence was measured with a Horiba Jovin Yvon Fluoromax-4 spectrofluorimeter. Absorbance measurements were made with a UNICAM Helios Gamma, using quartz cells.

2.5. Scope and System Boundaries

This was a cradle-to-grave study that aimed to compare the potential environmental impacts of the synthesis of CDs from either waste products (SCGs) from the coffee industry or typical precursors used at laboratory scale. More specifically, the aim of this LCA study was to obtain comparative information regarding the environmental impacts of using SCG samples or typical precursors to fabricate CDs, and not to obtain quantitative data. This work was focused on the manufacturing stage of the target CDs and considered the direct emissions from the production of CDs and indirect impacts associated with upstream resource extraction and energy generation.

This work used thermal carbonization of three different SCGs and one citric acid—urea mixture—as the synthetic strategy for the four studied CDs. A detailed description of the four routes can be found in Section 2.2. The environmental impacts were analyzed and compared in the first stage using a weight-based functional unit of 1 kg of CDs. This was made because the selection of a weight-based functional unit makes sense when comparing the production of an equivalent nanomaterial [33,34]. However, these impacts were normalized by the QY_{FL} of the different CDs (Table 2), in a later stage. Such an approach was required because weight-based functional units do not consider the functional benefits of the materials for which they were engineered, and so, they do not account for the possibility that a more resource-intensive synthesis may be later justified in the use stage (given the improved functionality). QY_{FL} was chosen as a normalization factor because, while CDs have many different applications, a high QY_{FL} is a required property in most, if not all, of these applications. In fact, a high QY_{FL} is essential for both imaging and sensing applications, which are the practical applications developed to date for SCG-based CDs.

2.6. Life Cycle Inventory Data

The assessment of the environmental impacts associated with the fabrication of CDs (via thermal carbonization) was based on inventory (foreground) data from laboratory-scale synthesis procedures performed by our group, as described in Section 2.2. The life cycle inventory data for the foreground system consisted of average data found on the Ecoinvent® 3.5 database for the synthesis procedures (primary data).

The present LCA model only included the carbonization step, given that this was a comparative study, and not a quantitative one. Subsequent purification steps were the same for all CDs, and so, they should have provided the same impacts for all synthesis procedures. Thus, our model only included the electricity used to power the oven during the carbonization step (10 kWh) and the number of precursors needed to produce 1 kg of CDs, which was calculated considering the synthesis yields described (Table 1). More specifically, CA@U-CDs required 7.61 kg of citric acid and 2.54 kg of urea (synthesis yield of 9.85% and a 3:1 ratio) to produce 1 kg of CA@U-CDs. As for the SCG samples, it should be noted that in LCA terms, their use as CD precursors should not generate environmental impacts associated with their production. Namely, SCG samples are produced irrespective of their subsequent use as CDs precursors, given their production results entirely from coffee brewing. Thus, any environmental impact resulting from the production of SCG samples should be attributed to the coffee industry, and not to the production of CDs. Nevertheless, all four CDs produced unspecified chemical waste that should be accounted for in the final waste flows: 9.2 kg for CA@U-CDs, 187.7 kg for decaf-CDs, 130.6 kg for rossio-CDs, and 50.0 kg for prestige-CDs. Thus, our LCA model regarding the synthesis of SCG-based CDs only contemplated the electricity used to power the oven and the final waste flows, while for CA@U-CDs the electricity, final waste flows and the amounts of citric acid/urea were considered.

Table 1. Atomic surface compositions (%) *, synthesis yields (%), fluorescence quantum yield (QY_{FL}) (%) for decaf-carbon dots (CDs), rossio-CDs, prestige-CDs and CA@U-CDs.

	Decaf-CDs	Rossio-CDs	Prestige-CDs	CA@U-CDs
C (%)	59.1	57.1	59.0	58.9
O (%)	35.3	34.9	34.1	27.2
N (%)	2.8	6.2	4.4	13.9
K (%)	2.7	1.8	2.5	-
Synthesis yield (%)	0.53	0.76	1.96	9.85
QY _{FL} (%)	4.3	2.9	5.8	22.5

* Obtained by X-ray photoelectron spectroscopy (XPS).

The different processes and chemicals included in this study were modeled with the following life cycle inventory (LCI) data: citric acid {GLO} | market for; Urea, as N {GLO} | market for; Electricity, medium voltage {PT} | market for; Chemical waste, unspecified. The amounts of citric acid and urea are described above in the “Fabrication of CDs” section. The electricity process refers to the electricity used by the oven (VWR DL 112 Prime) during the carbonization process (200 °C for 4 h). This oven possesses a capacity of 112 L and a nominal power consumption of 2500 W. This results in an electricity amount of 10 kWh for each synthesis.

2.7. Environmental Impact Assessment

LCA studies were performed using SimaPro 9.0.0.35 Multiuser software. Environmental impacts were modeled using the ReCiPe 2016 v1.1 LCIA, Hierarchist version [35]. The impact potentials evaluated according to the ReCiPe method were: global warming—human health (GW-HH), global warming—terrestrial ecosystems (GW-TE), global warming—freshwater ecosystems (GW-FE), stratospheric ozone depletion (SO), ionization radiation (IR), ozone formation—human health (OF-HH), fine particulate matter formation (FPM), ozone formation—terrestrial ecosystems (OF-TE), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial ecotoxicity (TE), freshwater ecotoxicity (TET), marine ecotoxicity (MET), human carcinogenic toxicity (HC), human non-carcinogenic toxicity (HNC), land use (LU), mineral resource scarcity (MR), fossil resource scarcity (FR), water consumption—human health (WC-HH), water consumption—terrestrial ecosystem (WC-TE) and water consumption—aquatic ecosystems (WC-AE).

2.8. Sensitivity Analysis

A sensitivity analysis was performed to help to better understand uncertainties associated with this study by considering “what if” scenarios [36]. That is, by varying the amount and type of materials and electricity employed in the synthesis of the CDs. In this way, it is possible to evaluate to what extent key assumptions affect LCA results.

3. Results and Discussion

3.1. Synthesis of CDs

The thermal carbonization of the three SCG samples led to the formation of nanoparticles with a relatively heterogenous size distribution (Figure 1). These measurements also allowed us to obtain the following average sizes for the SCG-based CDs ($\pm$ standard deviation): 2.1 ± 1.0 nm for decaf-CDs, 3.9 ± 1.0 nm for rossio-CDs and 2.3 ± 0.8 nm for prestige-CDs. These values are in line with the sizes between 1 and 5 nm obtained in previous studies focused on the preparation of CDs from SCGs [25–28]. The thermal carbonization of citric acid + urea lead to the formation of CDs with a homogeneous distribution, with an average size of 1.0 ± 0.3 nm (Figure 1).

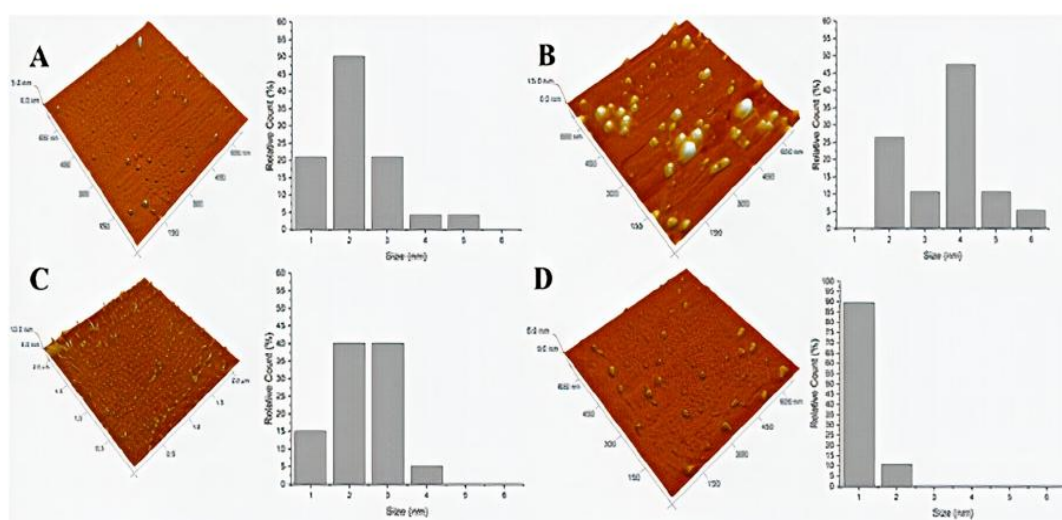


Figure 1. Atomic force microscopy (AFM) images and relative size distribution for (A) decaf-CDs, (B) rossio-CDs, (C) prestige-CDs and (D) CA@U-CDs.

Reaction yields (mass by mass) were obtained by measuring the weight of the freeze-dried powder relative to each sample, after steps of solubilization, centrifugation and dialysis. Unfortunately, the reaction yields for the reactions using the three SCG samples as precursors were not satisfactory: 0.53% for decaf-CDs, 0.76% for rossio-CDs and 1.96% for prestige-CDs (Table 1). Nevertheless, these values are in line with those obtained (2%) for the production of CDs from Nescafe® original instant coffee [26]. Furthermore, while the reaction yield for CA@U-CDs was higher (9.85%), this is still a rather poor yield (Table 1). Thus, it appears that in terms of reaction yield, the efficiency of the synthesis using SCGs is comparable to that of the same process using standard precursors. It should also be noted that the yields of the production of CDs are generally quite low [37–39]. Given this, the problem of low production yield cannot be ascribed specifically to SCG-based CDs, but to CDs in general. Thus, the main conclusion that can be obtained from these results is that SCG-based CDs present similar production yields to typical CDs.

In conclusion, these results demonstrate that the thermal treatment of SCG samples led to the formation of nanoparticles with a yield similar to that of using standard precursors. Further

evaluation and characterization were then done to assess if the resulting CDs possessed potentially advantageous characteristics.

3.2. Surface Characterization of CDs

An XPS analysis was done to analyze the surface composition of the four CD samples (Figures S1–S3). More specifically, the XPS atomic composition (at w %) of each sample was determined (Table 1). It was revealed that all types of sample (both SCG-derived and CA@U-CDs) were composed mostly of C (~57–58%) and O (~27–35%). The main differences were the presence of small amounts of K (~2–3%) in the SCG-derived CDs, and that the N molecule fraction in CA@U-CDs (~14%) was significantly higher than in the SCG-based CDs (~3–6%). This lower N (at w %) in the latter samples was related with higher amounts of O. A detailed scan for the internal levels of C 1s, O 1s and N 1s was subsequently done, towards deconvolution and chemical state and the quantitative analysis (Figures S1–S3).

C 1s spectra could be split into four peaks for all samples: at binding energies of ~284 eV (attributed to C-C/C-H groups) [40], ~286 eV (C-O/C-N groups) [41], ~287 eV (C=O groups) and ~288 eV (O-C=O) [42]. SCG-derived decaf-CD and prestige-CD samples presented a very similar composition, with a higher contribution from C-C/C-H groups (~47–48%), followed by C-O/C-N groups (~36–38%). The contribution by C=O groups was moderate (~11%), while that by O-C=O groups was negligible (~3%). This analysis might indicate a small degree of π -conjugation at the surface of these CDs, with potential impacts on the photoluminescence efficiency. The degree of π -conjugation should be slightly higher for SCG-derived rossio-CDs, given a reduced contribution of C-C/C-H groups (~40%), and the consequent higher contribution of C-O/C-N (~42%) and C=O (~16%) groups. Nevertheless, the contribution by C 1s was qualitatively very similar between all SCG-based CDs. This is not true, however, for CA@U-CDs. For these CDs, the contribution of π -conjugated groups (C=O and O-C=O) was 35%, with an emphasis on O-C=O groups (~25%), while that contribution was just ~15% for SCG-based CDs. This increase resulted from a decrease in the contribution of C-N/C-O groups (~17%), while the contribution by C-C/C-H groups remained predominant (~47%). C 1s analysis indicated that the degree of π -conjugation at the surface of CA@U-CDs should be significantly higher than at the surface of SCG-based CDs.

O 1s could be split into just two peaks: at binding energies of ~531 eV (C=O groups) and ~532 eV (C-O groups) [42]. The three SCG-based CDs presented identical compositions: predominant contributions by C-O groups (~67–74%), with moderate contributions from C=O groups (~25–32%). The composition of CA@U-CDs was quite different, however, given that the dominant contribution was from C=O groups (~84%), while the contribution by C-O groups was quite moderate (~16%). These results further support the notion that the degree of π -conjugation at the surface of CA@U-CDs was higher than at the surface of CA@U-CDs, with potential benefits for the former regarding their photoluminescent properties. Finally, N 1s could also be split into two peaks: at binding energies of ~400 eV (amine/amide groups) and ~401.4 eV (protonated amides). All four samples presented dominant contributions from amine/amide groups (~88–95%), with small/almost negligible contributions from protonated amines (~4–12%) [43].

The zeta potential of all samples was measured by DLS in aqueous solutions, revealing the following results: −24 mV for decaf-CDs, −12.5 mV for rossio-CDs, −18.1 mV for prestige-CDs and −0.5 mV for CA@U-CDs. This indicates the possibility that the SCG-based CDs were negatively charged, while CA@U-CDs should have presented a neutral state.

3.3. Fluorescent Characterization of CDs

The absorption, excitation and fluorescence spectra of the four samples can be found in Figure 2. The two latter spectra were obtained at the fluorescent and excitation wavelength maxima, respectively.

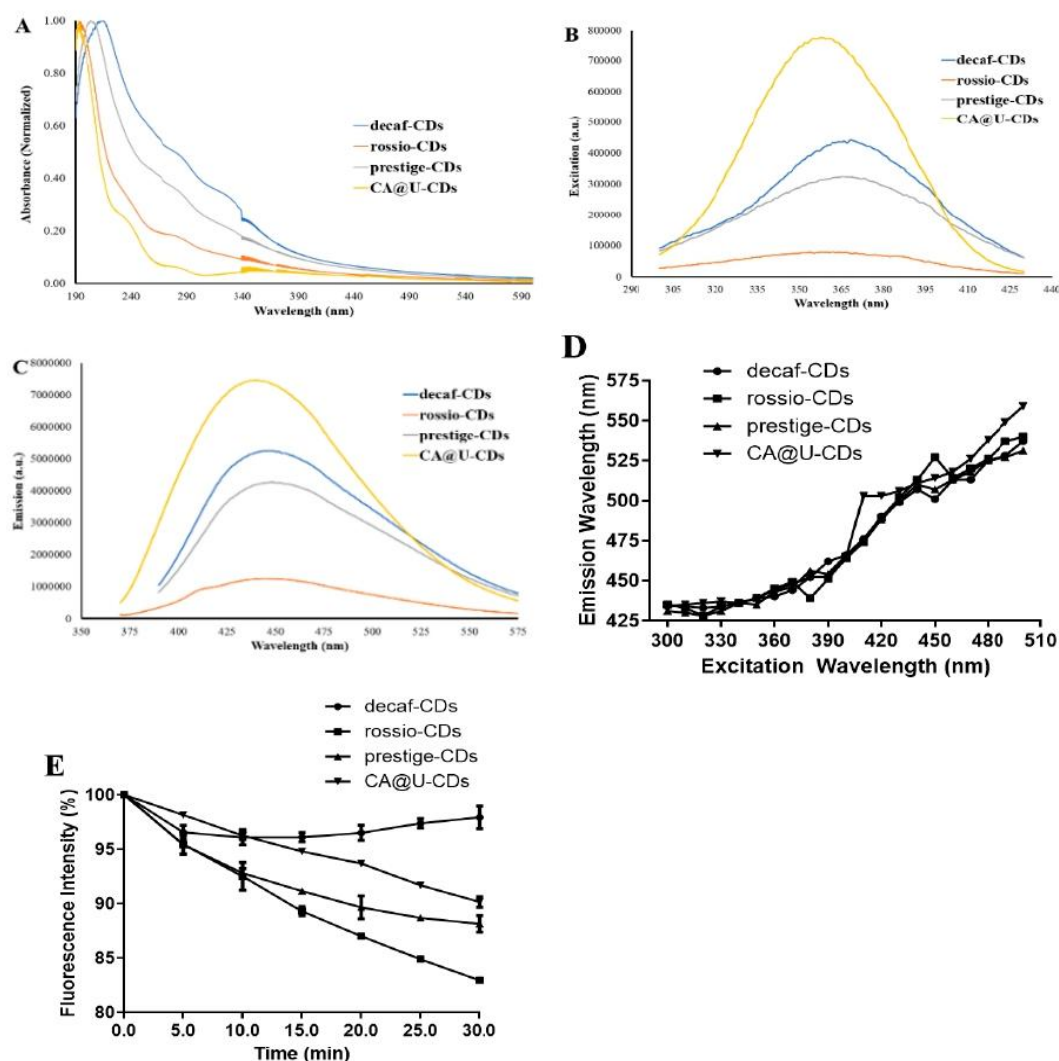


Figure 2. (A) Absorption, (B) excitation and (C) fluorescence spectra in aqueous solution for decaf-CDs, rossio-CDs, prestige-CDs and CA@U-CDs. (D) Emission wavelength (in nm) as a function of the excitation wavelength (in nm). (E) Photostability of the four CDs, measured as the variation of the fluorescence intensity as a function of irradiation time under a UV light source (365 nm).

All samples presented the typical absorption spectra of CDs (Figure 2A), with strong absorbance in the UV region and the absence of the comparable absorbance of the wavelengths of the excitation maxima [44,45]. All four CDs present quite similar excitation wavelength maxima (Figure 2B): 370 nm for decaf-CDs and prestige-CDs, 360 nm for rossio-CDs and 350 nm for CA@U-CDs. The same can also be said for their fluorescence maxima, given that all CDs generated blue emission (Figure 2C): 450 nm for decaf-CDs, 445 nm for rossio-CDs, 450 nm for prestige-CDs and 440 nm for CA@U-CDs. This blue emission was consistent with the fluorescence exhibited by other SCG-based CDs described in the literature [25–27]. This indicates that SCG samples can be used to produce CDs with comparable emission and excitation maxima to those obtained by using typical precursors. However, it is noticeable that the bands of CA@U-CDs were more well-resolved than those of SCG-derived CDs, especially considering their excitation spectra. The broader excitation bands of SCG-derived CDs could be attributed to their higher size heterogeneity.

Interestingly, all CD samples presented an excitation-dependent emission (Figure 2D), which is a typical feature of CDs [46,47]. Such a feature has also been found in the case of SCG-based

CDs [25–27]. Namely, the emission maxima of these four nanoparticles is in the blue region of the visible spectrum. However, increasing the excitation wavelength from 300 to 500 nm, leads to a quite significant ~100 nm redshift in the emission. Therefore, SCG-derived CDs can also emit green light, albeit with less efficiency than blue fluorescence.

Given the relative size heterogeneity of the SCG-based CDs (Figure 1), one could be tempted to attribute this excitation-dependence to quantum confinement effects [48], that is, particles of different sizes emit with different energies. However, the size homogeneity of CA@U-CDs do not support this hypothesis. Thus, it is possible that this excitation-dependence arises from sp² subregions in the carbon core and from functional groups on the surface, as hypothesized by Van Dam et al. [47]. Nevertheless, the luminescence of CDs should not be explained by just a single dominating mechanism, but rather by two or more processes (such as quantum size effects, bandgap transitions or defect-derived emission) [49].

Such a conclusion is supported by the excitation–emission (EEM) matrices of the three SCG-based CDs, which are presented as 2D color surfaces in Figure 3. These EEMs show only a well-defined luminescent region for each SCG-based CD, with a maximum in the blue region of the spectrum, and similar luminescent centers (which could be expected if the excitation-dependent emission was the result of different emitter species mixed in the solution).

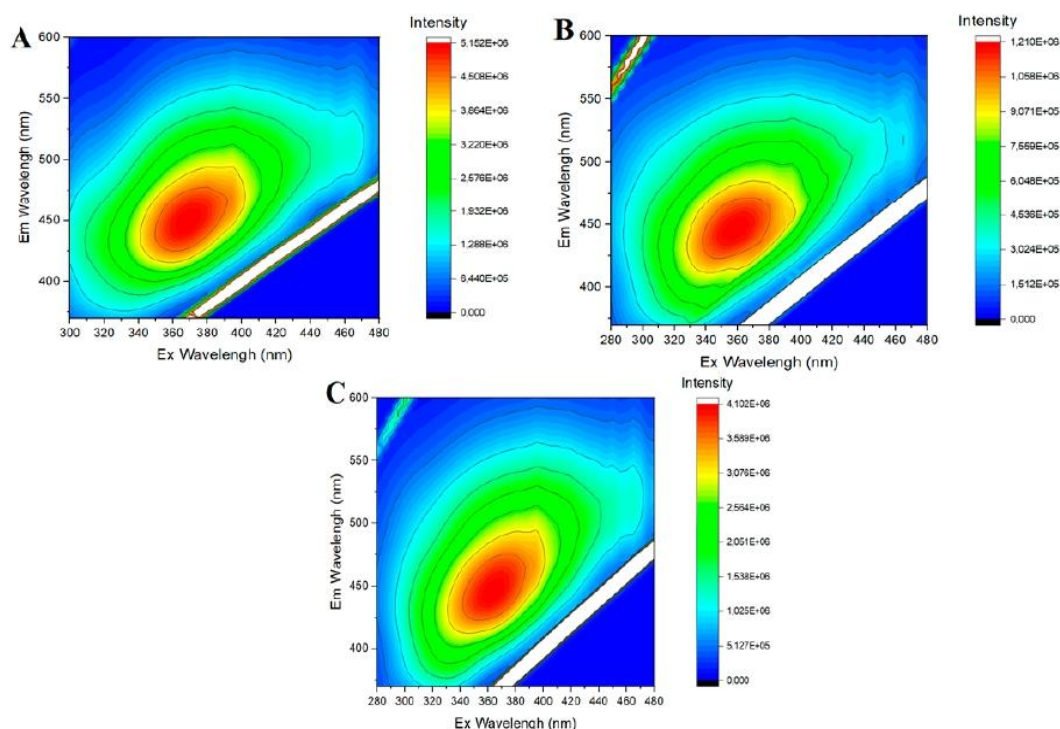


Figure 3. Two-dimensional excitation-emission matrices (EEMs) for decaf-CDs (A), rossio-CDs (B) and prestige-CDs (C).

The QY_{FL} for the four CD samples can be found in Table 1. The QY_{FL} obtained for SCG-based CDs is interesting (between 2.9 and 5.8%), thereby indicating that SCGs can be appropriate precursors for the fabrication of CDs. Such values are in line with those obtained for other SCG-based CDs [25–27], which were between 3.8% and 8%. However, SCG-based CDs appear to be at disadvantage when we compare them directly with CDs prepared with typical precursors. Namely, the QY_{FL} of CA@U-CDs was significantly higher (22.5%) than those recorded by CDs derived from SCGs. Two reasons can explain the higher QY_{FL} of CA@U-CDs: one, the higher π -conjugation of the surface of this type of CD as demonstrated by XPS analysis (Figures S1–S3); the second reason is the higher N XPS mole fraction

of this type of CD, also demonstrated by XPS analysis. More specifically, the N-doping of CDs has been demonstrated to generate a high QY_{FL} [50,51]. Additionally, the lower content of amide groups on the surface of CDs vs. CA@U-CDs (see XPS N content) may also account for the observed differences. In the synthesized model CDs, the abundance of amide groups on the surface favors photoinduced charge transfer between amide and carboxylic acid groups via hydrogen bond interactions, enabling a more rigid structure, with this being the origin of the high blue fluorescence observed in almost all CDs [52].

Figure 2E shows the photostability of the four CD samples, which was accounted for by measuring their fluorescence after subjecting them to UV radiation at 365 nm (low pressure UV lamp, 40 W). Decaf-CDs were the most photostable CDs, with their fluorescent intensity only decreasing by ~3% in a 30-min period. Prestige-CDs and CA@U-CDs presented similar photostability, as their fluorescence intensity decreased by ~10% in the same 30-min period. Rossio-CDs were shown to be the least photostable, with a decrease of ~20% in the measured time period. Given this, this analysis revealed that at least decaf-CDs and prestige-CDs CDs can be considered to be photostable (with decreases of just ~3% and ~10% when subjected to 30 min of UV irradiation). Moreover, these SCG-based CDs presented comparable photostability to that of typical CDs, such as CA@U-CDs.

3.4. Sensing of Fe³⁺

Arguably, one of the most common and well-studied applications for CDs is their use as fluorescent probes in the sensing of heavy metal cations [53–55]. The focus on this field results from the significant adverse health effects resulting from the presence of heavy metals in water resources. One of such metal cations is Fe³⁺ [53], as iron is an essential trace element for human metabolism, but elevated intake of this metal can lead to health hazards due to the production of reactive oxygen species (ROS) [53]. ROS can cause several diseases, such as Alzheimer's and Parkinson's diseases and cancer [56]. United States Environmental Protection Agency (USEPA) established a permissible limit of 0.3 mg/L (5.357 μM) in drinking water [53].

The potential Fe³⁺-induced quenching of SCG-based CDs was subsequently explored, with the emission profiles of CDs as a function of Fe³⁺ presented in Figure 4. Fe³⁺ induced a concentration-dependent quenching for all four CD samples, with quenching following a linear Stern–Volmer relationship. However, an R² of 0.82 for decaf-CDs indicates the inability of this type of SCG-based CDs to be used as a fluorescent probe for Fe³⁺. In contrast, an R² of 0.99 for the other CDs indicate their potential as fluorescent probes for this heavy metal. Fitting the data of decaf-CDs, rossio-CDs, prestige-CDs and CA@U-CDs to the standard Stern–Volmer equation revealed the following Stern–Volmer constants (K_{SV}): 5.80 × 10^{−3}, 1.82 × 10^{−2}, 6.71 × 10^{−3} and 1.39 × 10^{−2} μM^{−1}, respectively. These values correlate well with the obtained quenching for each CD: 15.8% for decaf-CDs, 34.8% for rossio-CDs, 26.5% for prestige-CDs and 36.0% for CA@U-CDs. The emission profiles (Figure 4) also allowed us to obtain limits of detection (LoD) for each CD: 14.11 μM for decaf-CDs, 3.51 μM for rossio-CDs, 4.58 μM for prestige-CDs, and 3.07 μM for CA@U-CDs.

These results pointed to SCG-based CDs (except for decaf-CDs) exhibiting a response to Fe³⁺ comparable to CA@U-CDs. Furthermore, their LoDs were below the permissible limit for Fe³⁺ (5.357 μM) in drinking water, as established by USEPA [52]. Both rossio-CDs and prestige-CDs have the potential to be employed as fluorescent probes of environmental relevance, with a response to Fe³⁺ comparable to that shown by CDs prepared with typical precursors (CA@U-CDs).

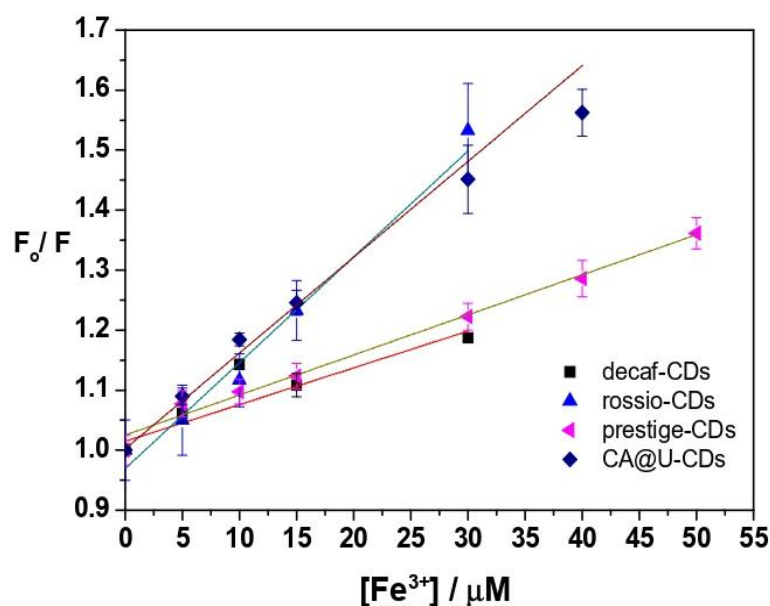


Figure 4. Emission profiles of (■) decaf-CDs, (◀) prestige-CDs, (♦) CA@U-CDs (▲) and rossio-CDs (d) as a function of the concentration of Fe³⁺ (in mM), in aqueous solution. F₀ and F are the relative fluorescence intensities in the absence and presence of Fe³⁺, respectively. R² = 0.85; 0.98; 0.98 and 0.96, respectively.

3.5. Comparative LCA Study

Another parameter that should be considered while evaluating the potential of certain samples as CD precursors is the environmental impact they cause by using them in the synthesis and development of CDs. That is, new precursors should lead to a sustainable synthesis strategy for this type of nanoparticle. This is particularly important since the production steps of engineered nanomaterials have been identified as being of environmental concern [57]. In fact, it has been found that the production of engineered nanomaterials can be orders of magnitude more energy- and material-consuming than for fine chemicals and pharmaceuticals [58]. Furthermore, LCA studies of engineered nanomaterials have shown that the energy and chemicals used during the synthesis of these nanomaterials can contribute a significant share of the environmental impacts generated during their life cycle.

To obtain comparative information regarding the sustainability of using SCG samples as CD precursors we have performed an LCA study, given that LCA aims to quantify the relevant environmental impacts of a given system during its life cycle [13,59–62]. To this end, the environmental impacts of synthesizing CDs with selected precursors (decaf-CDs, rossio-CDs and prestige-CDs) and typical reagents (CA@U-CDs) was analyzed and compared by using a mass-based functional unit of 1 kg of CDs. This was made because the selection of a weight-based functional unit (FU) makes sense to compare the production of an equivalent amount of nanomaterial [33]. However, these impacts were later normalized by the QY_{FL} of the CDs. Such an approach is needed because weight-based functional units do not consider the functional benefits of the engineered nanomaterials for which they were fabricated, and so might not account for the possibility that a more resource-intensive synthesis may be justified later in the use stage (given the improved functionality).

The relative environmental impacts associated with the synthesis of the four CDs are presented in Figure 5, by considering a weight-based functional. The analysis of the synthesis of CA@U-CDs (Figure 5A) shows that citric acid is the main agent responsible for the resulting environmental impacts in all categories, followed by urea. The contribution made by the use of electricity is always below 10%, except in the case of ionization radiation. The use of electricity is the only contributing parameter for the synthesis of SCG-based CDs (Figure 5B), given that it is the only input parameter in their

synthesis. This results from the fact that, as already referred to in Section 2.6, the use of SCGs as CD precursors should not generate environmental impacts associated with their production, given that their production results entirely from coffee brewing. More specifically, SCG samples are produced irrespective of their subsequent use as CD precursors, and so any environmental impact resulting from the production of SCG samples should be attributed to the coffee industry.

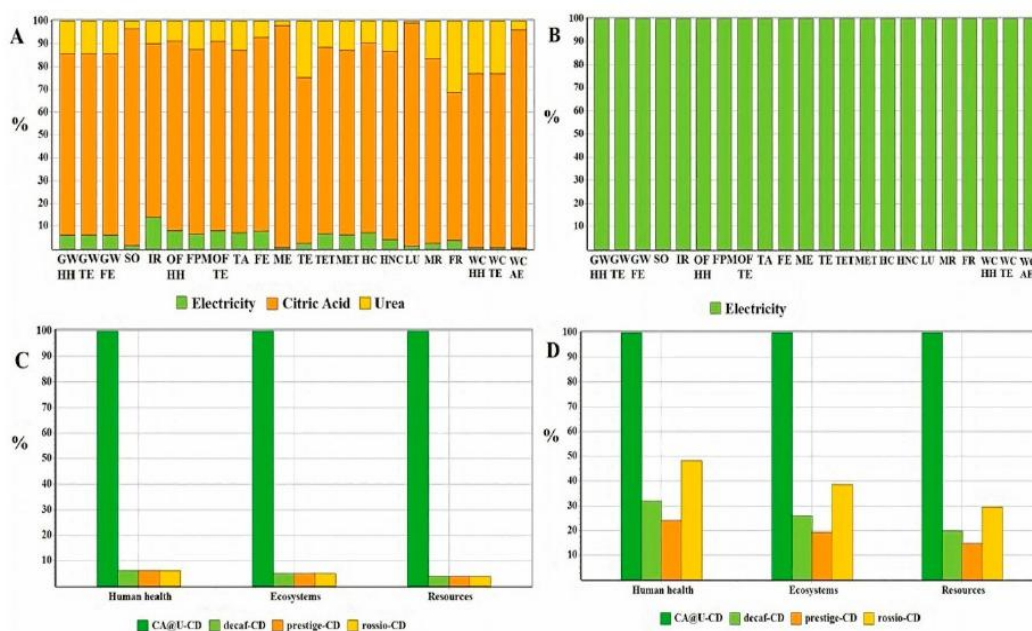


Figure 5. Relative environmental impacts of CA@U-CDs (A) and of spent coffee grounds (SCG)-based CDs (B) with the ReCiPe2016 as the life cycle impact assessment (LCIA) method. Comparative environmental (C,D) profiles for the four different routes for the synthesis of CDs. Environmental profiles were obtained using ReCiPe2016 as LCIA. Graphic C refers to an analysis using a weight-based functional unit of 1 kg of CDs, while Graphic D is re-scaled with respect to the QY_{FL} of the CDs.

All four CDs are compared in Figure 5C, with all weight-based environmental categories being divided into three main ones: human health, ecosystems and resources. It is clearly seen that CA@U-CDs produce significantly more environmental impacts in all categories than any of the SCG-derived CDs. This results from the significant use of citric acid and urea as precursors, while the latter CDs use a waste product of another industry as a precursor. Furthermore, this LCA assessment does not detect any difference regarding the environmental impacts produced by any SCG-based CD. This is not surprising, given that all CDs use the same amount of electricity.

The final step of this study was to assess the environmental impacts associated with each synthetic route, when rescaled with respect to the QY_{FL} of the resulting CDs (Table 2 and Figure 5D). This rescaling was done by considering the highest QY_{FL} (that of CA@U-CDs) as the reference QY_{FL}^{REF} . The QY_{FL} -normalized functional unit for each CD was then calculated as $\frac{QY_{FL}^{REF}}{QY_{FL}}$ (Table 2).

Table 2. Function-related functional unit for decaf-CDs, rossio-CDs, prestige-CDs and CA@U-CDs.

Function-Related Functional Unit	Decaf-CDs	Rossio-CDs	Prestige-CDs	CA@U-CDs
$\frac{QY_{FL}^{REF}}{QY_{FL}}$	5.2	7.8	3.9	1.0

There were not many qualitative differences with the rescaling, given that the fabrication of CA@U-CDs still produced significantly higher environmental impacts in all categories than any

of the SCG-based CDs, even when it was considered a function-based functional unit. However, the contributions from each SCG-based CD was significantly higher in all three categories: decaf-CDs (19.7–32.1%), rossio-CDs (29.6–48.1%) and prestige-CDs (14.8–24.1%). From the three categories, the one associated with higher impacts is that of human health, followed by ecosystems and then by resources. Finally, for a QY_{FL}-based functional unit, the SCG-based CDs that generated higher environmental impacts were rossio-CDs > decaf-CDs > prestige-CDs.

A sensitivity analysis was performed by changing the input of the electricity and raw materials (citric acid and urea) by $\pm 30\%$. The results for changing the input of citric acid to 70% and 130% of the original value are present in Figure S4. This variation did not lead to any significant qualitative change, given that it still was CA@U-CDs that produced the highest environmental impacts in all categories (by a wide margin). Nevertheless, these results indicate that changing the amount of citric acid does have a non-negligible quantitative impact, as reducing the amount of citric acid by 30% led to a $\sim 10\%$ approximation between the impacts produced by CA@U-CDs and SCG-based CDs. In turn, a 30% increase led to a $\sim 10\%$ distancing.

The comparative environmental profiles for all CDs when changing the input of urea to 70% and 130% of its original value is shown in Figure S5. Changing the amount of urea did not lead to either significant qualitative or quantitative effects. CA@U-CDs still led to the higher environmental impacts, and the contribution of SCG-based CDs was nearly unchanged in all three categories.

Finally, the comparative environmental profiles for different inputs of electricity can be found in Figure S6. Once again, changing the amount of electricity used did not change the fact that CA@U-CDs led to higher environmental impacts, when in comparison with the studied SCG-based CDs. Nevertheless, varying the amount of electricity did have a non-negligible effect in the three categories. This effect is similar to that exerted by varying citric acid, but in an opposite way. That is, decreasing the input of electricity by 30% led to a $\sim 10\%$ distancing between CA@U-CDs and SCG-based CDs (while the same variation of citric acid led to an approximation), while increasing the electricity by 30% led to a $\sim 10\%$ approximation (while the reverse was true for citric acid). Thus, decreasing the used electricity favored the sustainability of SCG-based CDs, while decreasing the amount of citric acid favored CA@U-CDs. Urea did not seem to provide a significant effect either way.

In summary, SCG samples, despite lower synthesis yields and QY_{FL}, are a better option than typical precursors (citric acid and urea) for the fabrication of CDs in terms of environmental impacts. This is true both when considering a weight-based and a function-related functional unit. Prestige-CDs should be the preferred option when considering their QY_{FL}.

4. Conclusions

The fabrication of fluorescent CDs by using either spent coffee grounds (SCGs, biomass waste material from the coffee industry) or standard precursors (citric acid and urea) as precursors was compared, both in terms of associated environmental impacts and the structural and luminescent properties of these nanoparticles. The one-pot and solvent-free carbonization of the different precursor samples led to the formation of nanoparticles with an average size of 1.0–3.9 nm and a similar blue emission. However, while SCG-based CDs present moderate quantum yields (2.9–5.8%) and quite low reaction yields, CDs made from citric acid and urea showed more a significant quantum yield (22.5%) and higher reaction yields. Nevertheless, an LCA study demonstrated that the fabrication of CDs with SCGs is more environmentally beneficial than using typical precursors, irrespective of using either a weight-based or function-related functional unit. Thus, while comparison with CDs generated with typical precursors indicates that SCG-based CDs could benefit from some improvement, the present study validates the use of SCGs (instead of more common organic molecules) as precursors as the most sustainable route for the fabrication of CDs. SCG-based CDs also demonstrated their potential as sensing probes for Fe³⁺ in water.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2079-4991/10/6/1209/s1>, Figure S1: XPS C 1s spectra for decaf-CDs (A), rossio-CDs (B), prestige-CDs (C) and CA@U-CDs (D), Figure S2: XPS O 1s for decaf-CDs (A), rossio-CDs (B), prestige-CDs (C) and CA@U-CDs (D), Figure S3: XPS N 1s spectra for decaf-CDs (A), rossio-CDs (B), prestige-CDs (C) and CA@U-CDs (D), Figure S4: Comparative environmental profiles for the synthesis of all CDs, rescaled with respect to the QY_{FL} of the CDs, by changing the input of citric acid to 70% (A) and to 130% (B) of the original value, Figure S5: Comparative environmental profiles for the synthesis of all CDs, rescaled with respect to the QY_{FL} of the CDs, by changing the input of urea to 70% (A) and to 130% (B) of the original value, Figure S6: Comparative environmental profiles for the synthesis of all CDs, rescaled with respect to the QY_{FL} of the CDs, by changing the input of electricity to 70% (A) and to 130% (B) of the original value.

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References

- Lim, S.Y.; Shen, W.; Gao, Z. Carbon quantum dots and their applications. *Chem. Soc. Rev.* **2015**, *44*, 362. [[CrossRef](#)] [[PubMed](#)]
- Barman, M.K.; Patra, A. Current status and prospects on chemical structure driven photoluminescence behaviour of carbon dots. *J. Photochem. Photobiol. C* **2018**, *37*, 1–22. [[CrossRef](#)]
- da Silva, J.C.G.E.; Gonçalves, H.M.R. Analytical and bioanalytical applications of carbon dots. *Trends Anal. Chem.* **2011**, *30*, 1327. [[CrossRef](#)]
- Kozák, O.; Datta, K.K.R.; Greplová, M.; Ranc, V.; Kaslik, J.; Zboril, R. Surfactant-derived amphiphilic carbon dots with tunable photoluminescence. *J. Phys. Chem. C* **2013**, *117*, 24991. [[CrossRef](#)]
- Wang, K.; Gao, Z.; Gao, G.; Wo, Y.; Wang, Y.; Shen, G.; Cui, D. Systematic safety evaluation on photoluminescent carbon dots. *Nanoscale Res. Lett.* **2013**, *8*, 122. [[CrossRef](#)]
- ESimões, F.C.; da Silva, J.C.G.E.; Leitão, J.M.M. Peroxynitrite and nitric oxide fluorescence sensing by ethylenediamine doped carbon dot. *Sens. Actuator B* **2015**, *220*, 1043. [[CrossRef](#)]
- Algarra, M.; Orfãos, L.; Alves, C.S.; Moreno-Tost, R.; Pino-González, M.S.; Jiménez-Jiménez, J.; Rodríguez-Castellón, E.; Eliche-Quesada, D.; Casto, E.; Luque, R. Sustainable Production of Carbon Nanoparticles from Olive Pit Biomass: Understanding Proton Transfer in the Excited State on Carbon Dots. *ACS Sustain. Chem. Eng.* **2019**, *7*, 10493. [[CrossRef](#)]
- Kang, Y.F.; Li, Y.H.; Fang, Y.W.; Xu, Y.; Wei, X.M.; Yin, X.B. Carbon Quantum Dots for Zebrafish Fluorescence Imaging. *Sci. Rep.* **2015**, *5*, 11835. [[CrossRef](#)]
- Joseph, J.; Anappara, A.A. White-Light-Emitting Carbon Dots Prepared by the Electrochemical Exfoliation of Graphite. *Chem. Phys. Chem.* **2017**, *18*, 292. [[CrossRef](#)]
- Travlou, N.A.; Giannakoudakis, D.A.; Algarra, M.; Labella, M.; Rodríguez-Castellón, E.; Badosz, T.J. Carbon Quantum Dot Surface-Chemistry-Dependent Ag Release Governs the High Antibacterial Activity of Ag-Metal–Organic Framework Composites. *Carbon* **2018**, *135*, 103. [[CrossRef](#)]
- Crista, D.M.A.; Mello, G.P.C.; Shevchuk, O.; Sendão, R.M.S.; Simões, E.F.C.; Leitão, J.M.M.; da Silva, L.P.; da Silva, J.C.G.E. 3-Hydroxyphenylboronic Acid-Based Carbon Dot Sensors for Fructose Sensing. *J. Fluoresc.* **2019**, *29*, 265. [[CrossRef](#)] [[PubMed](#)]
- Algarra, M.; Pérez-Martín, M.; Cifuentes-Rueda, M.; Jiménez-Jiménez, J.; da Silva, J.C.G.E.; Badosz, T.J.; Rodríguez-Castellón, E.; López-Navarrete, J.T.; Casado, J. Carbon dots obtained using hydrothermal treatment of formaldehyde. Cell imaging in vitro. *Nanoscale* **2014**, *6*, 9071. [[CrossRef](#)] [[PubMed](#)]

13. Sendão, R.M.S.; de Yuso, M.V.M.; Algarra, M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Comparative life cycle assessment of bottom-up synthesis routes for carbon dots derived from citric acid and urea. *J. Clean Prod.* **2020**, *254*, 120080. [\[CrossRef\]](#)
14. Rodríguez-Padrón, D.; Algarra, M.; Tarelho, L.A.C.; Frade, J.; Franco, A.; de Miguel, G.; Jiménez-Jiménez, J.; Rodríguez-Castellón, E.; Luque, R. Catalyzed Microwave-Assisted Preparation of Carbon Quantum Dots from Lignocellulosic Residues. *ACS Sustain. Chem. Eng.* **2018**, *6*, 7200. [\[CrossRef\]](#)
15. Wang, H.; Ning, G.; He, X.; Ma, X.; Yang, F.; Xu, Z.; Zhao, S.; Xu, C.; Li, Y. Carbon quantum dots derived by direct carbonization of carbonaceous microcrystals in mesophase pitch. *Nanoscale* **2018**, *10*, 21492. [\[CrossRef\]](#)
16. Song, Y.; Zhu, S.; Zhang, S.; Fu, Y.; Wang, L.; Zhao, X.; Yang, B. Investigation from chemical structure to photoluminescent mechanism: A type of carbon dots from the pyrolysis of citric acid and an amine. *J. Mater. Chem. C* **2015**, *3*, 5976. [\[CrossRef\]](#)
17. Kasprzyk, W.; Swiergosz, T.; Bednars, S.; Walas, K.; Bashmakova, N.V.; Bogdat, D. Luminescence phenomena of carbon dots derived from citric acid and urea—A molecular insight. *Nanoscale* **2018**, *10*, 13889. [\[CrossRef\]](#)
18. Sendão, R.M.S.; Crista, D.M.A.; Afonso, A.C.P.; de Yuso, M.V.M.; Algarra, M.; da Silva, J.C.G.E.; da Silva, L.P. Insight into the hybrid luminescence showed by carbon dots and molecular fluorophores in solution. *Phys. Chem. Chem. Phys.* **2019**, *21*, 20919. [\[CrossRef\]](#)
19. Zhang, B.; Liu, Y.; Ren, M.; Li, W.; Zhang, X.; Vajtal, R.; Ajayan, P.M.; Tour, J.M.; Wang, L. Sustainable Synthesis of Bright Green Fluorescent Nitrogen-Doped Carbon Quantum Dots from Alkali Lignin. *ChemSusChem* **2019**, *12*, 4202. [\[CrossRef\]](#)
20. Sharma, V.; Tiwari, P.; Mobin, S.M. Sustainable carbon-dots: Recent advances in green carbon dots for sensing and bioimaging. *J. Mater. Chem. B* **2017**, *5*, 8904. [\[CrossRef\]](#)
21. Xie, Y.; Cheng, D.; Liu, X.; Han, A. Green Hydrothermal Synthesis of N-doped Carbon Dots from Biomass Highland Barley for the Detection of Hg²⁺. *Sensors* **2019**, *19*, 3169. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Pedras, B.M.; Nascimento, M.; Sá-Nogueira, I.; Simões, P.; Paiva, A.; Barreiros, S. Semi-continuous extraction/hydrolysis of spent coffee grounds with subcritical water. *J. Ind. Eng. Chem.* **2019**, *72*, 453. [\[CrossRef\]](#)
23. Parenti, A.; Guerrini, L.; Masella, P.; Spinelli, S.; Calamari, L.; Spugnoli, P. Comparison of Espresso Coffee Brewing Techniques. *J. Food. Eng.* **2014**, *121*, 112. [\[CrossRef\]](#)
24. Campos-Vega, R.; Loarca-Pina, G.; Vergara-Castaneda, H.A.; Oomah, B.D. Spent coffee grounds: A review on current research and future prospects. *Trends Food Sci. Technol.* **2015**, *45*, 24. [\[CrossRef\]](#)
25. Hsu, P.C.; Shih, Z.Y.; Lee, C.H.; Chang, H.T. Synthesis and analytical applications of photoluminescent carbon nanodots. *Green Chem.* **2012**, *14*, 917. [\[CrossRef\]](#)
26. Jiang, C.; Wu, H.; Song, X.; Ma, X.; Wang, J.; Tan, M. Presence of photoluminescent carbon dots in Nescafe® original instant coffee: Applications to bioimaging. *Talanta* **2014**, *127*, 68. [\[CrossRef\]](#)
27. Wang, L.; Li, W.; Wu, B.; Li, Z.; Wang, S.; Liu, Y.; Pan, D.; Wu, M. Facile synthesis of fluorescent graphene quantum dots from coffee grounds for bioimaging and sensing. *Chem. Eng. J.* **2016**, *300*, 75. [\[CrossRef\]](#)
28. Zhang, X.; Wang, H.; Ma, C.; Niu, N.; Chen, Z.; Liu, S.; Li, J.; Li, S. Seeking value from biomass materials: Preparation of coffee bean shell-derived fluorescent carbon dots *via* molecular aggregation for antioxidation and bioimaging applications. *Mater. Chem. Front.* **2018**, *2*, 1269. [\[CrossRef\]](#)
29. Liu, M.L.; Chen, B.B.; Li, C.M.; Huang, C.Z. Carbon dots: Synthesis, formation mechanism, fluorescence origin and sensing applications. *Green Chem.* **2019**, *21*, 449. [\[CrossRef\]](#)
30. Lakowicz, J.R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 1999.
31. Williams, A.T.R.; Winfield, S.A.; Miller, J.N. Relative fluorescence quantum yields using a computer-controlled luminescence spectrometer. *Analyst* **1983**, *108*, 1067. [\[CrossRef\]](#)
32. Melhuish, W.H. Quantum efficiencies of fluorescence organic substances: Effect of solvent and concentration of the fluorescent solute. *J. Phys. Chem.* **1961**, *65*, 229. [\[CrossRef\]](#)
33. Feijoo, S.; González-García, S.; Moldes-Diz, Y.; Vazquez-Vazquez, C.; Feijoo, G.; Moreira, M.T. Comparative life cycle assessment of different synthesis routes of magnetic nanoparticles. *J. Clean. Prod.* **2017**, *143*, 528. [\[CrossRef\]](#)
34. Hirschier, R.; Walser, T. Life cycle assessment of engineered nanomaterials: State of the art and strategies to overcome existing gaps. *Sci. Total Environ.* **2012**, *425*, 271. [\[CrossRef\]](#) [\[PubMed\]](#)

35. Huijbregts, M.A.J.; Steinmann, Z.J.N.; Elshout, P.M.F.; Stam, G.; Verones, F.; Vieira, M.; Zijp, M.; Hollander, A.; van Zelm, R. ReCiPe2016: A harmonised life cycle impact assessment method at midpoint and endpoint level. *Int. J. Life Cycle Assess.* **2017**, *22*, 138. [[CrossRef](#)]
36. Pianosi, F.; Beven, K.; Freer, J.; Hall, J.W.; Rougier, J.; Stephenson, D.B.; Wagener, T. Sensitivity analysis of environmental models: A systematic review with practical workflow. *Environ. Model. Softw.* **2016**, *79*, 214. [[CrossRef](#)]
37. Tan, J.; Zou, R.; Zhang, J.; Li, W.; Zhang, L.; Yue, D. Large-scale synthesis of N-doped carbon quantum dots and their phosphorescence properties in a polyurethane matrix. *Nanoscale* **2016**, *8*, 4742. [[CrossRef](#)]
38. Wang, J.; Peng, F.; Lu, Y.; Zhong, Y.; Wang, S.; Xu, M.; Ji, X.; Su, Y.; Liao, L.; He, Y. Large-scale green synthesis of fluorescent carbon nanodots and their use in optics applications. *Adv. Opt. Mater.* **2015**, *3*, 103. [[CrossRef](#)]
39. Suryawanshi, A.; Biswal, M.; Mhamane, D.; Gokhale, R.; Patil, S.; Guin, D.; Ogale, S. Large scale synthesis of graphene quantum dots (GQDs) from waste biomass and their use as an efficient and selective photoluminescence on-off-on probe for Ag⁺ ions. *Nanoscale* **2014**, *6*, 11664. [[CrossRef](#)]
40. Greczynski, G.; Hultman, L. C 1s Peak of Adventitious Carbon Aligns to the Vacuum Level: Dire Consequences for Material's Bonding Assignment by Photoelectron Spectroscopy. *Chem. Phys. Chem.* **2017**, *18*, 1507. [[CrossRef](#)]
41. Marsden, A.J.; Brommer, P.; Mudd, J.J.; Dyson, M.; Cook, R.; Asensio, M.; Avila, J.; Levy, A.; Sloan, J.; Quigley, D.; et al. Effect of oxygen and nitrogen functionalization on the physical and electronic structure of graphene. *Nano Res.* **2015**, *8*, 2620. [[CrossRef](#)]
42. Moulder, J.F.; Stickle, W.F.; Sobol, P.E.; Bomben, K.D. *Handbook of X-ray Photoelectron Spectroscopy*, Perkin-Elmer Corporation; Physical Electronics Division: Eden Prairie, MN, USA, 1992.
43. Campos, B.B.; Contreras-Cáceres, R.; Bandosz, T.J.; Jiménez-Jiménez, J.; Rodríguez-Castellón, E.; da Silva, J.C.E.; Algarra, M. Carbon dots coated with vitamin B 12 as selective ratiometric nanosensor for phenolic carbofuran. *Sens. Actuat. B* **2017**, *239*, 553. [[CrossRef](#)]
44. Dekaliuk, M.O.; Viagin, O.; Malyukin, Y.V.; Demchenko, A.P. Fluorescent carbon nanomaterials: "quantum dots" or nanoclusters? *Phys. Chem. Chem. Phys.* **2014**, *16*, 16075. [[CrossRef](#)] [[PubMed](#)]
45. Malyulin, Y.; Viagin, O.; Maksimchuk, P.; Dekaliuk, M.; Demchenko, A.P. Insight into the mechanism of the photoluminescence of carbon nanoparticles derived from cryogenic studies. *Nanoscale* **2018**, *10*, 9320. [[CrossRef](#)] [[PubMed](#)]
46. Sharma, A.; Gadly, T.; Gupta, A.; Ballal, A.; Ghosh, S.K.; Kumbhakar, M. Origin of Excitation Dependent Fluorescence in Carbon Nanodots. *J. Phys. Chem. Lett.* **2016**, *7*, 3695. [[CrossRef](#)]
47. Dam, B.V.; Nie, H.; Ju, B.; Marino, E.; Paulusse, J.M.; Schall, P.; Li, M.; Dohnalová, K. Excitation-Dependent Photoluminescence from Single-Carbon Dots. *Small* **2017**, *13*, 1702098.
48. Jiang, K.; Sun, S.; Zhang, L.; Lu, Y.; Wu, A.; Cai, C.; Lin, H. Red, Green, and Blue Luminescence by Carbon Dots: Full-Color Emission Tuning and Multicolor Cellular Imaging. *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 5360. [[CrossRef](#)]
49. Sarswat, P.K.; Free, M.L. Light emitting diodes based on carbon dots derived from food, beverage, and combustion wastes. *Phys. Chem. Chem. Phys.* **2015**, *17*, 27642–27652. [[CrossRef](#)]
50. Zhou, X.; Zhao, G.; Tan, X.; Qian, X.; Zhang, T.; Gui, J.; Yang, L.; Xie, X. Nitrogen-doped carbon dots with high quantum yield for colorimetric and fluorometric detection of ferric ions and in a fluorescent ink, *Microchim. Acta* **2019**, *186*, 67. [[CrossRef](#)]
51. Huang, Q.; Li, Q.; Chen, Y.; Tong, L.; Lin, X.; Zhu, J.; Tong, Q. High quantum yield nitrogen-doped carbon dots: Green synthesis and application as "off-on" fluorescent sensors for the determination of Fe³⁺ and adenosine triphosphate in biological samples. *Sens. Actuator B* **2018**, *276*, 82. [[CrossRef](#)]
52. Vallan, L.; Urriolabeitia, E.P.; Ruiperez, F.; Matxain, J.M.; Canton-Vitoria, R.; Tagmatarchis, N.; Benito, A.M.; Maser, W.K. Supramolecular-enhanced charge transfer within entangled polyamide chains as the origin of the universal blue fluorescence of polymer carbon dots. *J. Am. Chem. Soc.* **2018**, *140*, 12862–12869. [[CrossRef](#)]
53. Devi, P.; Rajput, P.; Thakur, A.; Kim, K.H.; Kumar, P. Recent advances in carbon quantum dot-based sensing of heavy metals in water. *Trends Anal. Chem.* **2019**, *114*, 171. [[CrossRef](#)]
54. de Acha, N.; Elosua, C.; Corres, J.M.; Arregui, F.J. Fluorescent Sensors for the Detection of Heavy Metal Ions in Aqueous Media. *Sensors* **2019**, *19*, 599. [[CrossRef](#)] [[PubMed](#)]
55. Berlina, A.N.; Zherdev, A.V.; Dzantiev, B.B. Progress in rapid optical assays for heavy metal ions based on the use of nanoparticles and receptor molecules. *Microchim. Acta* **2019**, *186*, 172. [[CrossRef](#)] [[PubMed](#)]

56. Qiao, Y.; Liu, C.; Zheng, X. Enhancing the quantum yield and Cu²⁺ sensing sensitivity of carbon dots based on the nano-space confinement effect of silica matrix. *Sens. Actuators B* **2018**, *259*, 211. [[CrossRef](#)]
57. Pourzahedi, L.; Eckelman, M.J. Comparative life cycle assessment of silver nanoparticle synthesis routes. *Environ. Sci. Nano* **2015**, *2*, 361. [[CrossRef](#)]
58. Eckelman, M.J.; Zimmerman, J.B.; Anastas, P.T. Toward Green Nano. *J. Ind. Ecol.* **2008**, *12*, 316.
59. Hauschild, M.Z. Assessing Environmental Impacts in a Life-Cycle Perspective. *Environ. Sci. Technol.* **2005**, *39*, 81A–88A. [[CrossRef](#)]
60. Ramos, A.; Teixeira, C.A.; Rouboa, A. Assessment study of an advanced gasification strategy at low temperature for syngas generation. *Int. J. Hydrog. Energy* **2018**, *43*, 10155–10166. [[CrossRef](#)]
61. Ramos, A.; Teixeira, C.A.; Rouboa, A. Environmental Analysis of Waste-to-Energy—A Portuguese Case Study. *Energies* **2018**, *11*, 548. [[CrossRef](#)]
62. Christé, S.; da Silva, J.C.G.E.; da Silva, L.P. Evaluation of the Environmental Impact and Efficiency of N-Doping Strategies in the Synthesis of Carbon Dots. *Materials* **2020**, *13*, 504. [[CrossRef](#)]



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2.2. Supporting Information

SUPPORTING INFORMATION

Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots

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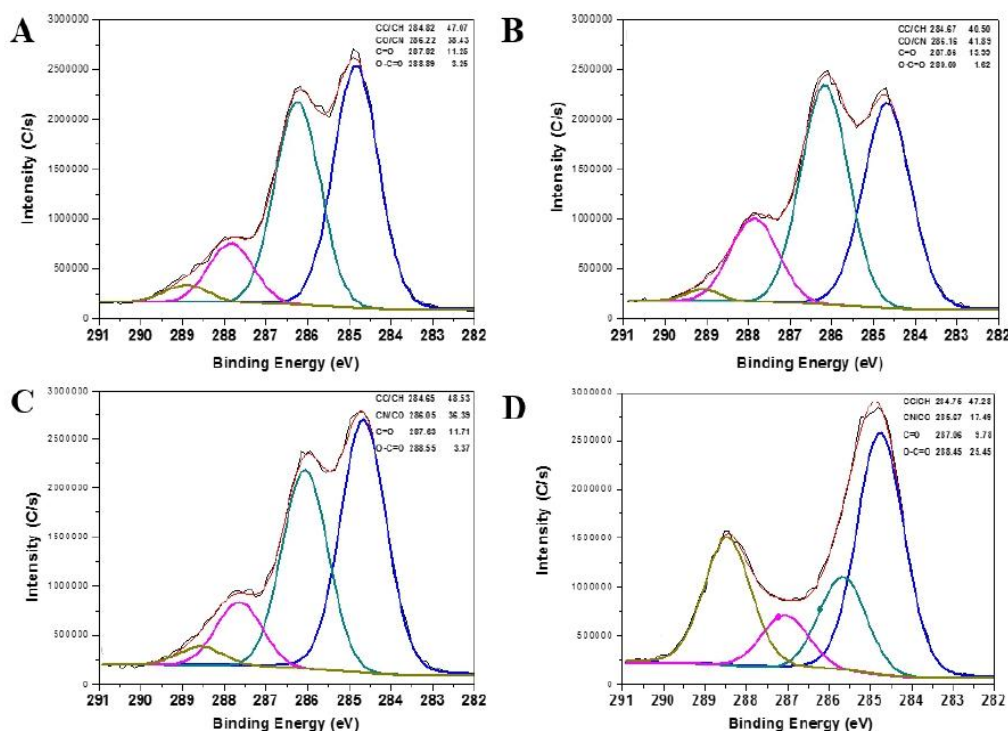


Figure S1 – XPS C 1s spectra for decaf-CDs (A), rossio-CDs (B), prestige-CDs (c) and CA@U-CDs (D).

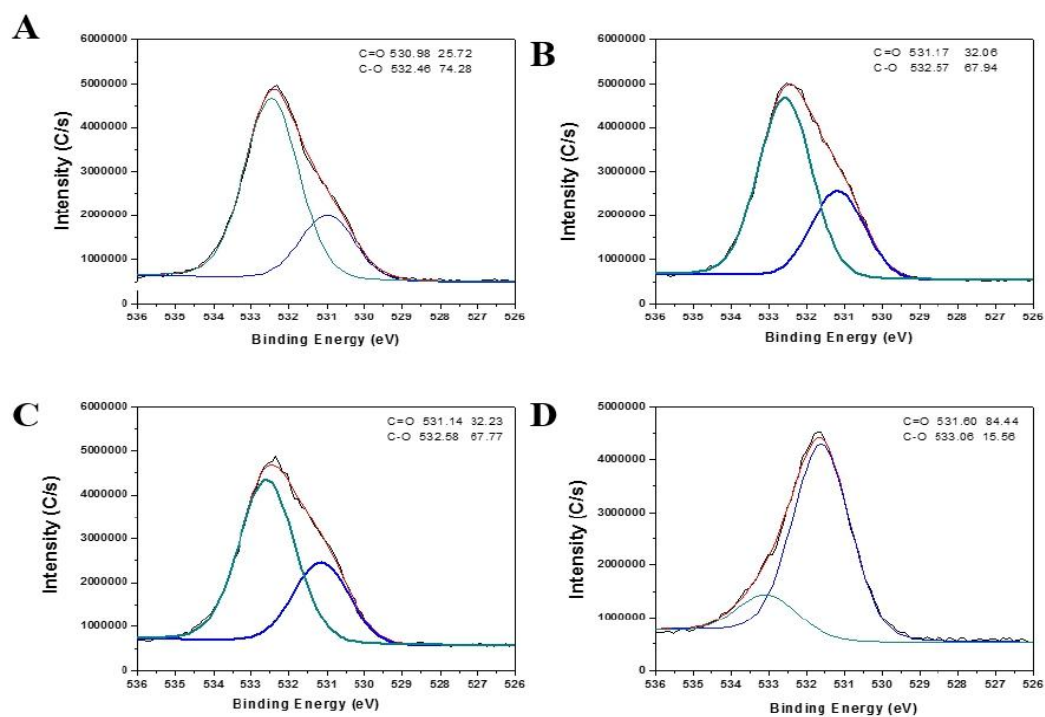


Figure S2 – XPS O 1s spectra for decaf-CDs (A), rossio-CDs (B), prestige-CDs (C) and CA@U-CDs (D).

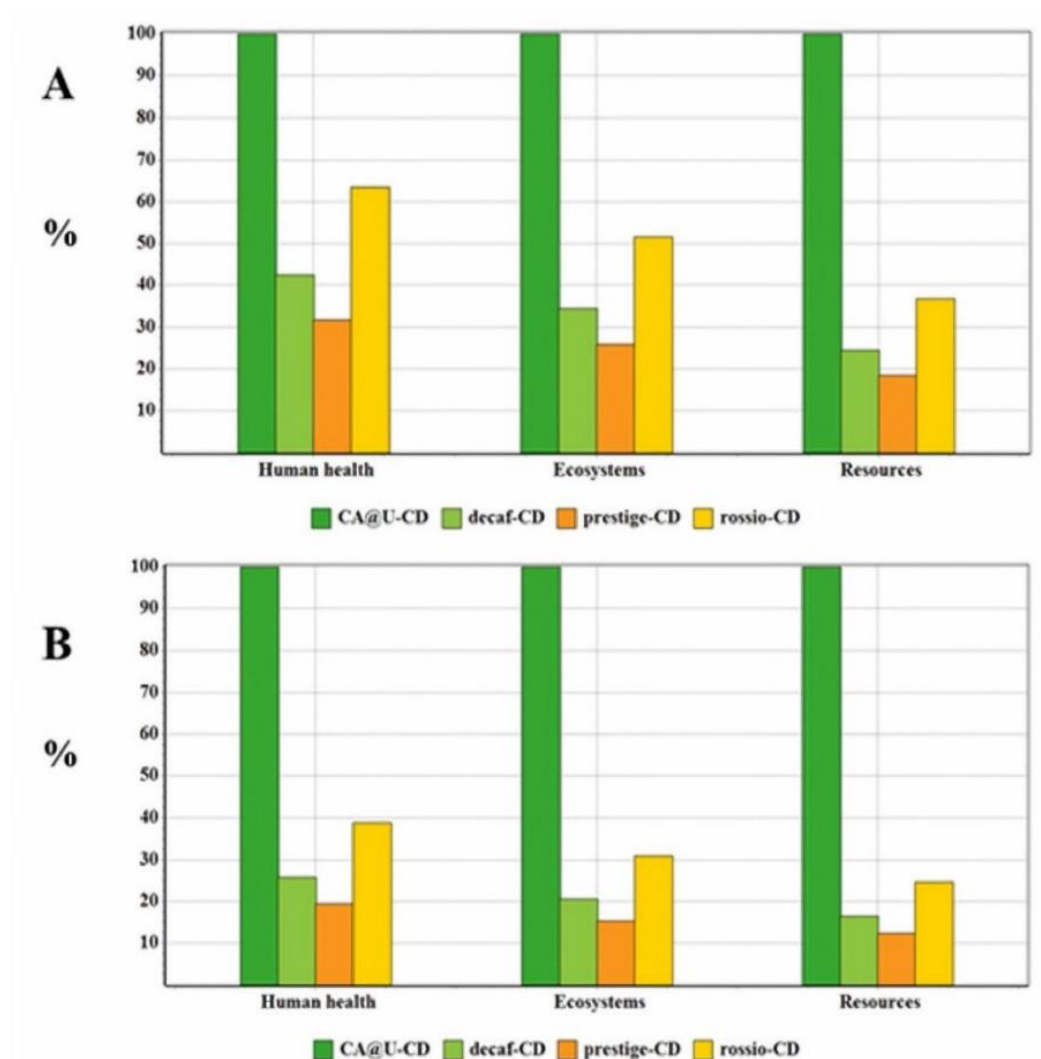


Figure S4. Comparative environmental profiles for the synthesis of all CDs, re-scaled with respect to the QY_{FL} of the CDs, by changing the input of citric acid to 70% (A) and to 130% (B) of the original value.

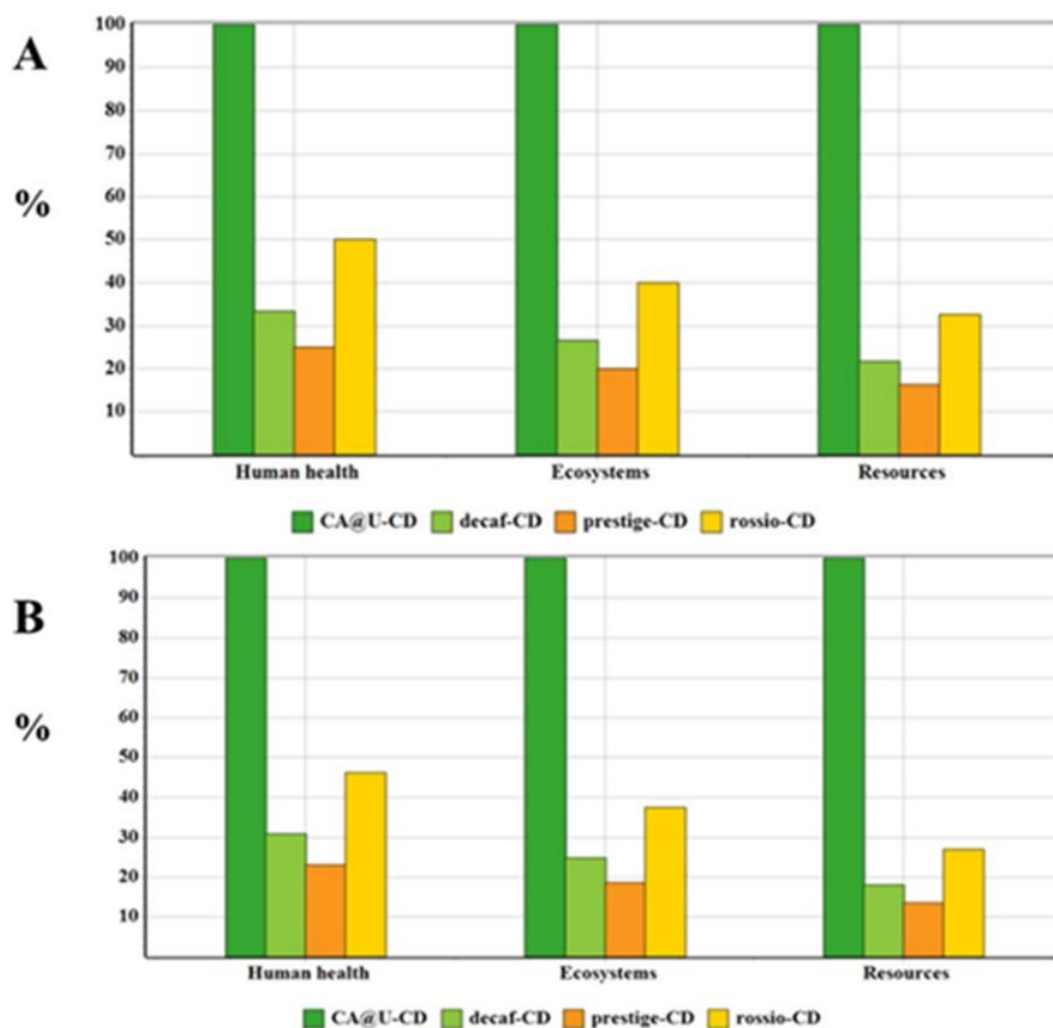


Figure S5. Comparative environmental profiles for the synthesis of all CDs, re-scaled with respect to the QY_{FL} of the CDs, by changing the input of urea to 70% (A) and to 130% (B) of the original value.

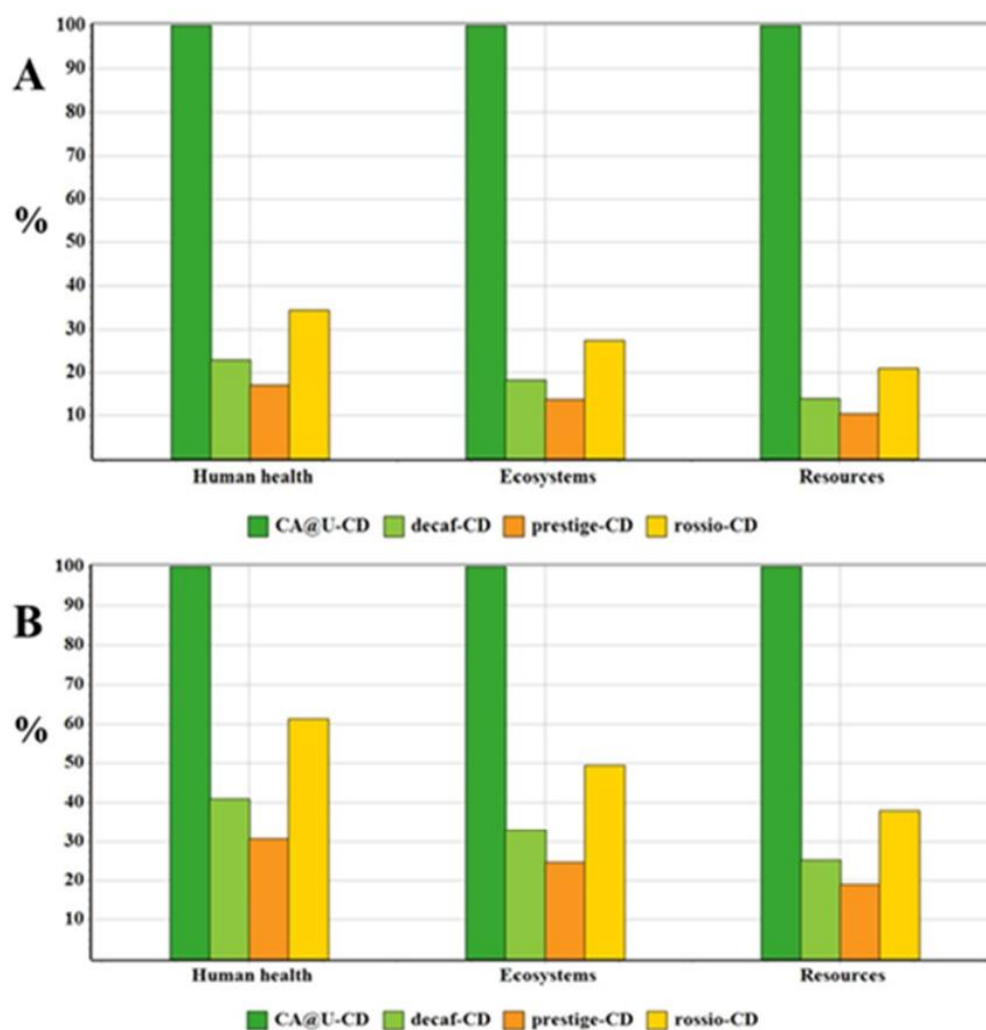


Figure S6. Comparative environmental profiles for the synthesis of all CDs, re-scaled with respect to the QY_{FL} of the CDs, by changing the input of electricity to 70% (A) and to 130% (B) of the original value.

2.3. Conclusions

The synthesis of fluorescent CDs utilizing either SCGs, a biomass waste material from the coffee industry, or conventional precursors such as citric acid and urea was evaluated, considering their environmental implications and the structural and luminescent characteristics of the resultant nanoparticles. The one-step, solvent-free carbonization process of the various precursor materials yielded nanoparticles with an average size ranging from 1.0 to 3.9 nm and similar blue emission properties. However, CDs derived from SCGs exhibited moderate quantum yields (2.9–5.8 %) and relatively low reaction yields, whereas those from citric acid and urea displayed significantly higher quantum yields (22.5 %) and reaction yields.

Nonetheless, a LCA revealed that the production of CDs using SCGs is more environmentally advantageous than conventional precursors, regardless of the chosen unit for evaluation (weight-based or function-related). Thus, although SCG-based CDs may require enhancements compared to those synthesized from typical precursors, this study underscores the sustainability of utilizing SCGs as precursors for CD fabrication. Furthermore, SCG-based CDs exhibited potential as sensing probes for Fe³⁺ in aqueous environments.

3. Carbon dots synthesis by three different bottom-up strategies

3.1. Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots

Article 2

Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots

Diana M.A.Crista, Joaquim C.G. Esteves da Silva and Luís Pinto da Silva, *Nanomaterials*, **2020**, 10, 1316.

3.1.1. Contextualization

CDs represent a class of carbon-based nanoparticles characterized by highly appealing luminescent properties. Their synthesis through bottom-up approaches offers considerable flexibility, as variations in reaction precursors and synthesis methodologies yield a diverse array of CDs with distinct properties and potential applications. However, this extensive variability complicates the characterization of the structural and optical features of these nanomaterials. In this chapter, we studied three representative bottom-up strategies (hydrothermal, microwave-assisted, and calcination) to elucidate their impact on the properties of CDs derived from identical precursors (citric acid and urea).

Our findings revealed that these synthesis routes produced nanoparticles of comparable sizes, displaying identical excitation-dependent blue-to-green emission and similar surface functionalization. Nonetheless, we observed that microwave and calcination methods exhibited superior efficiency in nitrogen-doping compared to hydrothermal synthesis, resulting in CDs with markedly higher fluorescence quantum yields. Additionally, the different synthesis strategies influenced the photoluminescence characteristics of the CDs, with hydrothermal-based nanoparticles demonstrating emission sensitivity to surface states, while microwave- and calcination-based CDs

showed emissions predominantly influenced by core states. Furthermore, calcination and microwave routes proved more conducive to high-yield synthesis (~27–29 %), whereas hydrothermal synthesis yielded negligible synthesis yields (~2 %). Finally, a LCA was conducted to evaluate the sustainability of these processes, highlighting microwave synthesis as the most favourable option for future investigations.

3.1.2. Contributions to this work

All synthesis of CDs, as well as optical characterization and LCA studies, was performed by me (Diana Crista) under the supervision of my supervisors. XPS analysis, AFM, and Zeta Potential measurements and discussion were conducted with my assistance.



Article

Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots

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Abstract: Carbon dots (CDs) are carbon-based nanoparticles with very attractive luminescence features. Furthermore, their synthesis by bottom-up strategies is quite flexible, as tuning the reaction precursors and synthesis procedures can lead to an endless number of CDs with distinct properties and applications. However, this complex variability has made the characterization of the structural and optical properties of the nanomaterials difficult. Herein, we performed a systematic evaluation of the effect of three representative bottom-up strategies (hydrothermal, microwave-assisted, and calcination) on the properties of CDs prepared from the same precursors (citric acid and urea). Our results revealed that these synthesis routes led to nanoparticles with similar sizes, identical excitation-dependent blue-to-green emission, and similar surface-functionalization. However, we have also found that microwave and calcination strategies are more efficient towards nitrogen-doping than hydrothermal synthesis, and thus, the former routes are able to generate CDs with significantly higher fluorescence quantum yields than the latter. Furthermore, the different synthesis strategies appear to have a role in the origin of the photoluminescence of the CDs, as hydrothermal-based nanoparticles present an emission more dependent on surface states, while microwave- and calcination-based CDs present an emission with more contributions from core states. Furthermore, calcination and microwave routes are more suitable for high-yield synthesis (~27–29%), while hydrothermal synthesis present almost negligible synthesis yields (~2%). Finally, life cycle assessment (LCA) was performed to investigate the sustainability of these processes and indicated microwave synthesis as the best choice for future studies.

Keywords: carbon dots; bottom-up synthesis; photoluminescence; life cycle assessment

1. Introduction

Carbon dots (CDs) are quasi-spherical carbon-based nanoparticles, with a core that can be either amorphous or nanocrystalline [1–4]. The core of these nanomaterials is expected to be composed mainly of graphitic sp² carbon connected by amorphous sp³ carbon atoms in between. The surface can present different functionalization degrees, depending on the chosen precursors and synthesis routes, and thus, different functional groups can be found (such as carboxylic acids, alcohols, and amines) [1,5].

Carbon dots belong to the broad family of carbon allotropes and related nanostructures and molecules. Carbon allotropes include diamond with sp³ hybridization, graphite, graphene, and carbon nanotubes with sp² hybridization, fullerenes with hybridization between sp² and sp³, as well as graphyne and carbynes with sp hybridization [6–8].

CDs have attracted significant attention from the research community due to their quite remarkable features, such as high photoluminescence [2,9,10], low toxicity [11], good water solubility [2,12], biocompatibility [13,14], and good physical–chemical and photochemical stability [12,15,16].

Therefore, it is not surprising that CDs have gained relevance in various fields, such as sensing [17–20], bioimaging [21], photocatalysis [10], drug delivery [22], solar cells [23], and photodynamic therapy [24].

CDs can be obtained by two main synthesis routes: top-down and bottom-up [3,25,26]. The former procedure is based on the breakdown of large graphitic materials into smaller carbon-based materials [27]. However, top-down approaches generally require harsh reaction conditions, expensive materials/equipment, and long processing times. By their turn, bottom-up routes are arguably the most widely used synthesis methods [3,25,26]. They generally consist on the pyrolysis of smaller organic molecules either in powder form (calcination) [28] or in solution via hydrothermal [25,29] or microwave-based approaches [16,30,31]. Bottom-up strategies have the advantage of being suitable for mass production, being eco-friendly, and of low cost [26].

While top-down strategies are restricted to using carbon “bulk” samples as precursors (such as graphite powders, graphene, and black carbon) [5], bottom-up procedures can use a myriad of molecular organic precursors, which range from smaller chemicals (as carbohydrates and acids) [1,3] to more heterogenous sources (as rice, coffee beans, and waste) [32–35]. Due to the structure, surface-functionalization and photoluminescence of the CDs are heavily dependent on the choice of precursors and synthesis procedures. This flexibility of bottom-up strategies allows for endless possibilities for tuning the properties of CDs and develop novel applications for them.

Among the several existent possibilities for organic precursors, citric acid (CA) is arguably the most popular one [1,3,5]. CA is readily available (being present even in several citrus fruits), cheap, nontoxic, and has a low carbonization temperature, thereby being the choice for several researchers. However, synthesis using just CA generally leads to CDs with low photoluminescence [36]. Therefore, researchers typically add also precursors containing appropriate heteroatoms (such as N, S, and P) to achieve heteroatom-doping of CDs [37], which generally enhances the fluorescence quantum yield (QY_{FL}) [38–40] of the CDs. Among these, N-doping is the most widely used for enhancing the QY_{FL} of CDs. N-doping is achieved by adding a nitrogen-containing small organic molecule as a nitrogen source to the carbon precursor in which urea [16,41] and ethylenediamine (EDA) [42,43] are common options.

The high flexibility of bottom-up strategies (both in terms of precursors and synthesis routes) has led to an explosion in the number of studies focusing on CDs, which resulted in countless CDs with different properties and/or applications. However, the large number of possible variables has made it difficult to understand the role of either the chosen precursors or the employed synthesis routes on the properties of the obtained CDs. Thus, at this moment it is not really possible to develop new CDs in a rational and target-oriented manner.

Moreover, significant efforts to determine the role of precursors in the structure, surface-functionalization, and photoluminescence of CDs have recently been made [1,5]. The effect exerted by the employed bottom-up route is still poorly understood due to the lack of systematic studies. Herein, in this study we have focused on evaluating the effect exerted by three representative bottom-up routes (hydrothermal, microwave-assisted, and calcination synthesis) on the properties of CDs obtained using the same precursors (CA and urea as carbon and nitrogen sources, respectively). To this end, the CDs were characterized by fluorescence, UV–Vis, and X-ray photoelectron (XPS) spectroscopy, as well as dynamic light scattering (DLS) and atomic force microscopy (AFM). A life cycle assessment (LCA) approach was also used to evaluate the sustainability and environmental impacts of each synthesis route [44–46].

2. Materials and Methods

2.1. Fabrication

The three types of studied CDs were prepared from the same set of precursors (urea and citric acid, CA), which were purchased from Sigma-Aldrich (St. Louis, MO, USA), via different bottom-up routes: hydrothermal-, microwave-, and calcination-assisted synthesis. The same amount of CA (0.75 g) and urea (0.25 g) was used in all syntheses. For the hydrothermal-assisted synthesis, CA and urea were mixed in 5 mL of deionized water. The reaction mixture was then placed in a Teflon-lined reactor, which was encased in a stainless-steel shell, and was heated at 200 °C for 2 h in an oven. In microwave-assisted synthesis, CA and urea were also mixed in 5 mL of deionized water. The reaction mixture solution was placed in a glass beaker and was subsequently subjected to microwave irradiation (700 W in a domestic microwave) for 5 min. Finally, calcination-assisted synthesis consisted on the one-pot thermal heating of a CA and urea powder mixture during 2 h in an oven at 200 °C placed in a glass petri box.

The synthesized CDs were subsequently suspended in water (5 mL) and purified by centrifugation (10 min at 12,000 rpm) to eliminate suspended impurities. The samples were purified by dialysis (Float-A-Lyzer® G2 Dialysis Device SPECTRUM® (New Brunswick, NJ, USA) (molecular weight cut-off of 500 Da)) for 24 h.

2.2. Characterization

Fluorescence analysis was measured in a 10 mm fluorescence quartz cell by using a Horiba Jobin Yvon Fluoromax spectrofluorimeter (Madrid, Spain) using 5 nm slit widths. AFM analysis was carried out using a Veeco (Plainview, NY, USA) Metrology Multimode/Nanoscope IVA by tapping mode, using a Bruker (Billerica, MD, USA) silicon probe (model TESP-SS, resonant frequency 320 kHz, nominal force constant 42 N/m, estimated tip radius 2 nm). The Zeta Potential was measured by using a particle analyzer Anton Paar Litesizer™ 500 (Graz, Austria) and a polycarbonate Omega Cuvette (Ref. 155765). X-ray photoelectron spectroscopy (XPS) analysis was recorded with a Fi Kratos Axis Ultra HAS-VISION (Manchester, UK), using a monochromatic Al-K α radiation (15 kV, 90 W). The samples were deposited in a silica plate. Spectra were analyzed by using the CasaXPS software (Teignmouth, UK).

2.3. Fluorescence Quantum Yield Calculation

Fluorescence quantum yield (QY_{FL}) was calculated with a standard procedure, based on the comparison of the integrated luminescence intensities and absorbance values of the synthesized CDs with a reference (which depends on the emission wavelength of the sample) quinine sulfate with the following equation:

$$QY_{FL}^{\text{Sample}} = QY_{FL}^{\text{Quinine Sulphate}} \times (\text{Grad}_{\text{Sample}}/\text{Grad}_{\text{Quinine Sulphate}}) \times (\eta^2_{\text{Sample}}/\eta^2_{\text{Quinine Sulphate}}) \quad (1)$$

where Grad is the gradient from the plot of integrated fluorescence intensity versus absorbance and η is the refractive index [47]. Quinine sulfate was chosen as a reference fluorophore of known quantum yield (QY_{FL} = 0.54) [48]. Absorbance measurements were made with a UNICAM Helios Gamma, using standard quartz cells.

2.4. Scope and System Boundaries

This cradle-to-gate study aims to quantify and compare the potential environmental impacts as well as characterize and evaluate the differences found in the three different methods for the synthesis of CDs: hydrothermal, calcination, and microwave-assisted synthesis. In this research we will study the laboratory-scale manufacturing stage of the target nanoparticles and consider the direct emissions from CDs production and the indirect impacts associated with upstream resource extraction and energy generation. Three different synthetic strategies were used. CA and urea were used as carbon and

nitrogen source, respectively. The environmental impacts were compared first by using a weight-based functional unit of 1 Kg of CDs. These impacts were also normalized by the QY_{FL} of the different CDs. This is needed because weight-based functional units do not consider functional benefits for which they were engineered for. The QY_{FL} was chosen as the normalization factor because, while CDs have many different applications, a high QY_{FL} is generally a very important property in most of these applications.

2.5. Life Cycle Inventory Data

The environmental impacts related to these three different synthesis routes were evaluated based on inventory data from laboratory-scale synthesis procedures found in the Ecoinvent[®] 3.5 database. The foreground system of the synthesis procedure consists of chemicals used as raw material and electricity used in the fabrication process and purification steps (oven, microwave, heating plate, centrifuge, stirring plate). The different processes and chemicals included in this study were modeled with the following data presented in the Ecoinvent[®] 3.5 database (GLO standing for global, RER for regional market for Europe, and PT for Portugal):

- Citric acid {GLO}|Market;
- Urea, as N {GLO}|Market;
- Water, deionized, from tap water, at user {Europe without Switzerland}|Market;
- Electricity, medium voltage|Market;
- Chemical Waste, unspecified.

The chemical amount used is described in Table S1 and was rescaled to the amount needed to produce 1 Kg of CDs. The dataset used for electricity describes the available electricity data on the medium voltage level in Portugal in 2014, as described in the Ecoinvent[®] 3.5 database. The electricity consumption considered here combines the electricity required for using either a domestic microwave (microwave-assisted synthesis) or oven (hydrothermal or calcination-based synthesis) with electricity used during the purification steps (centrifugation and dialysis). Microwave-assisted synthesis was made in an Electronia domestic microwave (model P70B17L-DE) with a power consumption of 700 W. The hydrothermal-assisted and calcination-based syntheses were made in a Furnace 47900 from Thermolyne with a power consumption of 1000 W. Centrifugation was made with a power consumption of 180 W. Stirring was done using a Jenway (Staffordshire, UK) model 1000 stirrer, with 500 W maximum power consumption.

2.6. Environmental Impact Assessment

This study is based on the cradle-to-gate approach, from the production of precursor materials to the fabrication of CDs. Environmental impacts were modeled using the ReCiPe 2016 V1.03 endpoint method, Hierarchist version, which evaluates three categories of potential impacts: Human Health, Ecosystems, and Resources. In the Human Health subsection the following is evaluated: global warming–human health (GW-HH), stratospheric ozone depletion (SO), ionization radiation (IR), ozone formation–human health (OF-HH), fine particulate matter formation (FPM), human carcinogenic toxicity (HC), human non-carcinogenic toxicity (HNC), and water consumption–human health (WC-HH). Ecosystems potential impacts evaluate the following: global warming–terrestrial ecosystems (GW-TE), global warming–freshwater ecosystems (GW-FE), ozone formation–terrestrial ecosystems (OF-TE), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial ecotoxicity (TET), freshwater ecotoxicity (FET), marine ecotoxicity (MET), land use (LU), water consumption–terrestrial ecosystem (WC-TE), and water consumption–aquatic ecosystems (WC-AE). In the Resources subsection the following is evaluated: mineral resource scarcity (MR), fossil resource scarcity (FR). LCA study was performed using the SimaPro 9.0.0.48 software (Amersfoort, The Netherlands).

3. Results and Discussion

3.1. Synthesis

AFM measurements (Figure 1) were performed to determine the size of the three types of obtained CDs, and to ensure that they were indeed nanosized. AFM results provided the following average nanoparticles sizes; 6.9 ± 2.0 nm for hydrothermal-CDs, 7.3 ± 1.7 nm for microwave-CDs, and 6.1 ± 1.7 nm for calcination-CDs.

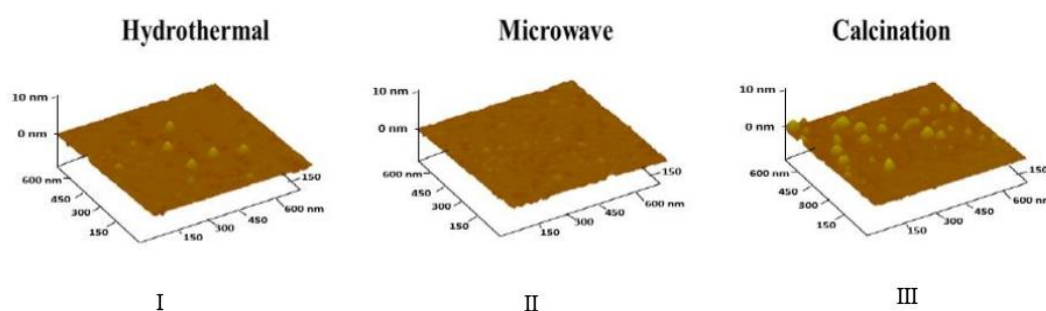


Figure 1. Atomic force microscopy (AFM) images for (I) hydrothermal-carbon dots (CDs), (II) microwave-CDs, and (III) calcination-CDs.

The synthesis yield (weight-by-weight, in %) (Table 1) was calculated considering the amount of final powder (after purification step) and initial precursors. The reaction yields obtained were satisfactory in the case of microwave- and calcination-assisted syntheses with 31.6% and 26.1%, respectively. In fact, the obtained synthesis yields for those two CDs are in line with values obtained in studies aiming to high-yield synthesis of CDs [49]. Unfortunately, the synthesis yield for hydrothermal-CDs was quite low (1.5%), which indicates that this type of bottom-up route is not appropriated for high-yield synthesis.

Table 1. Synthesis and fluorescence quantum yields (in %) and zeta potential (in mV) data for the three CDs.

	Hydrothermal	Microwave	Calcination
Synthesis yield/%	1.8 ± 0.4	28.5 ± 4.5	26.9 ± 1.1
Quantum yield/%	3.7	25.1	29.3
Zeta Potential/mV	−0.5	0.0	−0.1

3.2. Surface Characterization

The surface of the three types of CDs was characterized by XPS spectroscopy (Figures S1–S3), with the XPS atomic composition (at %) of each sample being presented in Table 2. The data showed that all CDs are composed by C (~60–62%), N (~9–13%), and O (~24–28%). Interestingly, we can see that the studied bottom-up routes can lead to different degrees of N- and O-incorporation in the surface of CDs. Namely, hydrothermal synthesis led to both the highest amount of O (28.8%), which was achieved with the lowest amount of N (9.1%). By its turn, microwave-assisted synthesis appears to be the most well-balanced route in this respect, with relatively high incorporation of both N (13.1%) and O (26.9%). These findings are in line with literature, as a recent study performed by our group also revealed that microwave-assisted synthesis led to higher N-incorporation than a hydrothermal route for the same set of precursors, at the expense of O-incorporation [25]. On the contrary, calcination achieved the highest incorporation of N (13.5%) at the expense of O (24.7%), as this type of synthesis was responsible for the lowest amount of the latter heteroatom.

Table 2. Atomic composition (%) obtained by XPS for the three different synthesis.

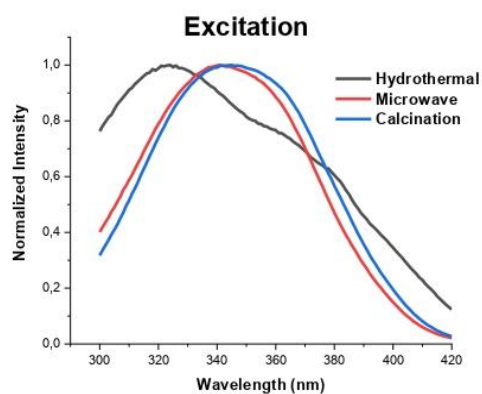
	Hydrothermal-CDs	Microwave-CDs	Calcination-CDs
C (%)	62.0	60.0	61.9
N (%)	9.1	13.1	13.5
O (%)	28.8	26.9	24.7

Functional groups present at the surface of the CDs were identified by performing a detailed scan for internal levels of C 1s, O 1s, and N 1s (Figures S1–S3). C 1s spectra could be split into three peaks (Figure S1) in the case of microwave and calcination synthesis: at binding energies of ~285 eV (attributed to C-C/C-H groups) [50], ~286 eV (C-O/C-N groups) [51], and ~288 eV (O-C = O) [52]. In the case of hydrothermal synthesis, four peaks were found instead: at binding energies of ~285 eV (attributed to C-C/C-H groups), ~286 eV (C-O/C-N groups), ~287 eV (C = O groups), and ~288 eV (O-C = O). Thus, all produced CDs presented a higher contribution from C-C/C-H groups (~56–60%), followed by O-C = O groups (~20–36%) and C-O/C-N groups (~9–12%). For hydrothermal-CDs, we also observed a contribution from C = O groups (~9%). O 1s could be split into two peaks at a binding energy of ~531 eV (C = O groups) and ~532 eV (C-O groups) [52]. The three synthesized CDs present a similar composition of almost 50% from both groups: C-O groups (~50.01%) and C = O groups (~49.99%). Finally, N 1s could also be split into two peaks at binding energies of ~400 eV (amine/amide groups) and ~401 eV (protonated amides). All three samples show the same contribution from protonated amines (~49.98%) and amine/amide groups (~49.98%). Thus, the three different routes appear to generate the same type of functional groups in the surface of the CDs. The main difference is then the apparently higher presence of C = O groups in the surface of hydrothermal-CDs, which is in line with their higher content of both C (62.0%) and O (28.8%).

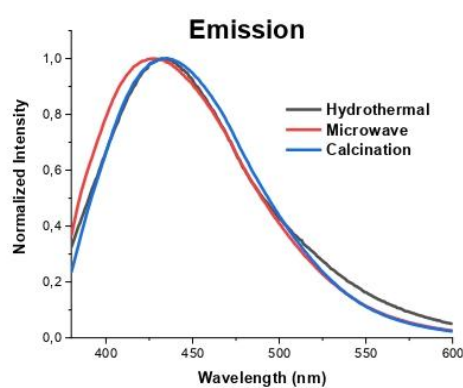
The zeta-potential of all CDs (Table 1) was measured by DLS in aqueous solutions: −0.5 mV for hydrothermal-CDs, 0.0 mV for microwave-CDs, and −0.1 mV for calcination-CDs. These results indicate that all three CDs present very a similar charge and can be considered virtually as neutral particles. This similarity is not surprising as XPS analysis revealed similar types of surface functional groups. The slightly higher negative charge for hydrothermal-CDs is also not unexpected, given the lower amount of N (9.1%) in comparison to the other two CDs (13.1–13.5%), which can lead to a lower amount of cationic functional groups (such as protonated amides). Nevertheless, the almost neutral values for all CDs indicate that these nanoparticles are prone to aggregation in solution.

3.3. Fluorescent Characterization

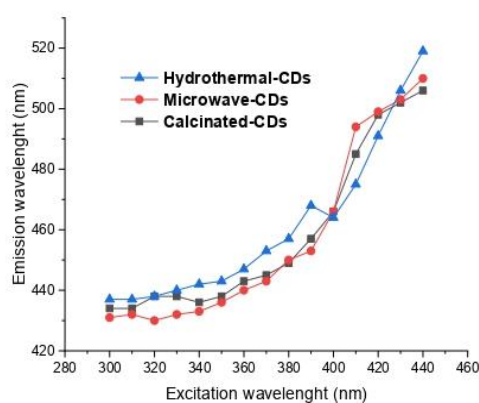
The absorption (Figure S4), excitation (Figure 2), and emission (Figure 2) spectra of the three CDs were obtained in aqueous solution. The absorption spectra for the CDs are very similar and present a main band at ~350 nm and a shoulder at ~245 nm, which can be attributed to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions [16]. In terms of fluorescence, the three bottom-up routes do not appear to induce significant differences, as all CDs present similar blue emission (433–438 nm) and UV excitation (320–340 nm). Nevertheless, it can be seen that hydrothermal-CDs present a blue-shifted excitation when comparing with other CDs. This can be attributed to the lower N-doping of the former CDs (Table 1), as N-doping is known to red-shift the excitation of CDs [53].



I



II



III

Figure 2. (I) Excitation- and (II) emission-spectra in aqueous solution from hydrothermal-, microwave-, and calcinated-CDs at 320, 340, and 330 nm excitation wavelength, respectively, and (III) emission wavelength (nm) as a function of the excitation wavelength (nm).

As shown in Figure 2II, all samples present an excitation-dependent emission which is a typical characteristic of CDs [54,55]. As we said before, all samples exhibit a blue fluorescence and when we increase the excitation wavelength from 300 to 440 nm, we observe a red-shift of ~80 nm towards green emission.

While the position of the fluorescence peak is identical for the three CDs, their intensity is not. Namely, both microwave-CDs (25.1%) and calcination-CDs (29.3%) present significantly higher QY_{FL} than hydrothermal-CDs (3.7%). Thus, hydrothermal synthesis is at a disadvantage when we compare it with microwave and calcination synthesis. As for the reason of the lower QY_{FL} for hydrothermal-CDs, it should be noted that N-doping strategies have been demonstrated to generate high QY_{FL} [56,57]. In fact, XPS analysis (Table 2) has demonstrated that hydrothermal synthesis leads to a lower degree of N-incorporation into the surface of CDs than the other two bottom-up routes, which may explain their lower QY_{FL}. Furthermore, previous research by Maser et al. showed that the abundance of amide groups in the surface of CDs can lead to higher photoluminescence by favoring a more rigid structure, due to photoinduced charge transfer between amide and carboxylic groups [58]. Therefore, it is possible that hydrothermal-CDs possess lower amide groups compared to the other CDs due to a lower N-content (Table 2), leading to lower QY_{FL}. In summary, our data revealed that while the three studied bottom-up routes lead to identical fluorescence wavelengths (probably due to generating similar surface functional groups), they can lead to significantly different QY_{FL} values, which can be attributed to their different efficiencies regarding heteroatom (O and N) doping.

Despite extensive research, the origin of photoluminescence of CDs is still a matter of debate. A particular focus of research in recent years is to determine the role and relevance of core and surface states to the emission of these nanomaterials [59–61]. Therefore, we have also analyzed the effect exerted by the external environment to the fluorescence of the three studied CDs. More specifically, we have measured their fluorescence spectra in different protic and aprotic solvents: water, ethanol (EtOH), methanol (MeOH), acetonitrile (ACN), and dimethylformamide (DMF). The obtained results can be observed in Figure 3.

Interestingly, the solvent affected the three CDs in different ways. Hydrothermal-CDs were the most affected nanoparticles as their fluorescent intensity varied widely with the solvent. Furthermore, the emission band of hydrothermal-CDs appears to be particularly influenced by hydrogen-bonding, as they presented the same emission maxima (~440) in the three protic solvents (water, ethanol, and methanol). However, their emission maxima underwent a ~35 nm red-shift (to ~475 nm) in the two studied aprotic solvents (DMF and acetonitrile). These results indicate that the fluorescent moieties of hydrothermal-CDs should be exposed to the external environment, and thus, their emission should have a relevant contribution from surface states. On the contrary, the solvent has limited influence on the emission of both microwave-based and calcination-CDs. More specifically, the solvent does not significantly affect their emission bands, with the maximum red-shift being just ~10 nm in DMF. The changes in fluorescence intensity are also similar between microwave-based and calcination-CDs, and less relevant for hydrothermal-CDs. Thus, we can conclude that the fluorescence moieties of the former CDs are shielded from the external environment, and thus, their emission should originate more from core states with more limited influence from surface states.

We have also analyzed the effect of the methyl viologen (a known electron acceptor) on the emission of the CDs (Figure S5). Methyl viologen did not induce any appreciable effect on the emission of the three CDs, which indicates that they are not electron-donors.

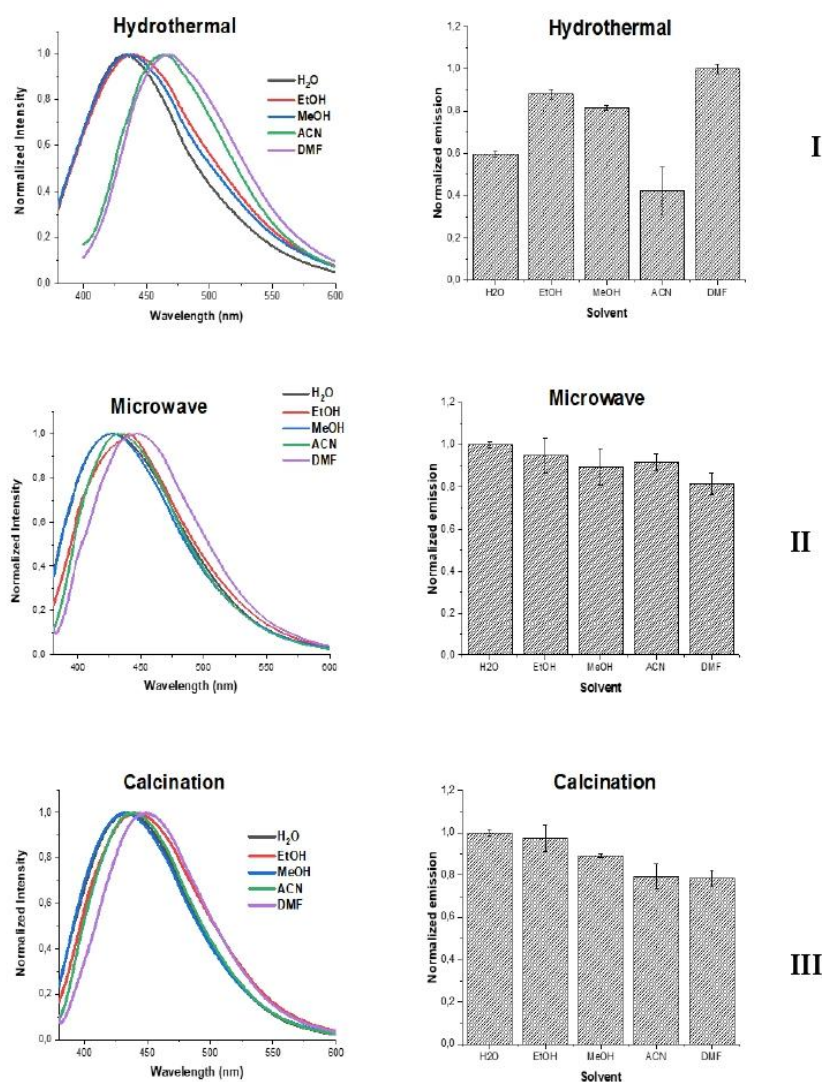


Figure 3. Normalized emission spectra for the three CDs: (I) hydrothermal-CDs, (II) microwave-CDs, and (III) calcination-CDs.

3.4. Comparative LCA Study

In this section, the three different synthesis routes were first evaluated individually to analyze their impact contributions in each category using a weight-based functional unit to produce 1 Kg of CDs. As in line with previous studies from our group [25,31], the use of CA accounts for most environmental impacts to all categories (Figures S5–S7). Urea is clearly the second highest contributor to the different categories for hydrothermal synthesis, especially for the Resources category (Figure S6). On the contrary, the environmental impacts of electricity consumption are relatively negligible for hydrothermal synthesis (Figure S6). The profile of environmental impacts for calcination and microwave synthesis is somewhat different (Figures S7 and S8). Namely, for these two synthesis routes, both urea and electricity consumption present more relevant contributions to the different categories, despite CA still being the highest one. Moreover, electricity is now the second highest contributor for the global categories of Human Health and Ecosystems (Figures S7 and S8), for both calcination and microwave synthesis. Water consumption presents negligible contributions for both hydrothermal and microwave synthesis, while being non-existent for the calcination route.

When we compare the relative impacts of the three synthesis routes, we can clearly see that the hydrothermal synthesis has the highest contribution for the environmental impact when compared to the other two syntheses (Figure S9 and Figure 4I). While microwave and calcination approaches provide similar impacts, we can identify the latter as the synthesis route with less environmental impacts, when we consider a weight-based functional unit. These results are easily explained by the synthesis yields (Table 1) of these synthetic strategies in which the yield for hydrothermal synthesis is almost negligible while the other two approaches provide more relevant yields (with microwave-assisted synthesis being the one with higher yield). Thus, microwave synthesis appears to be a better option due to its higher reaction yield, and consequently, lower need of chemicals per 1 Kg of CD production.

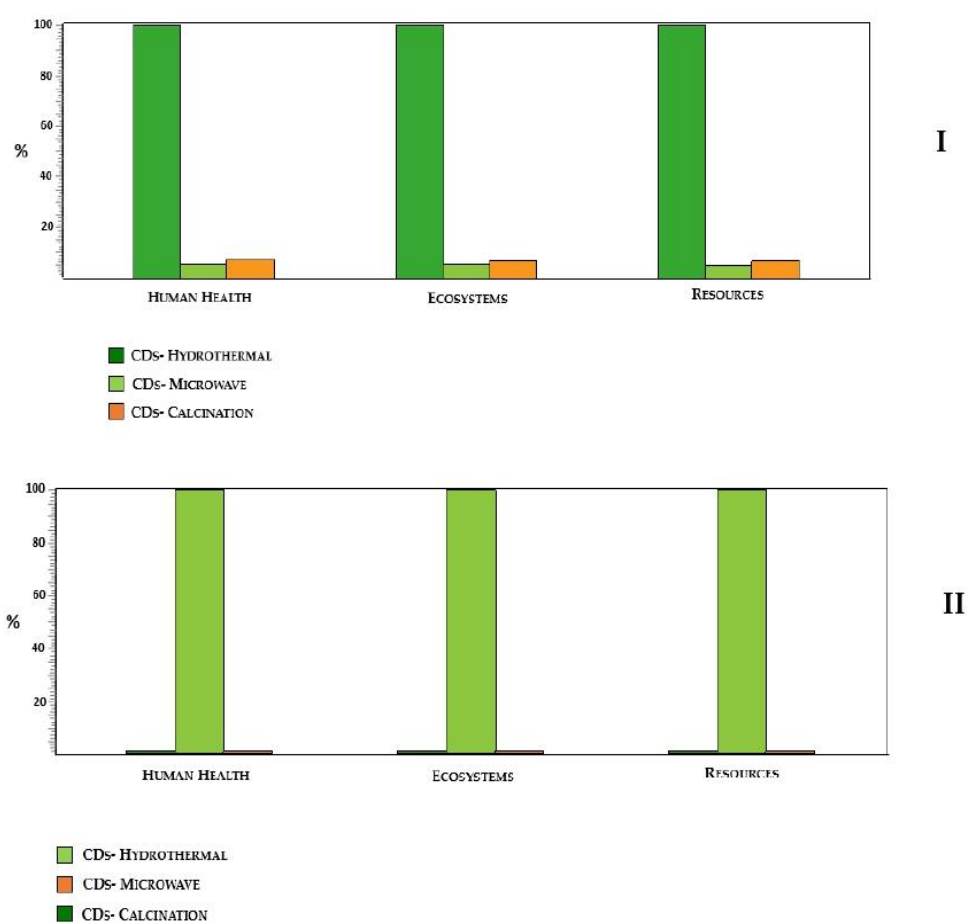


Figure 4. Comparative damage assessment for all three syntheses using (I) reaction yield and (II) quantum yield (QY) unit.

The sustainability of the three synthesis routes was also analyzed by rescaling the previous results with respect to the QY_{FL} of the three CDs (Table 3). This re-scaling was performed by considering the highest QY_{FL} (that of calcination-CDs) as the reference QY_{FL} (QY_{FL}^{REF}). The QY_{FL} -normalized functional unit for each CDs was calculated as QY_{FL}^{REF}/QY_{FL} (Table 3).

Table 3. Fluorescence quantum yields (QY_{FL}) and QY_{FL}-based functional units.

Carbon Dots	QY _{FL} (%)	QY _{FL} -Based Functional Unit (Using Microwave Synthesis as Reference)
Hydrothermal	3.7	7.9
Microwave	25.1	1.2
Calcination	29.3	1.0

This re-scaling did not affect the relative position of hydrothermal-CDs (Figure S9II and Figure 4II), as these CDs present both the lowest synthesis yield and QY_{FL} (Table 1). Interestingly, if we just compare just the environmental profiles of microwave-based and calcination-CDs (Figure S10), the latter nanoparticles are the ones that present the lowest environmental impacts, despite the former being the ones to present the highest QY_{FL}. This indicates that the better performance of calcination-CDs in terms of QY_{FL} is not enough to offset their higher requirement for initial precursors. In conclusion, our LCA analysis revealed that microwave-assisted synthesis should be the preferred bottom-up route when considering both a weight- and a function-based function, while hydrothermal synthesis is clearly the worst option.

4. Conclusions

We have performed a systematic study of the structural and optical properties of carbon dots (CDs) prepared by three representative bottom-up strategies (hydrothermal, microwave-assisted, and calcination synthesis), from the same precursors (citric acid and urea), with the objective of understanding the effect exerted by synthesis methods on the properties of novel CDs. Our results revealed that microwave and calcination are more suitable for high-yield synthesis, with the hydrothermal strategy providing quite low synthesis yields.

Further analysis revealed that the three synthesis strategies led to nanoparticles with similar sizes, identical excitation-dependent blue-to-green emission, and similar types of surface functional groups. Interestingly, despite these similarities, both microwave and calcination strategies are more efficient for nitrogen-doping than hydrothermal synthesis, which leads to significantly more efficient emission for the former than for the latter. Moreover, the different syntheses affect the origin of their photoluminescence, as hydrothermal-CDs present emission more susceptible to the external environment, which indicates that their emission has a higher contribution from surface states. On the contrary, both microwave and calcination strategies appear to generate CDs with emission more dependent on core states than on surface states.

Finally, a life cycle assessment (LCA) analysis was performed, considering both the synthesis and fluorescence quantum yields as functional units. These results demonstrated that microwave-assisted synthesis was the strategy with the least environmental impacts in both scenarios, and thus, should be the preferred synthesis route, followed closely by calcination.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2079-4991/10/7/1316/s1>, Table S1: Inputs required for the production of 1 Kg of CDs, Figure S1: XPS C 1s spectra for calcinated-, microwave-, and hydrothermal-based CDs, Figure S2: XPS O 1s spectra for calcinated-, microwave-, and hydrothermal-based CDs, XPS N 1s spectra for calcinated-, microwave-, and hydrothermal-based CDs, Figure S4: UV–Vis spectra of the three synthesized CDs, Figure S5: Normalized intensity of different CDs in the presence of different concentrations of methyl viologen, Figure S6: Relative environmental impacts and comparative damage assessment for hydrothermal synthesis, Figure S7: Relative environmental impacts and comparative damage assessment for microwave synthesis, Figure S8: Relative environmental impacts and comparative damage assessment for calcination synthesis, Figure S9: Relative environmental impacts for all synthesis using (I) weight unit and (II) QY unit, Figure S10: (I) Relative environmental impacts and (II) comparative damage assessment for microwave and calcination synthesis using QY unit.

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References

- Qu, D.; Sun, Z. The formation mechanism and fluorophores of carbon dots synthesized via a bottom-up route. *Mater. Chem. Front.* **2020**, *4*, 400–420. [[CrossRef](#)]
- Esteves da Silva, J.C.G. Analytical and bioanalytical applications of carbon dots. *Trends Anal. Chem.* **2011**, *30*, 1327–1336. [[CrossRef](#)]
- De Medeiros, T.V.; Manioudakis, J.; Noun, F.; Macairan, J.-R.; Victoria, F.; Naccache, R. Microwave-assisted synthesis of carbon dots and their applications. *J. Mater. Chem. C* **2019**, *7*, 7175–7195. [[CrossRef](#)]
- Dhenadhayalan, N.; Lin, K.-C.; Suresh, R.; Ramamurthy, P. Unravelling the Multiple Emissive States in Citric-Acid-Derived Carbon Dots. *J. Phys. Chem. C* **2016**, *120*, 1252–1261. [[CrossRef](#)]
- Carbonaro, C.M.; Corpino, R.; Salis, M.; Mocci, F.; Thakkar, S.V.; Olla, C.; Ricci, P.C. On the Emission Properties of Carbon Dots: Reviewing Data and Discussing Models. C—*J. Carbon Res.* **2019**, *5*, 60. [[CrossRef](#)]
- Jeong, S.; Pinals, R.L.; Dharmadhikari, B.; Song, H.; Kalluri, A.; Debnath, D.; Wu, Q.; Ham, M.H.; Patra, P.; Landry, M.P. Graphene Quantum Dot Oxidation Governs Noncovalent Biopolymer Adsorption. *Sci. Rep.* **2020**, *10*, 1–14. [[CrossRef](#)]
- Wang, Y.Z.; Yang, P.J.; Zheng, L.X.; Shi, X.W.; Zheng, H.J. Carbon nanomaterials with sp² or/and sp hybridization in energy conversion and storage applications: A review. *Energy Storage Mater.* **2020**, *26*, 349–370. [[CrossRef](#)]
- Liang, W.X.; Bunker, C.E.; Sun, Y.P. Carbon Dots: Zero-Dimensional Carbon Allotrope with Unique Photoinduced Redox Characteristics. *ACS Omega* **2020**, *5*, 965–971. [[CrossRef](#)]
- Xiong, Y.; Schneider, J.; Ushakova, E.V.; Rogach, A.L. Influence of molecular fluorophores on the research field of chemically synthesized carbon dots. *Nano Today* **2018**, *23*, 124–139. [[CrossRef](#)]
- Wang, R.; Lu, K.-Q.; Tang, Z.-R.; Xu, Y.-J. Recent progress in carbon quantum dots: synthesis, properties and applications in photocatalysis. *J. Mater. Chem. A* **2017**, *5*, 3717–3734. [[CrossRef](#)]
- Wang, K.; Gao, Z.; Gao, G.; Wo, Y.; Wang, Y.; Shen, G.; Cui, D. Systematic safety evaluation on photoluminescent carbon dots. *Nanoscale Res. Lett.* **2013**, *8*, 122. [[CrossRef](#)] [[PubMed](#)]
- Lim, S.Y.; Shen, W.; Gao, Z. Carbon quantum dots and their applications. *Chem. Soc. Rev.* **2015**, *44*, 362–381. [[CrossRef](#)] [[PubMed](#)]
- Baker, S.N.; Baker, G.A. Luminescent carbon nanodots: Emergent nanolights. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 6726–6744. [[CrossRef](#)]
- Sun, Y.P.; Zhou, B.; Lin, Y.; Wang, W.; Fernando, K.A.; Pathak, P.; Meziani, M.J.; Harruff, B.A.; Wang, X.; Wang, H.; et al. Quantum-sized carbon dots for bright and colorful photoluminescence. *J. Am. Chem. Soc.* **2006**, *128*, 7756–7757. [[CrossRef](#)] [[PubMed](#)]
- Kozák, O.; Datta, K.K.R.; Greplová, M.; Ranc, V.; Kašlík, J.; Zbořil, R. Surfactant-Derived Amphiphilic Carbon Dots with Tunable Photoluminescence. *J. Phys. Chem. C* **2013**, *117*, 24991–24996. [[CrossRef](#)]
- Sendão, R.M.S.; Crista, D.M.A.; Afonso, A.C.P.; Martínez de Yuso, M.d.V.; Algarra, M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Insight into the hybrid luminescence showed by carbon dots and molecular fluorophores in solution. *Phys. Chem. Chem. Phys.* **2019**, *21*, 20919–20926. [[CrossRef](#)]
- Crista, D.M.A.; Mello, G.P.C.; Shevchuk, O.; Sendao, R.M.S.; Simoes, E.F.C.; Leitao, J.M.M.; da Silva, L.P.; Esteves da Silva, J.C.G. 3-Hydroxyphenylboronic Acid-Based Carbon Dot Sensors for Fructose Sensing. *J. Fluoresc.* **2019**, *29*, 265–270. [[CrossRef](#)] [[PubMed](#)]

18. Shen, C.L.; Su, L.X.; Zang, J.H.; Li, X.J.; Lou, Q.; Shan, C.X. Carbon Nanodots as Dual-Mode Nanosensors for Selective Detection of Hydrogen Peroxide. *Nanoscale Res. Lett.* **2017**, *12*, 447. [\[CrossRef\]](#)
19. Simões, E.F.C.; Leitão, J.M.M.; Esteves da Silva, J.C.G. Carbon dots prepared from citric acid and urea as fluorescent probes for hypochlorite and peroxyxynitrite. *Microchim. Acta (Online)* **2016**, *183*, 1769–1777. [\[CrossRef\]](#)
20. Simões, E.F.C.; da Silva, L.P.; da Silva, J.C.G.E.; Leitão, J.M.M. Hypochlorite fluorescence sensing by phenylboronic acid-alizarin adduct based carbon dots. *Talanta* **2020**, *208*, 120447. [\[CrossRef\]](#)
21. Kang, Y.F.; Li, Y.H.; Fang, Y.W.; Xu, Y.; Wei, X.M.; Yin, X.B. Carbon Quantum Dots for Zebrafish Fluorescence Imaging. *Sci. Rep.* **2015**, *5*, 11835. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Hettiarachchi, S.D.; Graham, R.M.; Mintz, K.J.; Zhou, Y.; Vanni, S.; Peng, Z.; Leblanc, R.M. Triple conjugated carbon dots as a nano-drug delivery model for glioblastoma brain tumors. *Nanoscale* **2019**, *11*, 6192–6205. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Yan, L.; Yang, Y.; Ma, C.-Q.; Liu, X.; Wang, H.; Xu, B. Synthesis of carbon quantum dots by chemical vapor deposition approach for use in polymer solar cell as the electrode buffer layer. *Carbon* **2016**, *109*, 598–607. [\[CrossRef\]](#)
24. He, H.; Zheng, X.; Liu, S.; Zheng, M.; Xie, Z.; Wang, Y.; Yu, M.; Shuai, X. Diketopyrrolopyrrole-based carbon dots for photodynamic therapy. *Nanoscale* **2018**, *10*, 10991–10998. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Sendão, R.; Yuso, M.d.V.M.d.; Algarra, M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Comparative life cycle assessment of bottom-up synthesis routes for carbon dots derived from citric acid and urea. *J. Clean. Prod.* **2020**, *254*, 120080. [\[CrossRef\]](#)
26. Choi, Y.; Choi, Y.; Kwon, O.-H.; Kim, B.-S. Carbon Dots: Bottom-Up Syntheses, Properties, and Light-Harvesting Applications. *Chem. -Asian J.* **2018**, *13*, 586–598. [\[CrossRef\]](#)
27. Li, H.; Liu, R.; Kong, W.; Liu, J.; Liu, Y.; Zhou, L.; Zhang, X.; Lee, S.-T.; Kang, Z. Carbon quantum dots with photo-generated proton property as efficient visible light controlled acid catalyst. *Nanoscale* **2014**, *6*, 867–873. [\[CrossRef\]](#)
28. Chen, Y.; Wang, C.; Xu, Y.; Ran, G.; Song, Q. Red emissive carbon dots obtained from direct calcination of 1,2,4-triaminobenzene for dual-mode pH sensing in living cells. *New J. Chem.* **2020**, *44*, 7210–7217. [\[CrossRef\]](#)
29. Cailotto, S.; Mazzaro, R.; Enrichi, F.; Vomiero, A.; Selva, M.; Cattaruzza, E.; Cristofori, D.; Amadio, E.; Perosa, A. Design of Carbon Dots for Metal-free Photoredox Catalysis. *ACS Appl. Mater. Interfaces* **2018**, *10*, 40560–40567. [\[CrossRef\]](#)
30. Sri, S.; Kumar, R.; Panda, A.K.; Solanki, P.R. Highly Biocompatible, Fluorescence, and Zwitterionic Carbon Dots as a Novel Approach for Bioimaging Applications in Cancerous Cells. *ACS Appl. Mater. Interfaces* **2018**, *10*, 37835–37845. [\[CrossRef\]](#)
31. Christe, S.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Evaluation of the Environmental Impact and Efficiency of N-Doping Strategies in the Synthesis of Carbon Dots. *Materials* **2020**, *13*, 504. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Sharma, V.; Tiwari, P.; Mobin, S.M. Sustainable carbon-dots: Recent advances in green carbon dots for sensing and bioimaging. *J. Mater. Chem. B* **2017**, *5*, 8904–8924. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Hsu, P.-C.; Shih, Z.-Y.; Lee, C.-H.; Chang, H.-T. Synthesis and analytical applications of photoluminescent carbon nanodots. *Green Chem.* **2012**, *14*, 917–920. [\[CrossRef\]](#)
34. Kalita, H.; Mohapatra, J.; Pradhan, L.; Mitra, A.; Bahadur, D.; Aslam, M. Efficient synthesis of rice based graphene quantum dots and their fluorescent properties. *RSC Adv.* **2016**, *6*, 23518–23524. [\[CrossRef\]](#)
35. Crista, D.M.A.; El Mragui, A.; Algarra, M.; Esteves da Silva, J.C.G.; Luque, R.; Pinto da Silva, L. Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots. *Nanomaterials* **2020**, *10*, 1209. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Carbonaro, C.M.; Chiriu, D.; Stagi, L.; Casula, M.F.; Thakkar, S.V.; Malfatti, L.; Suzuki, K.; Ricci, P.C.; Corpino, R. Carbon Dots in Water and Mesoporous Matrix: Chasing the Origin of their Photoluminescence. *J. Phys. Chem. C* **2018**, *122*, 25638–25650. [\[CrossRef\]](#)
37. Zhou, J.; Zhou, H.; Tang, J.; Deng, S.; Yan, F.; Li, W.; Qu, M. Carbon dots doped with heteroatoms for fluorescent bioimaging: A review. *Microchim. Acta* **2017**, *184*, 343–368. [\[CrossRef\]](#)
38. Liao, J.; Cheng, Z.; Zhou, L. Nitrogen-Doping Enhanced Fluorescent Carbon Dots: Green Synthesis and Their Applications for Bioimaging and Label-Free Detection of Au³⁺ Ions. *ACS Sustain. Chem. Eng.* **2016**, *4*, 3053–3061. [\[CrossRef\]](#)

39. Martindale, B.C.M.; Hutton, G.A.M.; Caputo, C.A.; Reisner, E. Solar Hydrogen Production Using Carbon Quantum Dots and a Molecular Nickel Catalyst. *J. Am. Chem. Soc.* **2015**, *137*, 6018–6025. [[CrossRef](#)]
40. Xu, M.; He, G.; Li, Z.; He, F.; Gao, F.; Su, Y.; Zhang, L.; Yang, Z.; Zhang, Y. A green heterogeneous synthesis of N-doped carbon dots and their photoluminescence applications in solid and aqueous states. *Nanoscale* **2014**, *6*, 10307–10315. [[CrossRef](#)]
41. Kasprzyk, W.; Świergosz, T.; Bednarz, S.; Walas, K.; Bashmakova, N.V.; Bogdał, D. Luminescence phenomena of carbon dots derived from citric acid and urea—A molecular insight. *Nanoscale* **2018**, *10*, 13889–13894. [[CrossRef](#)]
42. Rao, L.; Tang, Y.; Lu, H.; Yu, S.; Ding, X.; Xu, K.; Li, Z.; Zhang, J.Z. Highly Photoluminescent and Stable N-Doped Carbon Dots as Nanoprobes for Hg²⁺ Detection. *Nanomaterials* **2018**, *8*, 900. [[CrossRef](#)] [[PubMed](#)]
43. Simões, E.F.C.; Esteves da Silva, J.C.G.; Leitão, J.M.M. Peroxynitrite and nitric oxide fluorescence sensing by ethylenediamine doped carbon dots. *Sens. Actuators B* **2015**, *220*, 1043–1049. [[CrossRef](#)]
44. Ramos, A.; Afonso Teixeira, C.; Rouboa, A. Environmental Analysis of Waste-to-Energy-A Portuguese Case Study. *Energies* **2018**, *11*, 548. [[CrossRef](#)]
45. Ramos, A.; Teixeira, C.A.; Rouboa, A. Assessment study of an advanced gasification strategy at low temperature for syngas generation. *Int. J. Hydrog. Energy* **2018**, *43*, 10155–10166. [[CrossRef](#)]
46. Fernandes, S.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Life Cycle Assessment of the Sustainability of Enhancing the Photodegradation Activity of TiO₂ with Metal-Doping. *Materials* **2020**, *13*, 1487. [[CrossRef](#)] [[PubMed](#)]
47. Eastman, J.W. Quantitative Spectrofluorimetry-The Fluorescence Quantum Yield of Quinine Sulfate. *Photochem. Photobiol.* **1967**, *6*, 55–72. [[CrossRef](#)]
48. Melhuish, W.H. Quantum Efficiencies of Fluorescence of Organic Substances: Effect of Solvent and Concentration of The Fluorescent Solute. *J. Phys. Chem.* **1961**, *65*, 229–235. [[CrossRef](#)]
49. Jing, S.; Zhao, Y.; Sun, R.-C.; Zhong, L.; Peng, X. Facile and High-Yield Synthesis of Carbon Quantum Dots from Biomass-Derived Carbons at Mild Condition. *ACS Sustain. Chem. Eng.* **2019**, *7*, 7833–7843. [[CrossRef](#)]
50. Greczynski, G.; Hultman, L. C 1s Peak of Adventitious Carbon Aligns to the Vacuum Level: Dire Consequences for Material's Bonding Assignment by Photoelectron Spectroscopy. *Chemphyschem* **2017**, *18*, 1507–1512. [[CrossRef](#)]
51. Marsden, A.; Brommer, P.; Mudd, J.; Dyson, A.; Cook, R.; Asensio, M.; Ávila, J.; Levy, A.; Sloan, J.; Quigley, D.; et al. Effect of oxygen and nitrogen functionalization on the physical and electronic structure of graphene. *Nano Res.* **2015**, *8*, 2620–2635. [[CrossRef](#)]
52. Moulder, J.F.; Stickle, W.F.; Sobol, P.E.; Bomben, K.D. *Handbook of X-ray Photoelectron Spectroscopy*; Chastain, J., Ed.; Perkin-Elmer Corp.: Eden Prairie, MN, USA, 1992.
53. Sarkar, S.; Sudolská, M.; Dubecký, M.; Reckmeier, C.J.; Rogach, A.L.; Zbořil, R.; Otyepka, M. Graphitic Nitrogen Doping in Carbon Dots Causes Red-Shifted Absorption. *J. Phys. Chem. C* **2016**, *120*, 1303–1308. [[CrossRef](#)]
54. Sharma, A.; Gadly, T.; Gupta, A.; Ballal, A.; Ghosh, S.K.; Kumbhakar, M. Origin of Excitation Dependent Fluorescence in Carbon Nanodots. *J. Phys. Chem. Lett.* **2016**, *7*, 3695–3702. [[CrossRef](#)] [[PubMed](#)]
55. Van Dam, B.; Nie, H.; Ju, B.; Marino, E.; Paulusse, J.M.J.; Schall, P.; Li, M.; Dohnalová, K. Excitation-Dependent Photoluminescence from Single-Carbon Dots. *Small* **2017**, *13*, 1702098. [[CrossRef](#)]
56. Huang, Q.; Li, Q.; Chen, Y.; Tong, L.; Lin, X.; Zhu, J. High quantum yield nitrogen-doped carbon dots: Green synthesis and application as “off-on” fluorescent sensors for the determination of Fe³⁺ and adenosine triphosphate in biological samples. *Sens. Actuators B* **2018**, *276*, 82–88. [[CrossRef](#)]
57. Zhou, X.; Zhao, G.; Tan, X.; Qian, X.; Zhang, T.; Gui, J.; Yang, L.; Xie, X. Nitrogen-doped carbon dots with high quantum yield for colorimetric and fluorometric detection of ferric ions and in a fluorescent ink. *Mikrochim Acta* **2019**, *186*, 67. [[CrossRef](#)]
58. Vallan, L.; Urriolabeitia, E.P.; Ruipérez, F.; Matxain, J.M.; Canton-Vitoria, R.; Tagmatarchis, N.; Benito, A.M.; Maser, W.K. Supramolecular-Enhanced Charge Transfer within Entangled Polyamide Chains as the Origin of the Universal Blue Fluorescence of Polymer Carbon Dots. *J. Am. Chem. Soc.* **2018**, *140*, 12862–12869. [[CrossRef](#)]
59. Sarswat, P.K.; Free, M.L. Light emitting diodes based on carbon dots derived from food, beverage, and combustion wastes. *Phys. Chem. Chem. Phys.* **2015**, *17*, 27642–27652. [[CrossRef](#)]

60. Ren, J.; Weber, F.; Weigert, F.; Wang, Y.; Choudhury, S.; Xiao, J.; Lauermann, I.; Resch-Genger, U.; Bande, A.; Petit, T. Influence of surface chemistry on optical, chemical and electronic properties of blue luminescent carbon dots. *Nanoscale* **2019**, *11*, 2056–2064. [[CrossRef](#)]
61. Das, A.; Roy, D.; De, C.K.; Mandal, P.K. “Where does the fluorescing moiety reside in a carbon dot?”—Investigations based on fluorescence anisotropy decay and resonance energy transfer dynamics. *Phys. Chem. Chem. Phys.* **2018**, *20*, 2251–2259. [[CrossRef](#)]



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3.2. Supporting Information

Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots

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Table S1. Inputs required for the production of 1 Kg of CDs.

Carbon dots	Carbon source (Kg)	Nitrogen source (Kg)	Deionized Water (Kg)	Electricity (KWh)
Hydrothermal	50	16	330	17
Microwave	2.4	0.8	20	12
Calcination	3	1	-	17

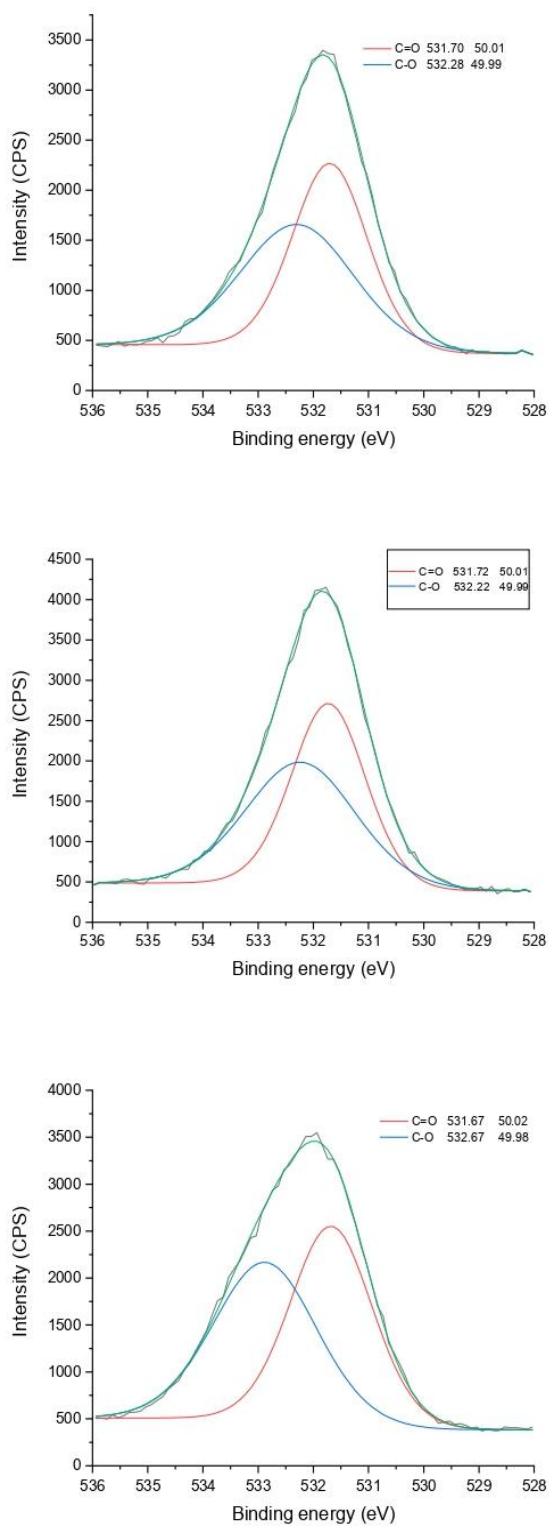


Figure S2. XPS O 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) CDs.

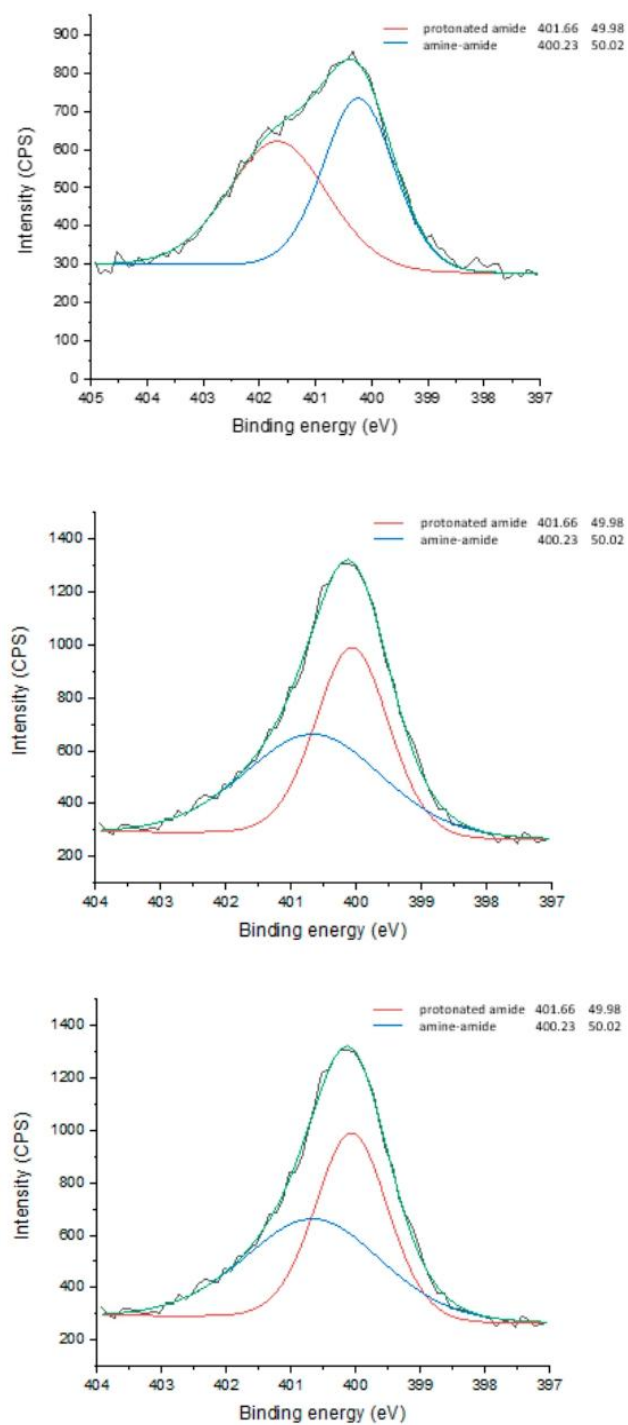


Figure S3. XPS N 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) CDs.

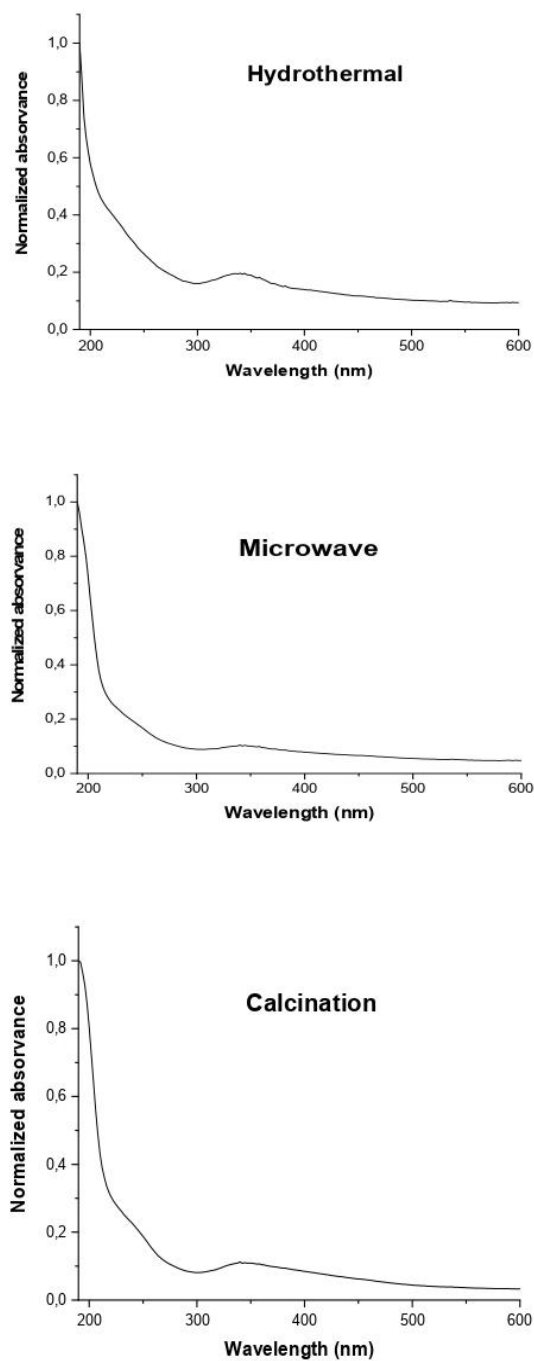


Figure S4. UV-Vis spectra of the three synthesized CDs.

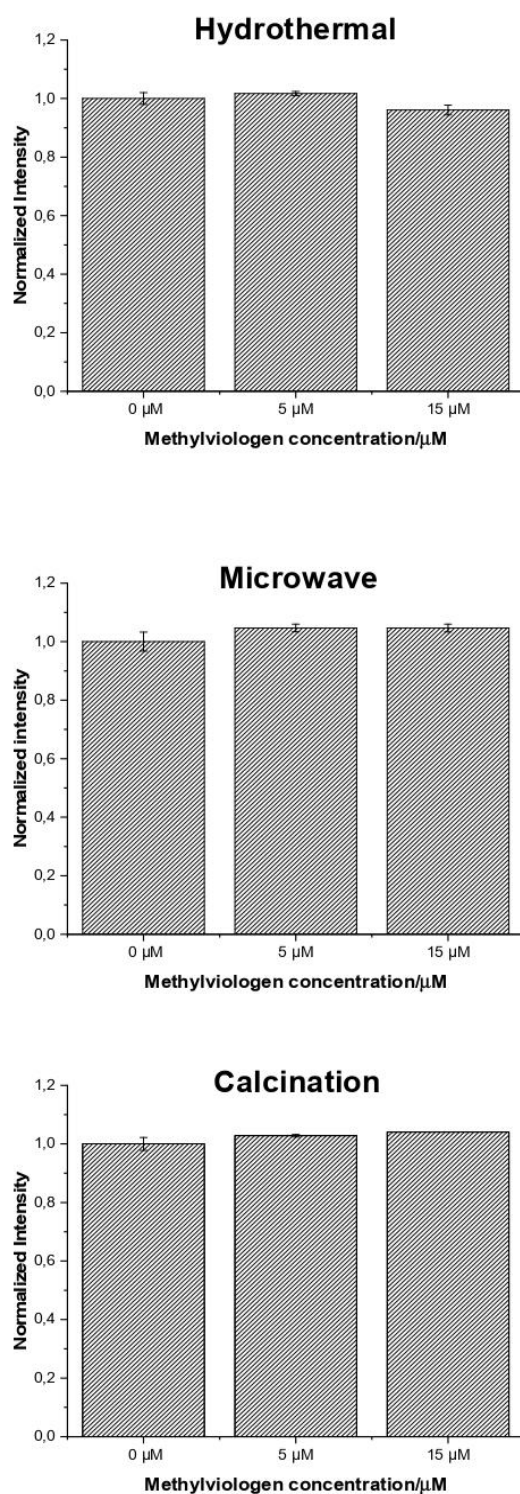


Figure S5. Normalized intensity of different CDs in the present of different concentrations of methyl viologen.

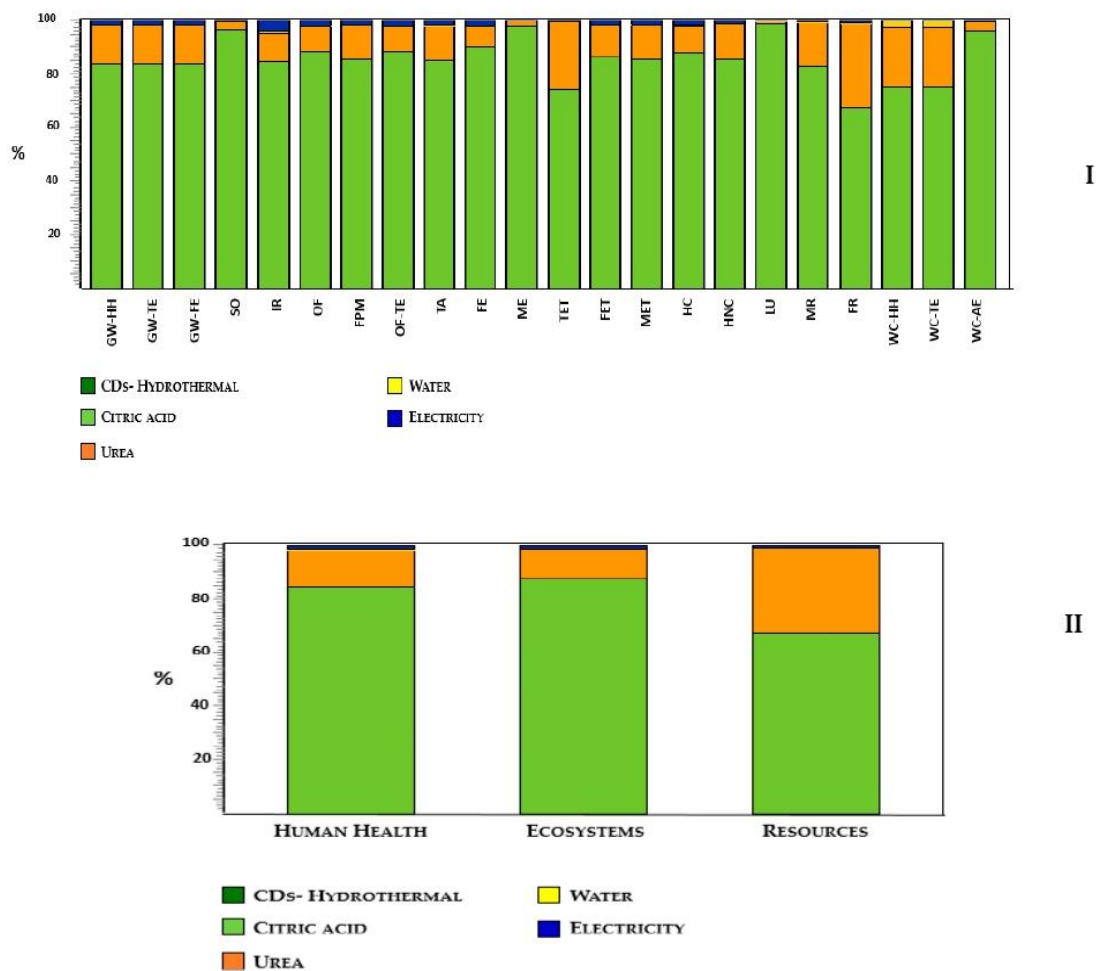


Figure S6. (I) Relative environmental impacts and **(II)** comparative damage assessment for hydrothermal synthesis.

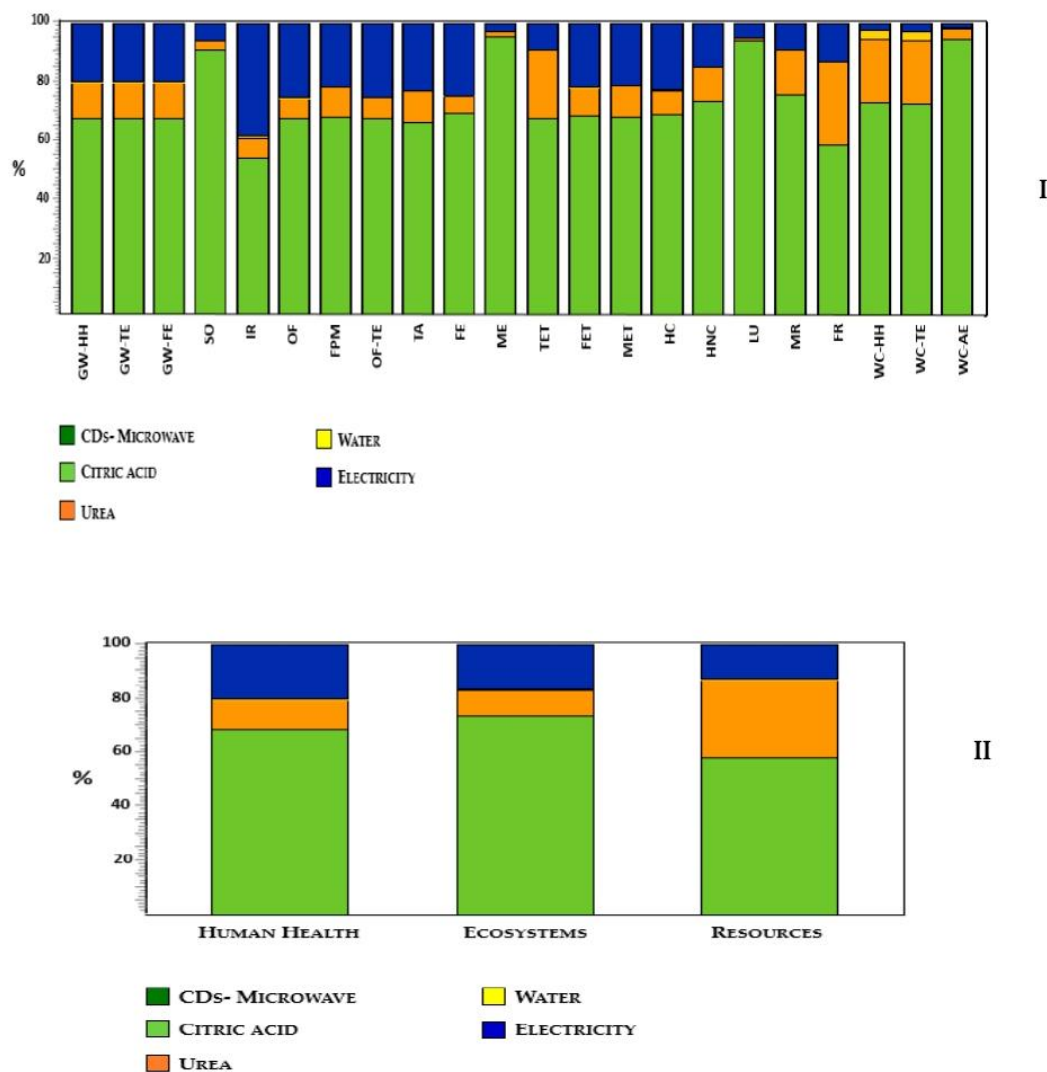


Figure S7. (I) Relative environmental impacts and (II) comparative damage assessment for microwave synthesis.

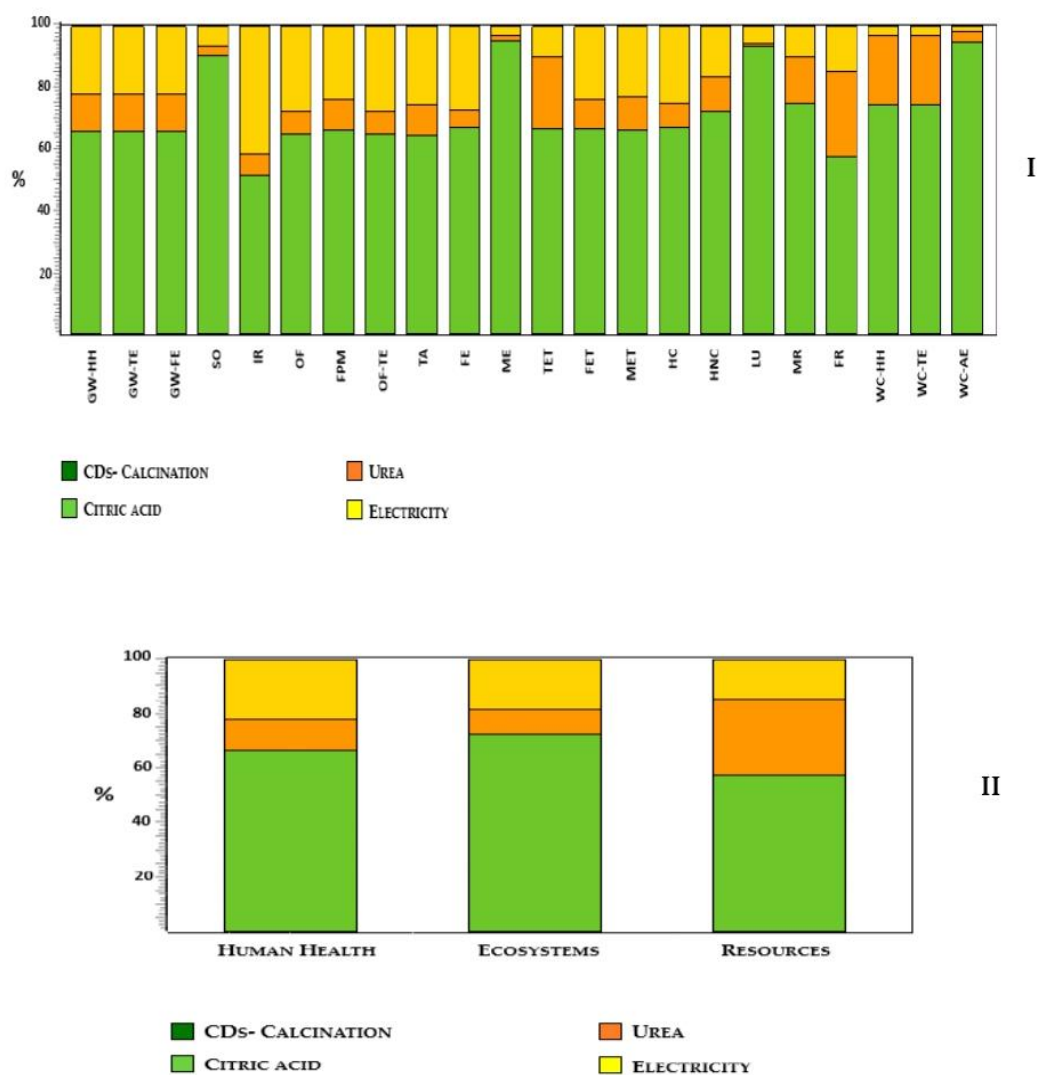


Figure S8. (I) Relative environmental impacts and (II) comparative damage assessment for calcination synthesis.

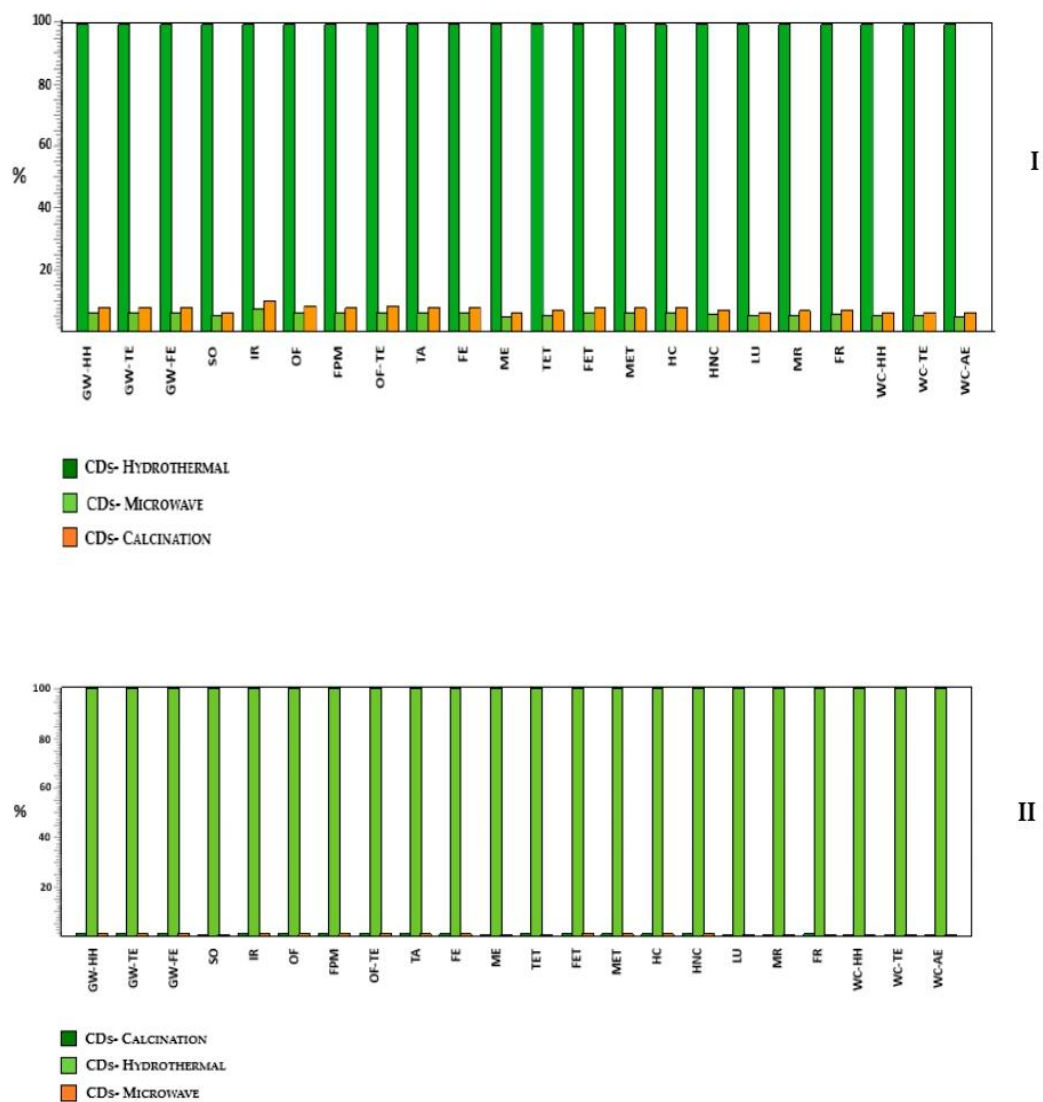


Figure S9. Relative environmental impacts for all synthesis using (I) weight unit and (II) QY unit.

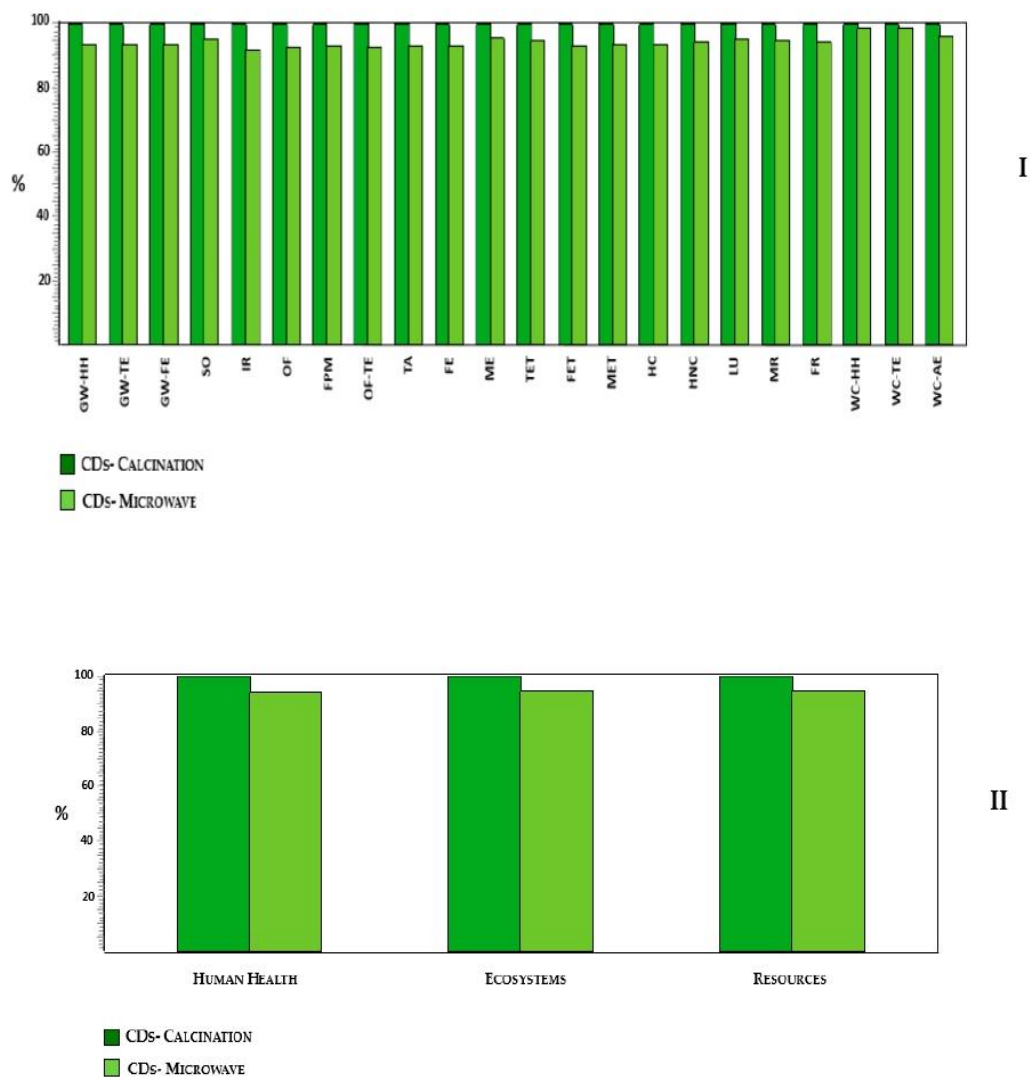


Figure S10. (I) Relative environmental impacts and (II) comparative damage assessment for Microwave- and Calcination synthesis using QY unit.

3.3. Conclusions

We conducted a comprehensive investigation into the structural and optical characteristics of CDs synthesized through three representative bottom-up methodologies: hydrothermal, microwave-assisted, and calcination synthesis. All CDs were derived from the same precursor materials, citric acid, and urea, with the aim of elucidating the influence of synthesis approaches on the properties of the resulting CDs. Our findings indicate that microwave and calcination techniques exhibit superior efficacy in yielding CDs with higher production rates, whereas the hydrothermal method yields comparatively lower synthesis yields.

Furthermore, our analysis revealed that despite similar sizes, excitation-dependent blue-to-green emission profiles, and surface functional group compositions across all synthesis routes, microwave and calcination methods demonstrate enhanced efficiency in nitrogen-doping compared to hydrothermal synthesis. Consequently, CDs produced via microwave and calcination routes exhibit significantly higher emission efficiency than those synthesized through hydrothermal means. Additionally, variations in synthesis methodologies influence the origin of photoluminescence, with hydrothermal-derived CDs showing emissions more susceptible to environmental factors, suggesting a greater contribution from surface states. Conversely, CDs synthesized via microwave and calcination routes exhibit emissions that are more reliant on core states than surface states.

Lastly, a LCA analysis was conducted, considering both synthesis and fluorescence quantum yields as functional units. Results indicate that microwave-assisted synthesis has the least environmental impact in both scenarios, making it the preferred synthesis route, followed closely by calcination.

4. Influence of Nitrogen Doping into Carbon Dots Properties

4.1. Investigation of the role of pH and the stoichiometry of the N-dopant in the luminescence, composition and synthesis yield of carbon dots

Article 3

Investigation of the role of pH and the stoichiometry of the N-dopant in the luminescence, composition and synthesis yield of carbon dots

Diana M.A.Crista, Manuel Algarra, Maria Valle Martínez de Yuso, Joaquim C.G. Esteves da Silva and Luís Pinto da Silva, Journal of Materials Chemistry B, **2023**, 11, 1131.

4.1.1. Contextualization

CDs represent carbon-based nanoparticles with highly appealing luminescent properties, and their straightforward and adaptable synthesis allows for the production of a myriad of CDs with diverse properties and applications. High QYFL are typically essential for various CD applications. A common strategy to enhance CD fluorescence properties involves heteroatom-doping, particularly nitrogen (N-doping), using precursors containing desired heteroatoms. Here, we detail the synthesis and systematic exploration of a range of N-doped CDs, derived from the dry heating of solid mixtures of glucose and urea at varying molar ratios, with two primary objectives: first, to investigate the influence of stoichiometry on the optical properties and composition of CDs, and second, to explore the formation of potential alkaline-responsive nanoparticles and the feasibility of this approach for achieving CDs with improved synthesis yields.

We conducted thorough characterization of the optical properties of this diverse array of glucose and urea-based CDs utilizing UV-Vis and fluorescence spectroscopies. Additionally, we examined the CDs using high-resolution transmission electron microscopy (HR-TEM) and X-ray photoelectron spectroscopy (XPS) and assessed the nanoparticles' thermal stability. Our findings revealed the generation of two types of CDs through this fabrication process: one readily soluble in water and the other soluble only at basic pH. The latter exhibited higher synthesis yields but lower QYFL and thermal stability compared to the former. Moreover, no correlation between the stoichiometry of the N-dopant and the QYFL of the resulting CDs was observed. This study presents novel insights that could inform the rational development of CDs with enhanced QYFL and synthesis yields in future endeavors.

4.1.2. Contributions to this work

All synthesis of CDs, as well as their optical characterization, was performed by me (Diana Crista) under the supervision of my supervisors. XPS and HR-TEM analysis was performed by the team of Dr. Manuel Algarra at the University of Malaga.



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Investigation of the role of pH and the stoichiometry of the N-dopant in the luminescence, composition and synthesis yield of carbon dots†

Diana Crista,^a Manuel Algarra,^b Maria Valle Martínez de Yuso,^c Joaquim C. G. Esteves da Silva^b and Luís Pinto da Silva^b

Carbon dots (CDs) are carbon-based nanoparticles with very attractive luminescence features, which simplicity and flexibility of their fabrication can lead to an endless number of CDs with distinct properties and applications. High fluorescence quantum yields (QY_{FL}) are generally a necessary feature for various applications of CDs. One commonly employed strategy to improve the fluorescence properties of CDs is heteroatom-doping using precursors containing desired heteroatoms (with focus on N-doping). In this work, we report the synthesis and systematic investigation of an array of N-doped CDs, obtained from the dry heating of solid mixtures of glucose and urea in different molar ratios with two main objectives: to study the role of stoichiometry in the optical properties and composition of CDs and to investigate the formation of possible alkaline-responsive nanoparticles and the potential of this procedure for obtaining CDs with higher synthesis yields. We have characterized the optical properties of this diverse array of glucose and urea-based CDs using both UV-Vis and fluorescence spectroscopies. In addition, we have also examined the CDs by using high-resolution transmission electron microscopy (HR-TEM) and X-Ray photoelectron (XPS) spectroscopy, as well as by assessing the thermal stability of the nanoparticles. We have found that this fabrication process generates two types of CDs, one readily soluble in water and other only soluble at basic pH. The latter was characterized by higher synthesis yields, and lower QY_{FL} and thermal stability, when compared with those of the former. Furthermore, the stoichiometry of the N-dopant does not appear to be correlated with the QY_{FL} of the obtained CDs. This study provides novel information that should be useful for the future rational development of CDs with higher QY_{FL} and synthesis yields.

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Introduction

Carbon dots (CDs) are fluorescent carbon-based nanoparticles with a quasi-spherical shape, typically sized below 10 nm, and with a core that can be either amorphous or nanocrystalline.^{1–3}

More specifically, CDs are generally thought to possess a core composed of sp² carbon connected by amorphous sp³ carbon atoms in between. Furthermore, their surface can present different functional groups (amines, carboxylic acids, alcohols, among others), which depend on the precursors and synthesis strategies employed.³

In recent years, there has been an explosion in the number of studies focusing on CDs, due to their quite interesting properties such as high (photo)stability, low toxicity, biocompatibility, tunable fluorescence, and remarkable water solubility.^{4–10} Another advantage of CDs is the simplicity and flexibility of their fabrication. Namely, they can be obtained *via* different bottom-up procedures (such as hydrothermal,^{11,12} microwave,^{13,14} and dry-heating^{11,15}), which are attractive green synthesis strategies as they are inexpensive, easy to implement, scalable and can be one-pot procedures.^{16,17} Furthermore, CDs can be prepared from a plethora of precursors, which range from smaller chemicals such as carbohydrates and acids (such as glucose and citric acid^{2,18,19})

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to more heterogeneous sources (such as different types of waste materials).^{15,20–22}

Given this, CDs can be exciting alternatives to molecular and inorganic fluorophores in different fields with commercial/industrial applications, such as in bioimaging, sensing, phototherapies and in the fabrication of light-emissive devices, among others.^{8,23–25}

For these applications, high fluorescence quantum yields (QY_{FL}) are generally a necessary feature of CDs. However, low-to-moderate QY_{FL} are still routinely obtained for the synthesized CDs.^{26,27} One commonly employed strategy to improve the fluorescence properties of the synthesized CDs is heteroatom-doping, in which the addition of appropriate precursors containing desired heteroatoms (such as N, S, B and P) leads to the incorporation of these elements into the structure of the nanoparticles.^{28–30} Among these strategies, N-doping is the most used strategy as nitrogen and carbon have comparable sizes, and nitrogen offers five valence electrons for bonding with carbon atoms.³¹ More importantly, it has been reported that N-doped CDs can indeed present quite high QY_{FL},^{28,32–36} while allowing for red-shifting the absorption and emission to longer wavelengths.^{37–39}

However, there are still unclarified issues that prevent the systematic and rational use of the N-doping strategy in fabricating suitable CDs for commercial/industrial applications. In fact, while N-doping has been reported to enhance the QY_{FL} of CDs, the mechanism by which this strategy can tune the properties of the CDs is still not identified/characterized.^{16,28} One of the difficulties in elucidating this matter is that there is no defined or unified synthetic route to produce representative CDs. In contrast, most of the new published studies basically produce new and unique CDs by taking advantage of the near-infinite combinations of synthesis procedures/parameters and the myriad of possible precursors.^{4–22} This production of countless CDs with different properties, combined with a large number of possible variables in their synthesis, makes it very difficult to understand and characterize the intrinsic properties of CDs in a systematic and rational manner.

More importantly, while the high QY_{FL} obtained by N-doped CDs has been attributed to N-doping, it is not clear if this enhancement is really due to this heteroatom-doping strategy. While different authors reported N-doped CDs with high QY_{FL}, most of them neither synthesized nor reported the corresponding non-N-doped CDs.^{8,26} Without this comparison, it is not really possible to attribute in a definitive manner the observed high QY_{FL} to just the inclusion of nitrogen heteroatoms in the structure of CDs. In fact, in recent studies performed by our group, we observed that there appears to exist little correlation between the N atomic content (in %) and the obtained QY_{FL}.^{11,40} For example, CDs with less than half N atomic content (6.9% versus ~15%) exhibited slightly higher QY_{FL} (3.5% versus 3.1–3.3%) than CDs with a higher N atomic content.⁴⁰ Other N-doped CDs also exhibited quite different QY_{FL} (3.1–29.3%) with a similar N atomic content (13.1–15.0%), with the CDs with a lower N atomic content exhibiting higher QY_{FL}.^{11,40} These results show a discrepancy between the expected benefits of N-doping and the obtained results.

The other relevant topic that casts some doubts in the matter is that, in contrast to what was initially expected, it has been found that bottom-up synthesis routes produce large amounts of highly fluorescent molecular impurities (besides CDs, in lesser amounts).^{13,41–44} These impurities can only be removed from solution by appropriate dialysis or chromatographic purification procedures and, more importantly, can mask or even alter the properties of the CDs.^{13,42} Despite this, different reports regarding N-doped CDs with high QY_{FL} still do not perform appropriate purification,^{38,45,46} and so, it is possible that the reported QY_{FL} values do not result from the CDs but from molecular fluorophores present in solution. If so, this would mean that the obtained QY_{FL} values are not due to N-doping but due to the formation of N-containing fluorophores.

Another less discussed, but still relevant topic, which can prevent a more large-scale use of CDs in commercial/industrial applications is that typical fabrication routes of these nanomaterials are plagued by quite low synthesis yields (below 10%).^{40,47,48} Recent studies have shown that one possible strategy to solve this problem is to perform an alkaline treatment of the solid carbonized fraction that results from bottom-up routes, leading to the formation of water-soluble particles in higher yields (~20–40%).⁴⁹ This means that while researchers typically focus on the readily water-soluble fraction that results from typical synthesis, these processes do generate alkaline-responsive particles that are relatively unexplored.

Herein, in this work, we report the synthesis and systematic investigation of an array of N-doped CDs, obtained from the dry-heating of solid mixtures of glucose (as carbon source) and urea (as a N-dopant) in different molar ratios. CDs prepared from just either glucose or urea were also prepared as controls. Our objective here is two-fold. By varying the molar ratio between the carbon source and the N-dopant, we first intend to gain insight into the role of stoichiometry in the optical properties and composition of CDs. This approach will then provide much needed information regarding the true impact of N-doping in the modulation of QY_{FL} for N-doped CDs. The second objective is to investigate the formation of possible alkaline-responsive nanoparticles and the potential of this procedure for obtaining CDs in higher yields. Such information is essential for devising more sustainable synthesis routes, by minimizing the production of waste. To this end, we have characterized the optical properties of this diverse array of glucose and urea-based CDs using both UV-Vis and steady-state fluorescence spectroscopies. In addition, we have also investigated the CDs by using high-resolution transmission electron microscopy (HR-TEM) and X-Ray photoelectron (XPS) spectroscopy, as well as by assessing the thermal stability of the nanoparticles.

Results and discussion

Investigation of the optical properties of water-soluble N-doped CDs

We started this study by investigating the role of stoichiometry in the optical properties of N-doped CDs, by preparing an array of nanoparticles *via* one-pot dry-heating (200 °C for 2 hours) of

powder mixtures of glucose (carbon source) and urea (N-dopant) at different reactant ratios. Glucose was chosen as a carbon source, as it is a commonly available carbohydrate with low carbonization temperature and low toxicity.^{50–52} Furthermore, life cycle assessment (LCA) studies have shown glucose to be a relatively sustainable choice for the carbon source, even better than other common options (as citric acid).^{17,53} Urea is also a very common choice for a N-dopant, which is here chosen as a good representative for N-doping agents.⁵⁴ Here, dry-heating was chosen as the preferred synthetic route as it has been found before to be capable of producing CDs, while being competitive with other bottom-up routes (as hydrothermal and microwave).¹¹ Also, by not requiring the presence of solvents, the dry-heating approach allows reducing the number of potential variables affecting the optical properties of the N-doped CDs. Thereby, this study allows focusing on the intrinsic features of the CDs and related N-doping, without the need to consider solvent effects that might affect the synthesis of the CDs.

An array of N-doped CDs were prepared by varying the molar ratio between glucose and urea. Namely, the amount of glucose was fixed at 3 mmol, while the amount of urea was varied between 0.9 and 6 mmol. This resulted in the synthesis of 6 N-doped CDs with the following glucose:urea ratios: 1:0.3 (CDs_{1:0.3}), 1:0.6 (CDs_{1:0.6}), 1:0.8 (CDs_{1:0.8}), 1:1 (CDs_{1:1}), 1:1.3 (CDs_{1:1.3}) and 1:2 (CDs_{1:2}). To ensure that the observed optical properties can indeed be (or not) attributed to N-doping, we have also produced as controls two other CDs. One of them was prepared from just glucose (CDs_{glucose}), and the other from just urea (CDs_{urea}).

It should be noted that in this section we focus on the study of standard CDs (that is, readily soluble in water), as obtained using typical bottom-up strategies. More specifically, after thermal treatment of the reaction mixture, the particles readily soluble in water were obtained by re-suspension of the carbonized reaction product in deionized water, followed by discarding insoluble particles by centrifugation, and followed by purification and dialysis. This class of CDs will be termed as water-soluble CDs from now on.

The synthesis yields (mass by mass, in %) were obtained by measuring the weight of the dried powder relative to each amount of used precursors, after steps of solubilization, centrifugation and dialysis. These results are presented in Table 1 and were in general quite low. More specifically, the obtained amounts of CDs_{1:0.3} and CDs_{urea} were too low to be quantified,

while the highest synthesis yield was for CDs_{1:2} (9.6%), which was still quite low. Interestingly, there does appear to be a trend in which increasing amounts of urea increases the synthesis yields of CDs. However, it should also be noted that CDs_{glucose} exhibit by themselves higher synthesis yields than most of the glucose:urea-based ones. Finally, it should also be mentioned once again that while the synthesis yields are low, they are in line with typical synthesis yields obtained for CDs (generally below ~10%).^{40,47,48}

The absorption spectra of all water-soluble CDs can be found in Fig. 1. The absorption spectrum of CDs_{glucose} (Fig. 1) is composed of a main band at ~280 nm (attributed to π - π^* transitions), and two shoulders at ~230 (attributed also to π - π^*

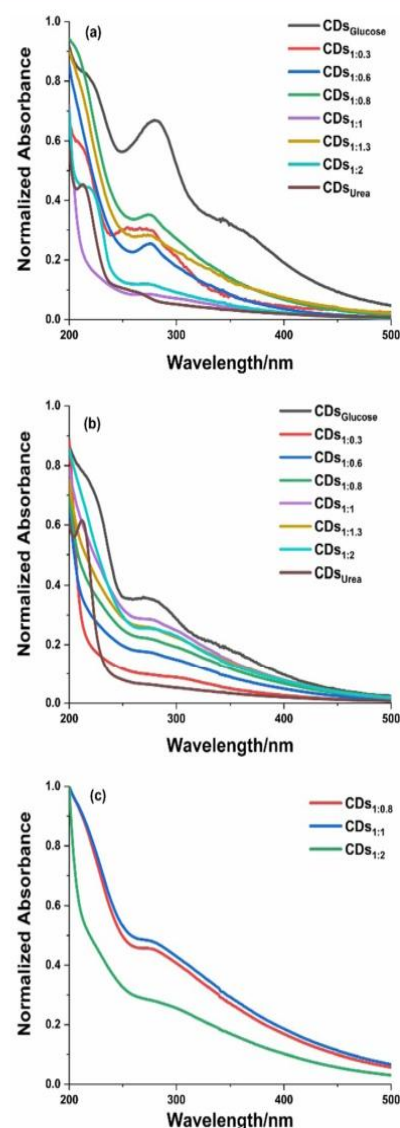


Fig. 1 Absorption spectra of (a) water-soluble CDs, (b) water-hydroxide-soluble CDs and (c) hydroxide-soluble CDs with a concentration of 100 $\mu\text{g mL}^{-1}$ in water.

Table 1 Synthesis yields (%) of all water- and water/hydroxide-soluble CDs. N.Q. means "not quantified"

	Water-soluble CDs	Water/hydroxide-soluble CDs
CDs _{glucose}	3.1	18.0
CDs _{1:0.3}	N.Q.	1.9
CDs _{1:0.6}	1.5	10.5
CDs _{1:0.8}	1.4	19.5
CDs _{1:1}	2.6	25.0
CDs _{1:1.3}	5.8	13.5
CDs _{1:2}	9.6	15.4
CDs _{urea}	N.Q.	0.5

transitions) and ~ 350 nm (attributed to $n-\pi^*$ transitions).⁵⁵ For CDs_{urea}, we can observe a main band at ~ 220 nm. Interestingly, we see that CDs obtained with glucose:urea ratios between 1:0.3 and 1:1.3 show a band at ~ 280 nm, like the one found in glucose-based CDs. This indicates that this band should result from electronic transitions associated with chromophoric moieties related to the use of glucose as the carbon source. In turn, the CDs_{1:2} ratio shows a main band at ~ 220 nm, similar to the one found in urea-based CDs. So, this band should be associated with chromophoric moieties only formed with the addition of higher amounts of urea as the N-dopant. This analysis also revealed that by increasing the amount of urea in the synthesis, we have a decrease of the band at ~ 280 nm and an increase in the band at ~ 220 nm. Thus, we can observe an effect of N-doping in the absorption spectra of these CDs. Finally, the absence of bands attributed to $n-\pi^*$ transitions in the glucose:urea-based CDs indicated that there is no presence of molecular fluorophores in these solutions.⁵⁵

The 2D excitation–emission matrixes (EEMs) of CDs_{glucose}, CDs_{1:0.3}, CDs_{1:2} and CDs_{urea} are shown in Fig. 2, while the EEMs of all studied particles are found in the ESI† (Fig. S1). It should be noted that basically all glucose:urea-based CDs present overlapped emission spectra with an emission maximum at ~ 430 nm. The exception is CDs_{1:0.3} (Fig. 2(b)), whose emission maximum is blue-shifted to 400 nm. Thus, we can conclude that tuning the molar ratio of urea, as the N-dopant, has limited influence on the emission band of glucose:urea-based CDs. Nevertheless, our results do show that the inclusion of urea to the reaction mixture results in a blue-shift in the emission spectra, when compared with non-N-doped CDs, as the emission wavelength maximum of CDs_{glucose} is of ~ 470 nm. Finally, CDs_{urea} presents a blue-shifted emission (~ 440 nm) when compared with CDs_{glucose}. However, this emission is still red-shifted when compared with glucose:urea-based CDs, which are the most blue-shifted ones. So, these results do not support the common notion that N-doping tends to red-shift the emission of N-doped CDs.^{37–39}

The measured QY_{FL} of glucose:urea-based CDs, CDs_{glucose} and CDs_{urea} are presented in Table 2. The results are interesting as they, in one way, support the notion that N-doping enhances the QY_{FL} of N-doped CDs. More specifically, the QY_{FL} of CDs_{glucose} (1.3%) is significantly lower than that of the N-doped CDs (6.3–20.0%). The obtained QY_{FL} of the N-doped CDs, while not being in “high” range, are still in a “moderate” range, supporting the benefits for N-doping. Also, it is worthy of note that just using the N-dopant as the precursor is not enough to obtain relevant QY_{FL}, as this parameter for CDs_{urea} is the lowest measured value (just 0.8%).

Having said that, it is also clear that there is no obvious relationship between the glucose:urea stoichiometry and the measured QY_{FL} of the N-doped CDs (Table 2). The higher QY_{FL} (20.0%) was found for the CDs with the lowest urea ratio (CDs_{1:0.3}), followed by the CDs with an immediately higher urea ratio (CDs_{1:0.6}, with QY_{FL} of 15.3%). Following the trend of the glucose:urea ratio, we just observe some variations in the measured QY_{FL}, without any clear relationship. Thus, our results indicate that while the introduction of N-heteroatoms

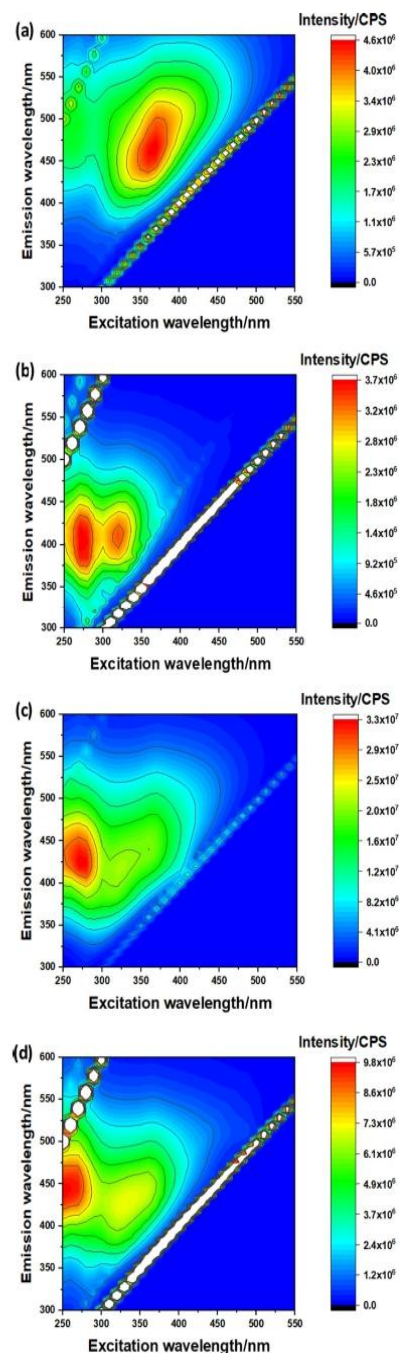


Fig. 2 EEMs of water-soluble CDs: (a) CDs_{glucose}, (b) CDs_{1:0.3}, (c) CDs_{1:2} and (d) CDs_{urea} with a concentration of 100 $\mu\text{g mL}^{-1}$ in water.

in CDs does have some benefits in the QY_{FL}, when compared with the corresponding non-N-doped CDs, there are more factors than just the stoichiometry of the N-dopant that govern the emission of N-doped CDs.

This is in line with previous works carried out by our group, in which a clear relationship between the QY_{FL} and the N

Table 2 Quantum yields (%) of all water- and water/hydroxide-soluble CDs

	Water-soluble CDs	Water/hydroxide-soluble CDs	Hydroxide-soluble CDs
CDs _{glucose}	1.3	2.0	
CDs _{1:0.3}	20.0	7.9	
CDs _{1:0.6}	15.3	1.8	
CDs _{1:0.8}	7.0	1.8	1.2
CDs _{1:1}	10.2	7.0	1.1
CDs _{1:1.3}	6.3	5.2	
CDs _{1:2}	11.8	9.5	5.4
CDs _{urea}	0.8	6.4	

atomic content was not found.^{11,40} Recently, Chahal *et al.* also tried to evaluate the impact of N-doping in the emission of CDs, and also found this heteroatom-doping strategy to be insufficient to enhance the QY_{FL} of CDs.¹⁶

In summary, our results and the previous reported data indicate that N-doping, by itself, is not a viable strategy for the rational and systematic enhancement of the QY_{FL} of CDs. That is, these results show that the introduction of the N-heteroatom into the structure of CDs does not guarantee the enhancement of QY_{FL}, indicating that there are other factors at play that can be more important than N-doping. Therefore, future studies on highly emissive N-doped CDs should be careful before attributing this high emission to just the introduction of N-dopant/N-enriched carbon sources.

The other matter of debate in the study of the origin of the photoluminescence of CDs is the relative relevance of core and surface states in the emission of these nanoparticles.^{56–58} To obtain further information in the subject, we have also investigated the effect exerted by the external environment to the fluorescence of CDs_{1:0.8}, CDs_{1:1} and CDs_{1:2}. More specifically, we have measured their fluorescence emission spectra in different protic and aprotic solvents: water (H₂O), ethanol (EtOH), methanol (MeOH) and dimethylformamide (DMF).¹¹ The obtained results are shown in Fig. 3.

The variation of the solvents affected all CDs in a similar way, with the major change being the relevant fluorescence enhancement of all CDs in the following order: H₂O < EtOH < MeOH < DMF. It should be noted, nevertheless, that the impacts of EtOH and MeOH are generally identical for all CDs. This means that the fluorescent moieties are not shielded from the external environment, and so, their emission should originate more from surface states than from core states. Interestingly, while all CDs present a small red-shifted emission in DMF (~10 nm), while increasing the glucose:urea ratio eliminates this shift in both EtOH and MeOH. Furthermore, the enhancement in emission between H₂O and DMF appears to increase slightly with the increasing glucose:urea ratio. Given these results, it is possible to conclude that increasing the amount of urea modifies/affects the surface group composition of the CDs, thereby affecting the emission of surface states. In fact, given the order of the fluorescent enhancement, which increases by decreasing the H-bond donor strength of the solvent and even more by going to an aprotic solvent, it is reasonable to assume that the surface of the CDs is composed of H-bond donor groups. This could lead to an enhanced emission in a medium that promotes H-bonding

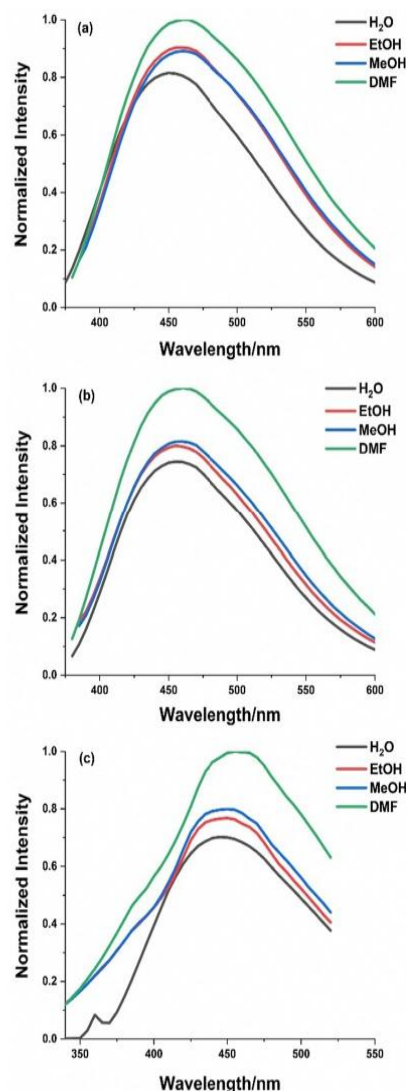


Fig. 3 Normalized emission spectra of the water-soluble CDs with different solvents: (a) CDs_{1:0.8}, (b) CDs_{1:1} and (c) CDs_{1:2}.

between the CDs (as donor) and the solvent (as acceptor). Following this reasoning, it is not unexpected that increasing the glucose:urea ratio leads to higher enhancement in DMF as high amounts of urea should lead to a higher presence of H-bond donor groups (as amines).

A similar analysis was also performed in aqueous solutions at different pH values for CDs_{1:0.8}, CDs_{1:1} and CDs_{1:2} (Fig. S2, ESI†). For all tested CDs, the emission intensity is higher at pH 3 and decreases with an increasing pH. However, this decrease is significantly higher for CDs_{1:0.8} and CDs_{1:1} and is less relevant at higher glucose:urea ratios. The other finding of note is that for all tested CDs it appears to exist an inflection point at pH 5–7 regarding the emission intensity. At this pH range, the emission intensity starts to increase once again with increasing pH. However, the emission intensity at pH 10 is still

lower than at pH 3. Nevertheless, this difference is lower for CDs_{1:2}. Finally, while for CDs_{1:0.8} and CDs_{1:1} there is no shift in the peak position, there is a red shift with increasing pH for CDs_{1:2}. In conclusion, these results show once again that the emissive centres of water-soluble CDs are not shielded from the external environment, and so, their emission should originate more from surface states. In this particular case, the pH-dependency of the tested CDs could be attributed to the reversible protonation and deprotonation of ionizable functional groups on their surface.⁵⁹ Given this, these results indicate that increasing the amount of urea should decrease the amount of ionizable surface functional groups on the produced CDs.

Characterization of water-soluble N-doped CDs

The HR-TEM image (Fig. 4) of water-soluble CDs_{1:1} presented a typical aggregation behaviour, which can be explained by the typical surface composition of these materials (as indicated below). Namely, by the presence of typical carboxyl, amine and carbonyl groups, with the subsequent formation of electrostatic interactions among them, as well as hydrogen-bonding. This has been seen for both N-CDs and other types of nanomaterials.^{60,61} The size observed of the N-CDs in the HR-TEM image confirms the size range at around 100 nm. CDs_{1:1} was evaluated by HR-TEM as a representative nanoparticle in the group of water-soluble CDs.

Subsequently, XPS analysis (Table 3) was subsequently performed to investigate the surface composition of CDs_{1:1}, once again as a representative nanoparticle for water-soluble CDs. This analysis revealed the following surface composition (in atom concentrations, in %): 71% of C, 18.1% of O, and 10.6% of N. The C 1s core spectra (Fig. 5(a)), after the deconvolution process, showed contributions at 284.5 eV (50.61%) assigned to the adventitious carbon (C-C/C-H); 285.2 eV (21.99%) assigned to C-O functionals groups, mainly alcohols; at 286.2 eV (18.66%) for C-N, mainly amine groups and 288.1 eV (8.7%) for the -C=O/-COOH groups.

The O 1s and N 1s core level spectra of CDs_{1:1} were also obtained and analysed. The O 1s spectra (Fig. 5(b)), which

Table 3 Atom composition (%) obtained by XPS of water-soluble and hydroxide-soluble CDs at three different glucose : urea ratios

	Ratio glucose : urea	C (%)	O (%)	N (%)
Water-soluble CDs	1:0.8	71.4	20.1	8.5
	1:1	71.2	18.1	10.6
	1:2	68.7	19.7	11.6
Hydroxide-soluble CDs	1:0.8	80.5	12.3	7.2
	1:1	81.3	12.4	6.3
	1:2	79.1	11.9	9.0

confirm previous discussion, showed two signals at 531.1 and 530.5 eV. These were assigned to C-O/C-OH and C=O groups, respectively. For the N 1s spectra (Fig. 5(c)), it also showed two peaks, with a main contribution at 398.7 eV, which was assigned to C-N from amine groups. The minor contribution was found at 399.8 eV and was attributed to protonated N groups.

Very similar results (Table 3 and Fig. S3 and S4) were found for other water-soluble CDs with different ratios (1:0.8 and 1:2). This indicates that qualitatively, the stoichiometry of the N-dopant should not affect the chemical surface composition of the resulting CDs. However, quantitatively, we could see a trend of decreasing C and increasing N contents with the increasing urea ratio (Table 3). However, there does not appear to be a clear relationship between the O content and the stoichiometry of the N-dopant. In fact, there appears to be instead an interplay between the C, O and N contents of the different CDs, which does not appear to be correlated with the stoichiometry of the N-dopant.

Investigation of the optical properties of water/hydroxide-soluble CDs

Having analysed the optical properties and chemical composition of the water-soluble CDs, it is also important to note that after their synthesis (besides these nanoparticles) large amounts of carbonized material that was not readily water-soluble also resulted. This formation in high amounts of the solid insoluble material is one of the reasons for the low synthesis yields that plague bottom-up routes.^{40,47,48} Thus, if this material could be converted to CDs, it could help increasing the synthesis yields of these nanomaterials.

Interestingly, recently some authors have indeed reported that CDs can be obtained after treating this solid material with alkaline solutions.⁴⁹ Given this, we have reported another variation of the synthesis of CDs_{1:0.3}, CDs_{1:0.6}, CDs_{1:0.8}, CDs_{1:1}, 1:1.3 CDs_{1:1.3}, CDs_{1:2}, CDs_{glucose} and CDs_{urea}. The main difference relative to the previous report is that, after the synthesis procedures, the particles were re-suspended in NaOH 0.1 M aqueous solution (instead of deionized water). This group of CDs will be termed as water/hydroxide-soluble CDs from now on.

The use of the NaOH solution to re-suspend the CDs was shown to be capable of dissolving a higher amount of particles. More specifically, in Table 1, the synthesis yields of the water-hydroxide-soluble CDs are also presented. When compared

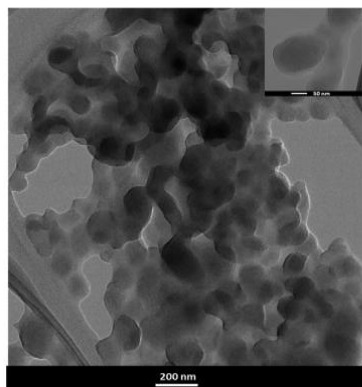


Fig. 4 TEM image of the obtained water-soluble CDs_{1:1}. The inset shows the 50 nm scale.

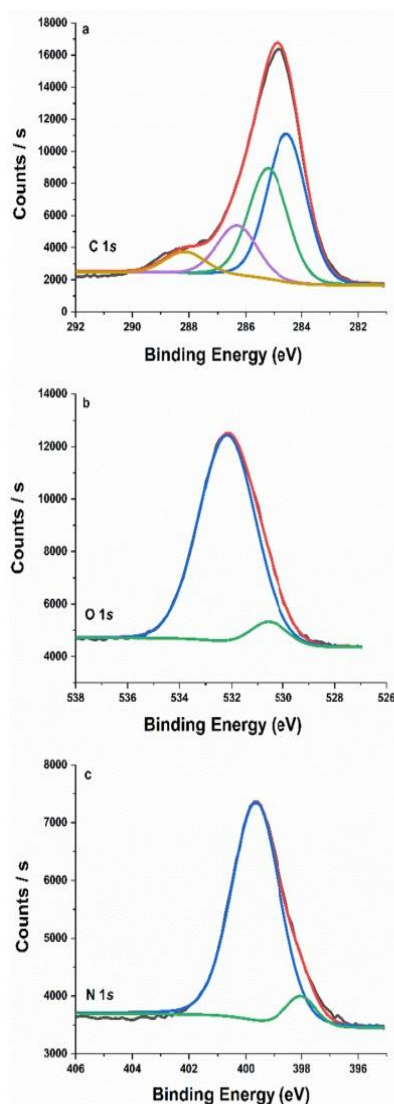


Fig. 5 XPS core level spectra of water-soluble CDs 1:1: (a) C 1s, (b) O 1s and (c) N 1s.

with those obtained for the water-soluble CDs (Table 1), these synthesis yields are quite satisfactory.

Namely, the synthesis yields of water/hydroxide-soluble CDs were ~2–14 times higher than those of their corresponding water-soluble versions. These results do support previous reports that state that the alkaline treatment of the carbonized product of bottom-up synthesis could be the solution to increase the yields of the obtained CDs.

Focusing on individual results, we can see that both CDs_{1:0.3} (1.9%) and CDs_{urea} (0.5%) still present negligible synthesis yields, despite the inclusion of an alkaline solution. It was also observed that CDs_{glucose} presented the third highest synthesis yield (18.0%). This is like what was observed for the group of water-soluble CDs, which means that by itself glucose is enough as the carbon source to produce CDs in a relatively

relevant amount (at least when compared with the corresponding N-doped CDs). It should also be noted that while for water-soluble CDs the increase in glucose : urea ratio increased the synthesis yield, there is now no such relationship for water/hydroxide-soluble CDs. For the latter group, there is an increase in the synthesis yield in the following order for the N-doped CDs: CDs_{1:0.3} < CDs_{1:0.6} < CDs_{1:1.3} < CDs_{1:2} < CDs_{1:0.8} < CDs_{1:1}. Thus, the stoichiometry of the reactants does not appear to govern the synthesis yields of water/hydroxide-soluble N-doped CDs.

The absorption spectra of all water/hydroxide-soluble CDs can be found in Fig. 1. The CDs were analysed at the same mass concentration. There are some similarities with water-soluble CDs. For instance, the spectrum of CDs_{glucose} is also dominated by three bands/shoulders at ~230, ~280 and ~350 nm. Nevertheless, the last two bands/shoulder are less well-resolved/intense than for its water-soluble version. The spectrum of CDs_{urea} also presents a band at ~220 nm. However, for the N-doped CDs, there are more noticeable differences. For instance, there is no discernible band/shoulder at either ~220 or ~230 nm. Furthermore, the band found at ~280 nm for nearly all N-doped CDs is less intense than that was found for their corresponding water-soluble CDs. Finally, now there is also no observable trend between the absorption spectra and the glucose : urea ratio for the water/hydroxide-soluble N-doped CDs.

According to the fluorescence EEMs of water/hydroxide CDs (Fig. S5, ESI[†]), all the nanoparticles present blue emission with maxima at ~450 nm. Once again, we can conclude that tuning the molar ratio of urea, as the N-dopant, has limited influence in the emission band of glucose : urea-based CDs. In Fig. 6, we can see the fluorescence EEMs of four water/hydroxide-soluble CDs (CDs_{glucose}, CDs_{1:0.3}, CDs_{1:2} and CDs_{urea}). Comparing these results with water-soluble CDs, where we observed two emissive centers with different excitation wavelengths (Fig. S1, ESI[†]), with water/hydroxide CDs, this only occurs when we have high amounts of urea (CDs_{1:2}) (Fig. 6(c)). Finally, CDs_{urea} presents only one emissive center, with a different excitation wavelength (~270 nm) different from CDs_{glucose} (excitation at ~350 nm).

The QY_{FL} values were also measured for all water/hydroxide-soluble CDs and are presented in Table 2. Interestingly, re-suspension in NaOH solution led to some increase in the QY_{FL} for both CDs_{glucose} and CDs_{urea}, especially for the later. However, it led to an opposite behaviour for the N-doped CDs, which all presented relevantly lower QY_{FL} than their corresponding water-soluble versions. More importantly, there is once again no clear relationship between the stoichiometry of the N-dopant and the obtained QY_{FL}. These results further indicate that N-doping, by itself, should not be responsible for the enhancement of the QY_{FL} of N-doped CDs.

At this point, it should be emphasized that this optical analysis indicates that re-suspension of the solid reaction product in NaOH solution do allow for obtaining higher amounts of luminescent and solubilized particles, than just re-suspension in deionized water. However, one question arises from the analysis of both water-soluble and water/hydroxide-

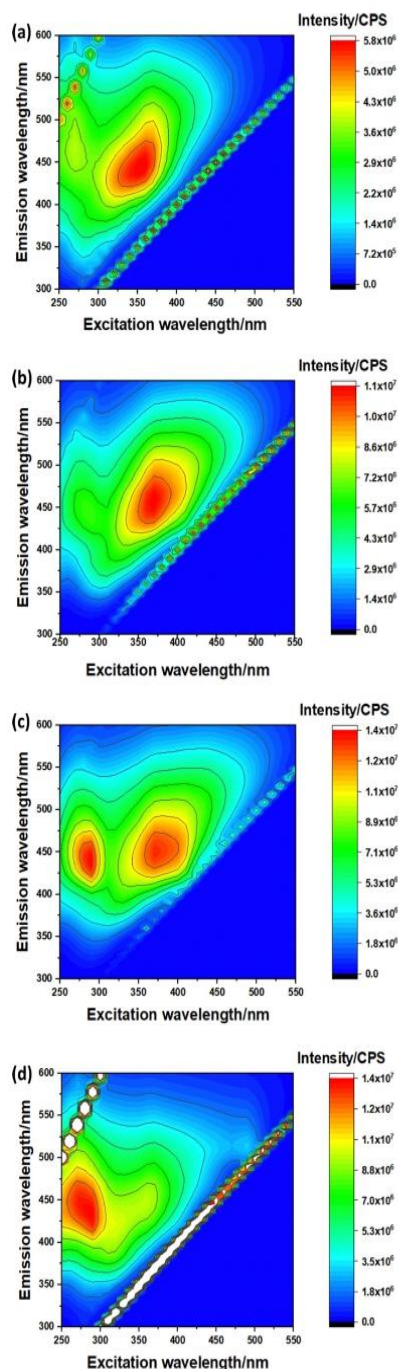


Fig. 6 EEMs of water/hydroxide-soluble CDs: (a) CDs_{glucose:urea}, (b) CDs_{1:0.3}, (c) CDs_{1:2} and (d) CDs_{urea} with a concentration of 100 $\mu\text{g mL}^{-1}$ in water.

soluble CDs. Are they the same CDs but simply with poor water solubility at acidic-neutral pH, thereby needing to be solubilized at higher pH values to be obtained in higher yields? Or are they two types of CDs, one readily soluble in water and another only soluble at basic pH? The fact that water-soluble and water/hydroxide soluble CDs present different properties (such as

absorption spectra and QY_{FL}) do indicate that the correct answer is the second one (two types of CDs). However, we did find that water-soluble CDs present pH-dependency (Fig. S2, ESI[†]), and so, these different properties could just be the result of water/hydroxide-soluble CDs being analysed at basic pH. Thus, further investigation is needed.

Investigation of the optical properties of hydroxide-soluble CDs

To address this question, we have then synthesized yet another version of CDs_{1:0.8}, CDs_{1:1} and CDs_{1:2}, which will be termed as hydroxide-soluble CDs from now on. To clarify if the water-soluble and water/hydroxide-soluble CDs are the same type of particles or not, we needed to further analyse them and guarantee that we study the purified versions of them. However, as we prepared the water/hydroxide-soluble CDs by re-suspending the solid product directly into NaOH aqueous solution, it is possible that the resulting solution possesses both CDs readily soluble in water and CDs only soluble at a basic pH. So, to remove readily water-soluble CDs, we synthesized the hydroxide-soluble CDs once again by one-pot dry-heating of glucose and urea. After this, we re-suspended first the solid product in deionized water, followed by centrifugation. The supernatant, containing readily soluble CDs, was discarded. The precipitate was then re-suspended again, but now in NaOH 0.1 M solution (followed by centrifugation to remove not-soluble particles). The resulting solution should then only contain CDs soluble at a basic pH. The hydroxide-soluble CDs were then further analysed after dialysis.

The absorption spectra of the three hydroxide-soluble CDs can be found in Fig. 1. The obtained spectra of hydroxide-soluble CDs are similar to those obtained for the corresponding water/hydroxide-soluble CDs, with a small band at ~ 280 nm. While no band is clearly defined at ~ 230 nm (associated with glucose-derived electronic transitions, as seen before), the absorption intensity in this region is higher for CDs_{1:0.8|1:1} than that for CDs_{1:2}. This does show the decreasing importance of glucose-derived transitions with increasing glucose:urea ratios.

The fluorescence EEMs of the hydroxide-soluble CDs (Fig. 7) show a common emission with maxima at ~ 450 nm between the analysed ratios (CDs_{1:0.8}, CDs_{1:1} and CDs_{1:2}). Comparing the results for the same ratios (CDs_{1:0.8}, CDs_{1:1} and CDs_{1:2}) with the water-soluble CDs, we can see that hydroxide-soluble CDs present a lower fluorescence intensity. On the other hand, in the fluorescence EEMs of hydroxide-soluble CDs, we can see two emissive centers at two different excitation wavelengths ~ 270 nm and ~ 370 nm (Fig. 7(b) and (c)). Additionally, hydroxide-soluble CDs showed an emission wavelength between 450 and 460 nm which means a red-shift around 30 nm when compared with respective water-soluble CDs (Fig. S1, ESI[†]).

To further investigate the optical properties of the hydroxide-soluble CDs, we have measured their fluorescence spectra in different protic and aprotic solvents (Fig. 8): H₂O, EtOH, MeOH and DMF. For comparison, the same fluorescence spectra from the water-soluble versions of these CDs are shown in Fig. 3. For both CDs_{1:0.8} and CDs_{1:1} the trends are the same in both water- and hydroxide-soluble versions, with the

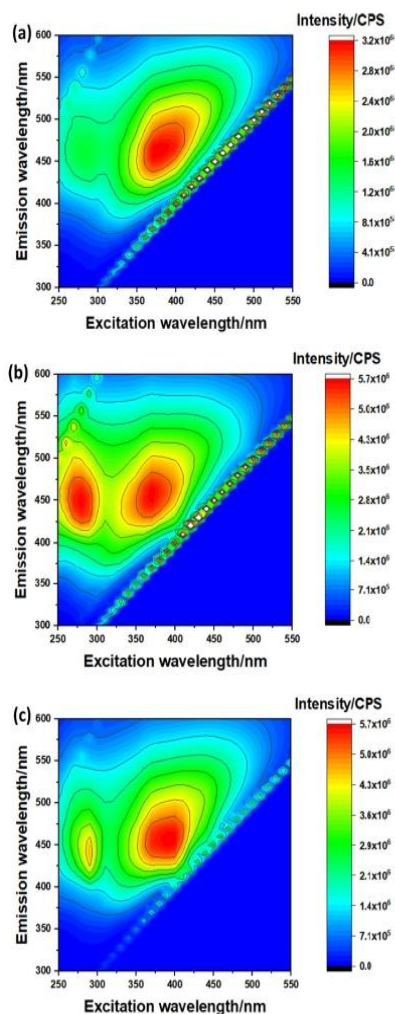


Fig. 7 EEMs of hydroxide-soluble CDs: (a) CD_{S1:0.8}, (b) CD_{S1:1} and (c) CD_{S1:2} with a concentration of 100 $\mu\text{g mL}^{-1}$ in water.

emission increasing with the general solvent order of $\text{H}_2\text{O} < \text{MeOH}/\text{EtOH} < \text{DMF}$. However, this fluorescence enhancement is significantly higher in the hydroxide-soluble versions. This is a relevant difference between versions that point to them being different CDs, and not just the same CDs at different pH values. This conclusion is further supported by comparing the results for the water- and hydroxide-soluble versions of CD_{S1:2}. While in the former there is fluorescence enhancement in the order of $\text{H}_2\text{O} \rightarrow \text{DMF}$, for the latter we have an opposite effect with the highest difference in the emission intensity for both solvents in the tested CDs. This is a relevant proof regarding water- and hydroxide-soluble CDs being two different types of CDs. In fact, and following our previous discussion, these results do indicate that while the surface of water-soluble CD_{S1:2} should be more composed of H-bond donors and that of hydroxide-soluble CD_{S1:2} should be more composed of H-bond acceptors.

Further analysis was also performed at different pH values for hydroxide-soluble CDs (Fig. S6, ESI[†]), with the corresponding

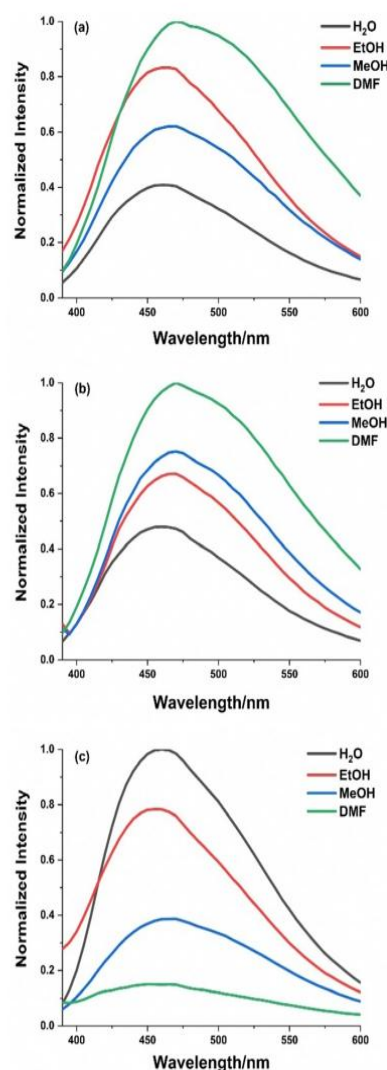


Fig. 8 Normalized emission spectra of the hydroxide-soluble CDs with different solvents: (a) CD_{S1:0.8}, (b) CD_{S1:1} and (c) CD_{S1:2}.

results from water-soluble CDs shown in Fig. S2 (ESI[†]). The results show again relevant differences for the studied versions of the CDs. Namely, for hydroxide-soluble CD_{S1:0.8} and CD_{S1:1}, while the highest emission intensity is also found at pH 3 (as in their water-soluble versions), and the difference for other pH values is not so relevant as in their water-soluble versions.

Thus, while we do see a relevant pH-dependency in both versions of CD_{S1:0.8} and CD_{S1:1}, there are appreciable differences that point to them being different types of CDs. For CD_{S1:2}, the differences between water- and hydroxide-soluble versions are even more notorious. More specifically, while for the latter the pH-dependency is quite negligible, for the former it does affect the emission intensity in a more relevant manner. Thus, it is possible that the latter version has less ionizable surface functional groups than the former.

The QY_{FL} values of hydroxide-soluble CDs (Table 2) were also determined. Interestingly, the obtained QY_{FL} values were relevantly lower than those of the corresponding water-soluble CDs, with rather weak fluorescence (in particular, $CDs_{1:0.8}$ and $CDs_{1:1}$).

In conclusion, the optical analysis here performed do support the hypothesis that the water- and hydroxide-soluble CDs are in fact different types of nanoparticles with distinct properties.

Characterization of hydroxide-soluble N-doped CDs

Hydroxide-soluble CDs were also evaluated by HR-TEM. More specifically, hydroxide-soluble $CDs_{1:1}$ was evaluated as a representative nanoparticle. TEM images were obtained and observed the similar aggregation process and explained as previously. The presence of nitrogenous groups enhances the possibility of forming electrostatic interactions, which can be justify in detail by the below XPS analysis. In the depicted TEM image (Fig. 9), it is possible to observe the 30 nm size.

XPS spectroscopy was once again employed to characterize the surface composition of CDs, in this case hydroxide-soluble $CDs_{1:1}$ (Table 3 and Fig. 10). Its surface was found to be composed (in atom concentrations, in %) by 81.3% of C, 12.4% of O and 6.3% of N (Table 3). Compared with its corresponding water-soluble version (Table 3), there is a relevant increase in the C content, which could be reflected in terms of the graphitic core. This higher C content leads to a lower content of both O and N, and not just of either one of these heteroatoms. This higher content of C, with the corresponding lower atom concentrations of N and O, is also found for hydroxide-soluble $CDs_{1:0.8}$ and $CDs_{1:2}$ (Table 3). These results are then helpful in determining that water-soluble and hydroxide-soluble nanoparticles are different types of CDs, as their surface composition is relevantly different. In fact, the XPS analysis (Table 3) indicates that hydroxide-soluble CDs are characterized by higher C content, and the lower incorporation of O/N heteroatoms in their structure.

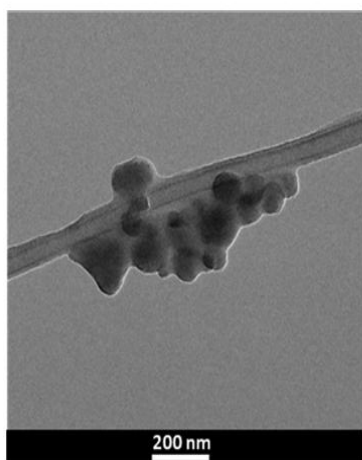


Fig. 9 TEM image of hydroxide-soluble CDs (ratio 1:1).

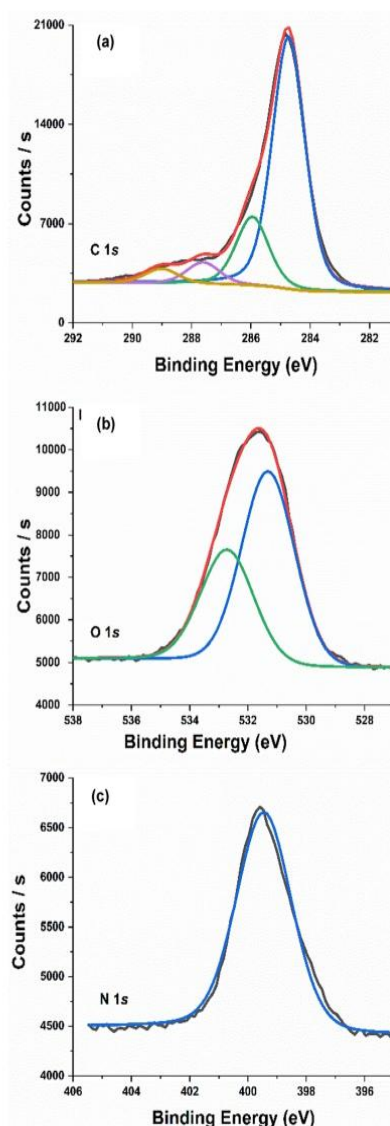


Fig. 10 XPS core level spectra of N-CDs hydroxide-soluble (po) (a) C 1s, (b) O 1s and (c) N 1s.

The C 1s core spectra of hydroxide-soluble $CDs_{1:1}$ (Fig. 10(a)) showed a qualitatively identical spectra than for its corresponding water-soluble version, with four main contributions: at 284.84 eV (76.9%), which was assigned to the C-C/C-H graphitic core; 286.2 eV (11.9%), assigned to C-O/C-OH groups; 287.4 eV (4.14%), assigned mainly to amine groups; and 288.69 eV, clearly assigned to C=O and -COHH groups.

In support of these results, the analysis of the O 1s core level spectra (Fig. 10(b)) showed two main contributions at 531.9 eV, assigned to C-O/C-OH, and at 532.7 eV, assigned to C=O from carboxyl groups. It is noticeable the increase of the C-O contribution in hydroxide-soluble $CDs_{1:1}$, when in comparison with its water-soluble version (Fig. 5(b)). The N 1s core level spectra of hydroxide-soluble $CDs_{1:1}$ (Fig. 10(c)) were similar to

those of water-soluble CDs_{1:1} (Fig. 5(c)) as, after the deconvolution process, two contributions were found: at 398.2 eV (assigned to C–N from amine groups) and at 399.5 eV (attributed to protonated N groups). Similar results were also found for hydroxide-soluble CDs_{1:0.8} (Fig. S7, ESI†) and CDs_{1:2} (Fig. S8, ESI†).

We have also evaluated the thermal stability of both water- and hydroxide-soluble CDs at glucose : urea ratios of 1 : 0.8, 1 : 1 and 1 : 2 (Fig. S9, ESI†). This evaluation was performed by calculating the percentage of the mass loss of the CDs under heating at 400 °C for 1 hour. With this approach, we intend to see if there are relevant differences regarding the loss of mass under heating for both water-soluble and hydroxide-soluble CDs, which could be qualitatively and comparatively explained by differences in the (organic) surface group content. In fact, we can see such differences between the two types. For one, the loss of mass for hydroxide-soluble CDs is always higher than that for their corresponding water-soluble ratios (Fig. S9, ESI†). Also, for water-soluble CDs, the loss of mass increases with the increasing urea ratio. For hydroxide-soluble CDs, the loss of mass occurs with the following trend: CDs_{1:0.8} > CDs_{1:2} > CDs_{1:1}. Thus, these data provide more evidence that the chemical composition of water- and hydroxide-soluble CDs is different between these groups, which means that they are two different types of CDs.

We have also investigated if the luminescence properties of water-soluble and hydroxide-soluble CDs change over the time. To this end, we measured the fluorescence spectra of the water-soluble and hydroxide-soluble versions of CDs_{1:1} over time (right after synthesis and after 60 days), which can be found in Fig. S10 (ESI†). There was no noticeable change in the shape of the spectra. However, we observed a fluorescence enhancement over time of 17% and 28% for water-soluble and hydroxide-soluble versions (respectively). This indicates that these CDs do experience some modification over time (potentially by oxidation). Furthermore, while the differences over time are qualitatively identical, the higher enhancement for hydroxide-soluble CDs indicates that these are indeed different nanoparticles.

Given this, it becomes clear that solubilization of the carbonized reaction product in alkaline solution provides access to a different type of CDs (associated with higher synthesis yields and lower QY_{FL}) than the typically studied water-soluble kind.

Experimental section

CD synthesis

CDs were prepared from the same precursors (glucose (Glu) and urea) which were purchased from Sigma Aldrich (St. Louis, MO USA) *via* one-pot and solvent-free dry heating method. All CDs were fabricated according to the same procedure. Namely, 3 mmol of Glu and varying amounts of urea (0.9–6 mmol) were placed in a Teflon-lined reactor, which was encased in a stainless-steel shell and was heated at 200 °C for 2 hours in an oven. The synthesized CDs were suspended in 10 mL of deionized water or 10 mL of 0.1 M of an NaOH aqueous solution.

Purification was initially performed by centrifuging the aqueous solutions at 12 000 rpm for 20 minutes to eliminate suspended impurities. The CDs were further purified by dialysis (Float-A-Lyzer® G2 Dialysis Device, MWCO: 500 Da), which ran continuously for 24 hours.

Characterization

Fluorescence analysis was performed in a 10 nm fluorescence quartz cell by using a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer (Madrid, Spain). Spectra were obtained with 1 nm interval and 5 nm slit widths. Optical absorption spectra were recorded using a VWR® UV-3100PC spectrophotometer by using quartz cells. The morphology of CDs was evaluated by high-resolution transmission electron microscopy (HR-TEM) using a FEI Talos F200X. Thermal stability of the nanoparticles was studied using a type F47900 Furnace (1000 W). The surface chemical characterization of the studied film was carried out by X-ray photoelectron spectroscopy (XPS). XPS spectra were recorded with a Physical Electronics PHI 5700 spectrometer with X-ray MgK_α radiation as the excitation source (300 W, 15 kV, 1253.6 eV). High-resolution spectra were recorded using a concentric hemispherical analyzer operating in the constant pass energy mode at 29.35 eV and using a diameter analysis area of 720 μm. The residual pressure in the analysis chamber was maintained below 10^{−9} torr during data acquisition. Accurate ±0.1 eV binding energies were determined with respect to the position of the adventitious C 1s peak at 284.8 eV. A PHI ACCESS ESCA-V6.0F software package was used for acquisition and data analysis. Atomic concentration percentages (A.C. %) of the characteristic sample elements were determined after subtraction of a Shirley-type background considering the corresponding area sensitivity factor for the different measured spectral regions.⁶²

Fluorescence quantum yield calculations

QY_{FL} was calculated with a standard procedure, which is based on the comparison of the integrated luminescence intensities and absorbance values of the synthesized CDs with a reference fluorophore, with the following equation:

$$QY_{FL}^{Sample} = QY_{FL}^{Quinine\ sulphate} \times \frac{Grad_{Sample}}{Grad_{Quinine\ sulphate}} \times \frac{\eta_{Sample}^2}{\eta_{Quinine\ sulphate}^2} \quad (1)$$

where Grad is the gradient from the plot of integrated fluorescence intensity *versus* absorbance and η is the refractive index.⁶³ Quinine sulphate was chosen as a reference fluorophore of the known quantum yield (QY_{FL} = 0.54).⁶⁴

Conclusions

In this work, the synthesis and systematic investigation of N-doped CDs were performed, which were fabricated from the dry heating of solid mixtures of glucose (as the carbon precursor) and urea (as the N-dopant). The investigation of the obtained materials revealed that these procedures generate two types of CDs. One is readily soluble in water, which is the commonly

obtained type of CDs in most studies. The other was composed of nanoparticles only soluble at basic pH. Moreover, the latter type was characterized by higher synthesis yields, and lower QY_{FL} and thermal stability, compared with those of the former type. More importantly, this study revealed that this type of bottom-up procedure can generate a relatively unexplored type of CDs, with different properties and compositions than typical readily water-soluble CDs. We have also investigated the possible correlation between N-doping and QY_{FL}, by controlling the stoichiometry of the N-dopant. Interestingly, a correlation between the stoichiometry of urea and the QY_{FL} was not observed, which limits N-doping as an efficient strategy for obtaining CDs with high QY_{FL}. This study provides new information that should be useful in the rational development of CDs with higher synthesis yields and QY_{FL}.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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References

- M. K. Barman and A. Patra, *Journal*, 2018, **37**, 1–22.
- J. C. G. Esteves da Silva and H. M. R. Gonçalves, *TrAC, Trends Anal. Chem.*, 2011, **30**, 1327–1336.
- S. Y. Lim, W. Shen and Z. Gao, *Chem. Soc. Rev.*, 2015, **44**, 362–381.
- O. Kozák, K. K. R. Datta, M. Greplová, V. Ranc, J. Kašlík and R. Zbořil, *J. Phys. Chem. C*, 2013, **117**, 24991–24996.
- M. J. Molaei, *Talanta*, 2019, **196**, 456–478.
- E. F. C. Simões, J. C. G. Esteves da Silva and J. M. M. Leitão, *Sens. Actuators, B*, 2015, **220**, 1043–1049.
- K. Wang, Z. Gao, G. Gao, Y. Wo, Y. Wang, G. Shen and D. Cui, *Nanoscale Res. Lett.*, 2013, **8**, 122.
- Z. Yi, X. Li, H. Zhang, X. Ji, W. Sun, Y. Yu, Y. Liu, J. Huang, Z. Sarshar and M. Sain, *Talanta*, 2021, **222**, 121663.
- A. C. P. Afonso, A. S. Correia, D. Duarte, A. T. S. C. Brandão, M. D. de Yuso, J. Jiménez-Jiménez, N. Vale, C. M. Pereira, M. Algarra and L. Pinto da Silva, *Chemosensors*, 2021, **9**, 191.
- N. Vale, S. Silva, D. Duarte, D. M. A. Crista, L. Pinto da Silva and J. C. G. Esteves da Silva, *RSC Med. Chem.*, 2021, **12**, 245–253.
- D. M. A. Crista, J. C. G. Esteves da Silva and L. Pinto da Silva, *Nanomaterials*, 2020, **10**(7), 1316.
- P. K. Yadav, V. K. Singh, S. Chandra, D. Bano, V. Kumar, M. Talat and S. H. Hasan, *ACS Biomater. Sci. Eng.*, 2019, **5**, 623–632.
- R. M. S. Sendão, D. M. A. Crista, A. C. P. Afonso, M. d V. M. d Yuso, M. Algarra, J. C. G. E. d Silva and L. P. d. Silva, *Phys. Chem. Chem. Phys.*, 2019, **21**, 20919–20926.
- B. Zhi, Y. Cui, S. Wang, B. P. Frank, D. N. Williams, R. P. Brown, E. S. Melby, R. J. Hamers, Z. Rosenzweig, D. H. Fairbrother, G. Orr and C. L. Haynes, *ACS Nano*, 2018, **12**, 5741–5752.
- D. M. A. Crista, A. El Mragui, M. Algarra, J. C. G. Esteves da Silva, R. Luque and L. Pinto da Silva, *Nanomaterials*, 2020, **10**, 1209.
- S. Chahal, N. Yousefi and N. Tufenkji, *ACS Sustainable Chem. Eng.*, 2020, **8**, 5566–5575.
- R. Sendão, M. d V. M. d Yuso, M. Algarra, J. C. G. Esteves da Silva and L. Pinto da Silva, *J. Cleaner Prod.*, 2020, **254**, 120080.
- T. V. de Medeiros, J. Manioudakis, F. Noun, J.-R. Macairan, F. Victoria and R. Naccache, *J. Mater. Chem. C*, 2019, **7**, 7175–7195.
- D. Qu and Z. Sun, *Mater. Chem. Front.*, 2020, **4**, 400–420.
- P.-C. Hsu, Z.-Y. Shih, C.-H. Lee and H.-T. Chang, *Green Chem.*, 2012, **14**, 917–920.
- H. Kalita, J. Mohapatra, L. Pradhan, A. Mitra, D. Bahadur and M. Aslam, *RSC Adv.*, 2016, **6**, 23518–23524.
- V. Sharma, P. Tiwari and S. M. Mobin, *J. Mater. Chem. B*, 2017, **5**, 8904–8924.
- M. Algarra, L. d Orfãos, C. S. Alves, R. Moreno-Tost, M. S. Pino-González, J. Jiménez-Jiménez, E. Rodríguez-Castellón, D. Eliche-Quesada, E. Castro and R. Luque, *ACS Sustainable Chem. Eng.*, 2019, **7**, 10493–10500.
- D. M. A. Crista, G. P. C. Mello, O. Shevchuk, R. M. S. Sendão, E. F. C. Simões, J. M. M. Leitão, L. P. da Silva and J. C. G. Esteves da Silva, *J. Fluoresc.*, 2019, **29**, 265–270.
- Y.-F. Kang, Y.-H. Li, Y.-W. Fang, Y. Xu, X.-M. Wei and X.-B. Yin, *Sci. Rep.*, 2015, **5**, 11835.
- Y. Liu, L. Jiang, B. Li, X. Fan, W. Wang, P. Liu, S. Xu and X. Luo, *J. Mater. Chem. B*, 2019, **7**, 3053–3058.
- Y. Zhan, T. Geng, Y. Liu, C. Hu, X. Zhang, B. Lei, J. Zhuang, X. Wu, D. Huang, G. Xiao and B. Zou, *ACS Appl. Mater. Interfaces*, 2018, **10**, 27920–27927.
- J. Liao, Z. Cheng and L. Zhou, *ACS Sustainable Chem. Eng.*, 2016, **4**, 3053–3061.
- B. C. M. Martindale, G. A. M. Hutton, C. A. Caputo and E. Reisner, *J. Am. Chem. Soc.*, 2015, **137**, 6018–6025.
- M. Xu, G. He, Z. Li, F. He, F. Gao, Y. Su, L. Zhang, Z. Yang and Y. Zhang, *Nanoscale*, 2014, **6**, 10307–10315.
- J. Zhou, H. Zhou, J. Tang, S. Deng, F. Yan, W. Li and M. Qu, *Microchim. Acta*, 2017, **184**, 343–368.
- Y. Li, Y. Zhao, H. Cheng, Y. Hu, G. Shi, L. Dai and L. Qu, *J. Am. Chem. Soc.*, 2012, **134**, 15–18.
- H. Liu, Y. Zhang, J. H. Liu, P. Hou, J. Zhou and C. Z. Huang, *RSC Adv.*, 2017, **7**, 50584–50590.

- 34 W. Liu, R. Zhang, Y. Kang, X.-Y. Zhang, H.-J. Wang, L.-H. Li, H.-P. Diao and W.-L. Wei, *New Carbon Mater.*, 2019, **34**, 390–402.
- 35 Q. Xu, T. Kuang, Y. Liu, L. Cai, X. Peng, T. Sreenivasan Sreepasad, P. Zhao, Z. Yu and N. Li, *J. Mater. Chem. B*, 2016, **4**, 7204–7219.
- 36 X. Zhou, G. Zhao, X. Tan, X. Qian, T. Zhang, J. Gui, L. Yang and X. Xie, *Microchim. Acta*, 2019, **186**, 67.
- 37 X. Chen, Q. Jin, L. Wu, C. Tung and X. Tang, *Angew. Chem., Int. Ed.*, 2014, **53**, 12542–12547.
- 38 D. Qu, M. Zheng, L. Zhang, H. Zhao, Z. Xie, X. Jing, R. E. Haddad, H. Fan and Z. Sun, *Sci. Rep.*, 2014, **4**, 5294.
- 39 Y.-Q. Zhang, D.-K. Ma, Y. Zhuang, X. Zhang, W. Chen, L.-L. Hong, Q.-X. Yan, K. Yu and S.-M. Huang, *J. Mater. Chem.*, 2012, **22**, 16714–16718.
- 40 S. Christé, J. C. G. Esteves da Silva and L. Pinto da Silva, *Materials*, 2020, **13**, 504.
- 41 C.-Y. Chen, Y.-H. Tsai and C.-W. Chang, *New J. Chem.*, 2019, **43**, 6153–6159.
- 42 J. B. Essner, J. A. Kist, L. Polo-Parada and G. A. Baker, *Chem. Mater.*, 2018, **30**, 1878–1887.
- 43 W. Liang, P. Wang, M. J. Mezziani, L. Ge, L. Yang, A. K. Patel, S. O. Morgan and Y.-P. Sun, *Nanoscale Adv.*, 2021, **3**, 4186–4195.
- 44 K. Mishra, S. Koley and S. Ghosh, *J. Phys. Chem. Lett.*, 2019, **10**, 335–345.
- 45 P. Liu, C. Zhang, X. Liu and P. Cui, *Appl. Surf. Sci.*, 2016, **368**, 122–128.
- 46 J. Zheng, Y. Xie, Y. Wei, Y. Yang, X. Liu, Y. Chen and B. Xu, *Nanomaterials*, 2020, **10**(1), 82.
- 47 J. Tan, R. Zou, J. Zhang, W. Li, L. Zhang and D. Yue, *Nanoscale*, 2016, **8**, 4742–4747.
- 48 Y. Yang, J. Cui, M. Zheng, C. Hu, S. Tan, Y. Xiao, Q. Yang and Y. Liu, *Chem. Commun.*, 2012, **48**, 380–382.
- 49 Y. Zhao, S. Jing, X. Peng, Z. Chen, Y. Hu, H. Zhuo, R. Sun and L. Zhong, *Cellulose*, 2020, **27**, 415–428.
- 50 S. Hill and M. C. Galan, *Beilstein J. Org. Chem.*, 2017, **13**, 675–693.
- 51 H.-I. Hwang, T. G. Hartman, R. T. Rosen, J. Lech and C.-T. Ho, *J. Agric. Food Chem.*, 1994, **42**, 1000–1004.
- 52 K. M. Klinger, F. Liebner, I. Fritz, A. Potthast and T. Rosenau, *J. Agric. Food Chem.*, 2013, **61**, 9004–9014.
- 53 S. Fernandes, J. C. G. Esteves da Silva and L. Pinto da Silva, *NanoImpact*, 2021, **23**, 100332.
- 54 B. Vercelli, R. Donnini, F. Ghezzi, A. Sansonetti, U. Giovannella and B. La Ferla, *Electrochim. Acta*, 2021, **387**, 138557.
- 55 V. Hinterberger, C. Damm, P. Haines, D. M. Guldi and W. Peukert, *Nanoscale*, 2019, **11**, 8464–8474.
- 56 A. Das, D. Roy, C. K. De and P. K. Mandal, *Phys. Chem. Chem. Phys.*, 2018, **20**, 2251–2259.
- 57 J. Ren, F. Weber, F. Weigert, Y. Wang, S. Choudhury, J. Xiao, I. Laueremann, U. Resch-Genger, A. Bande and T. Petit, *Nanoscale*, 2019, **11**, 2056–2064.
- 58 P. K. Sarswat and M. L. Free, *Phys. Chem. Chem. Phys.*, 2015, **17**, 27642–27652.
- 59 S. Qu, H. Chen, X. Zheng, J. Cao and X. Liu, *Nanoscale*, 2013, **5**, 5514–5518.
- 60 M. Algarra, A. L. Cuevas, M. V. M. de Yuso and J. Benavente, *J. Colloid Interface Sci.*, 2020, **579**, 714–722.
- 61 A. Cuevas, B. B. Campos, R. Romero, M. Algarra, M. I. Vázquez and J. Benavente, *Carbohydr. Polym.*, 2019, **206**, 238–244.
- 62 D. Briggs, *Auger and X-ray photoelectron spectroscopy*, Wiley, Chichester, 1994, vol. 1.
- 63 J. W. Eastman, *Photochem. Photobiol.*, 1967, **6**, 55–72.
- 64 W. H. Melhuish, *J. Phys. Chem.*, 1961, **65**, 229–235.

4.2. Supporting Information

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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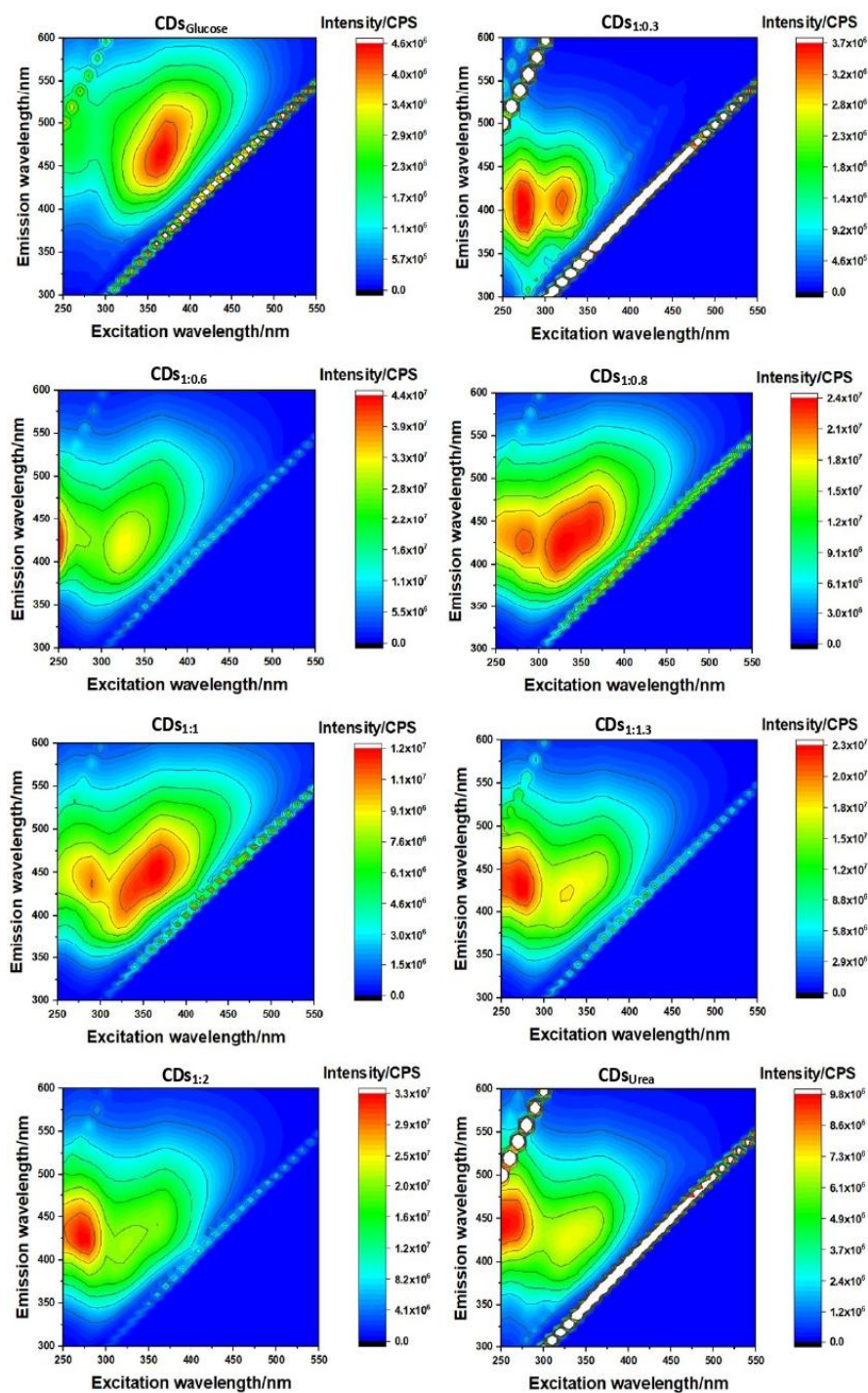


Figure S1. Excitation/Emission Matrix for all water-soluble CDs with a concentration of 100 µg/mL in water.

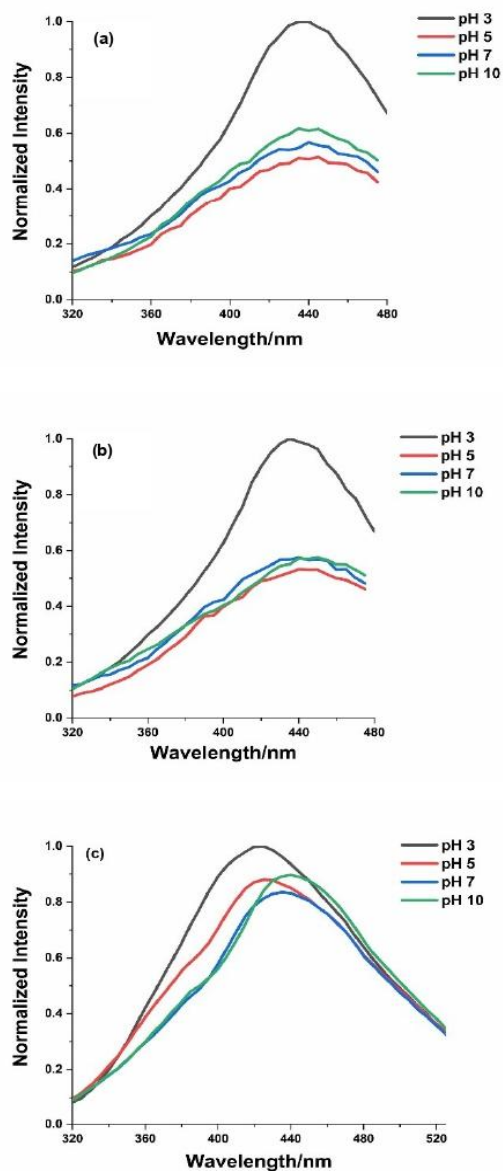


Figure S2. pH-dependence emission spectra for the water-soluble CDs: **(a)** CDs_{1.0.8}, **(b)** CDs_{1.1} and **(c)** CDs_{1.2} with a concentration of 100 µg/mL. In water. pH adjust made using 0.1M of HCl and NaOH aqueous solution.

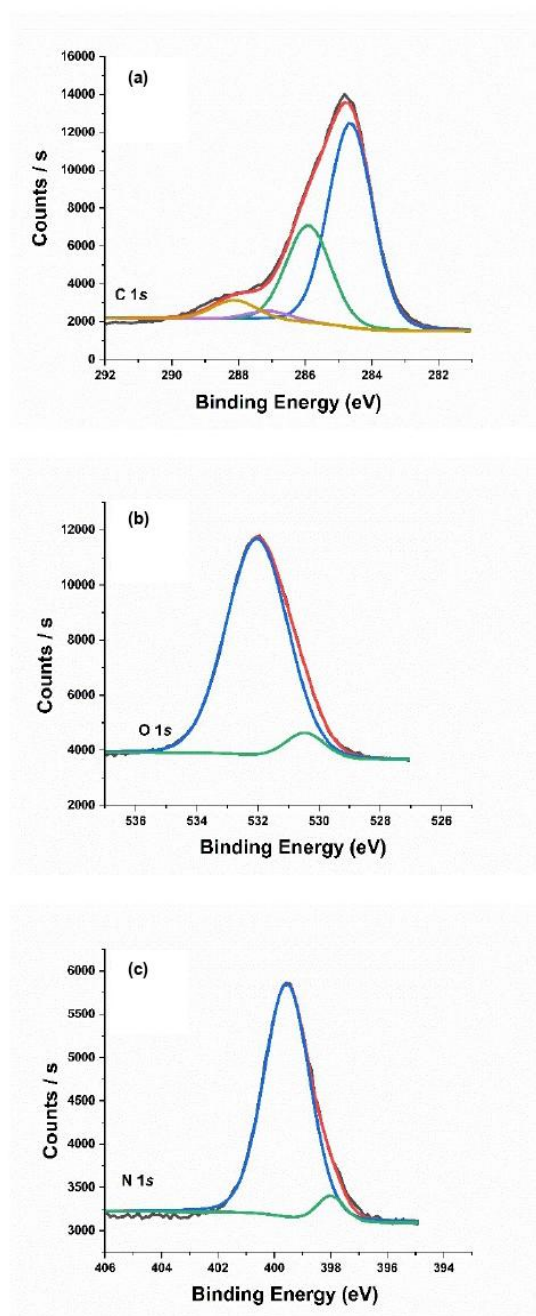


Figure S3. XPS core level spectra of water-soluble CDs_{10.8} (a) C 1s; (b) O 1s and (c) N 1s.

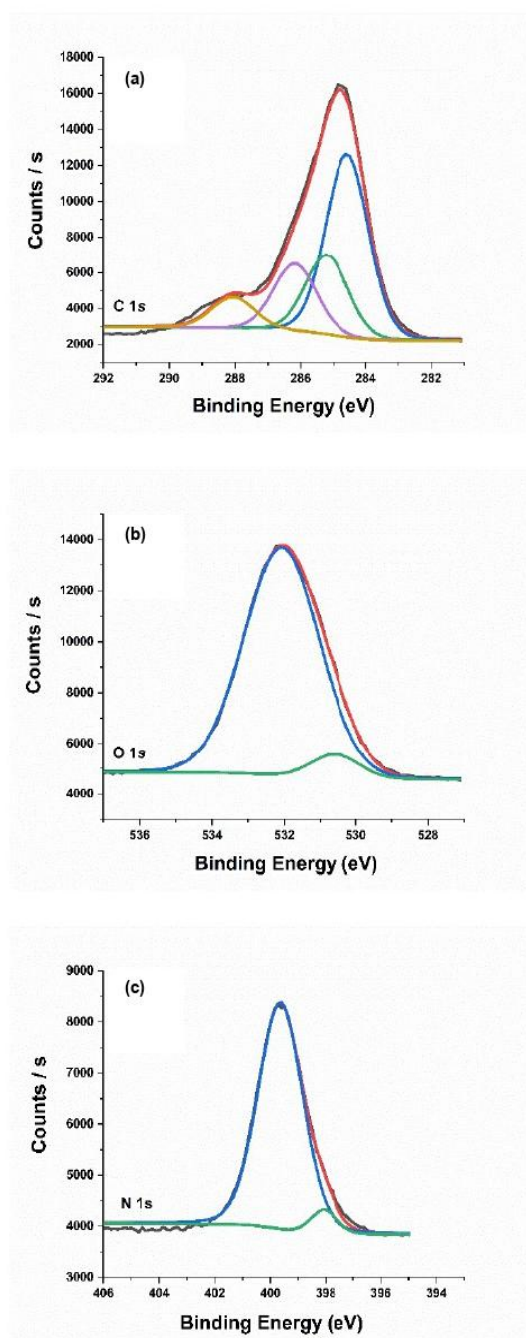


Figure S4. XPS core level spectra of water-soluble CDs_{1:2} (a) C 1s; (b) O 1s and (c) N 1s.

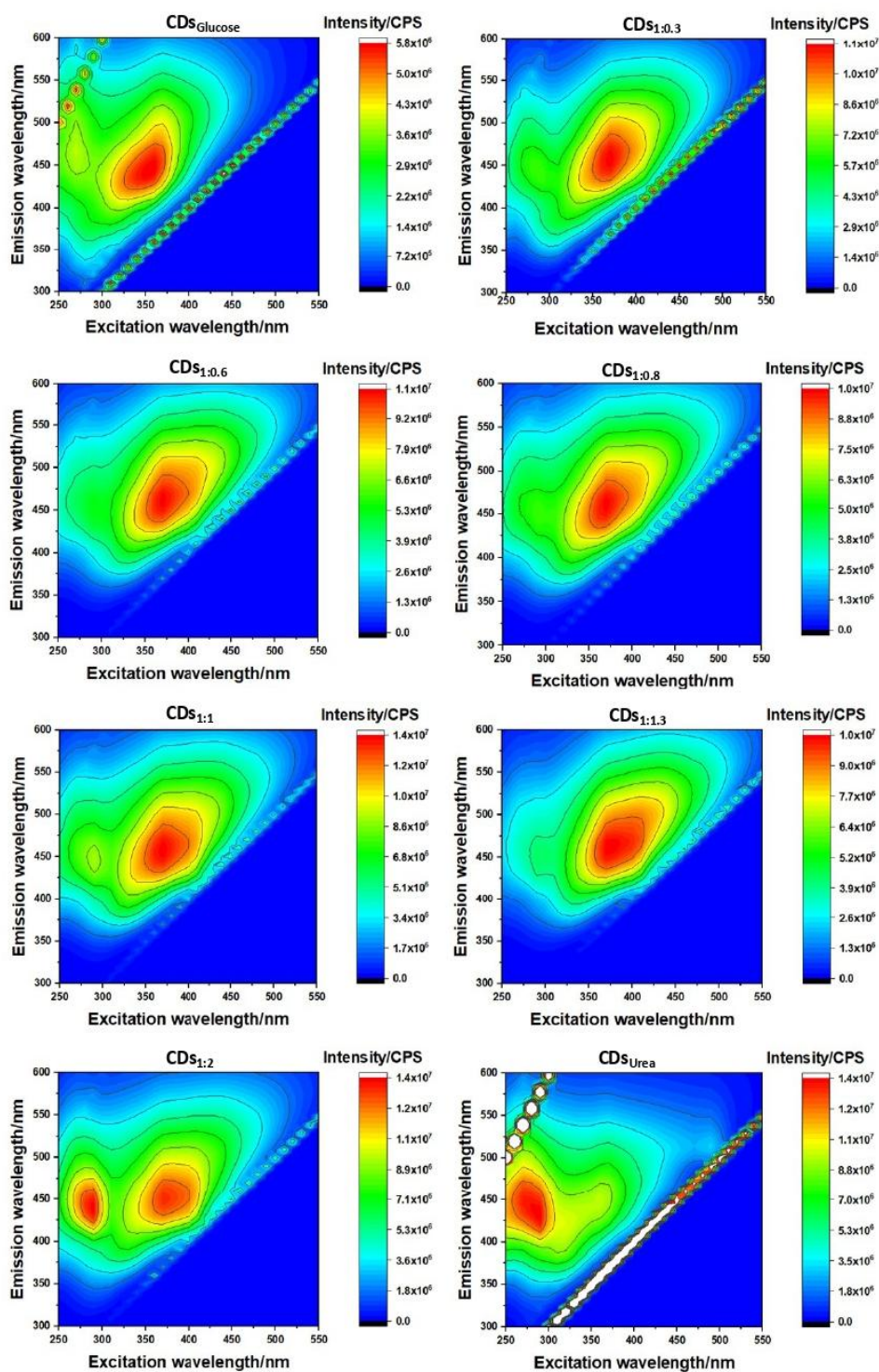


Figure S5. Excitation/Emission Matrix for all water/hydroxide-soluble CDs with a concentration of 100µg/mL in water.

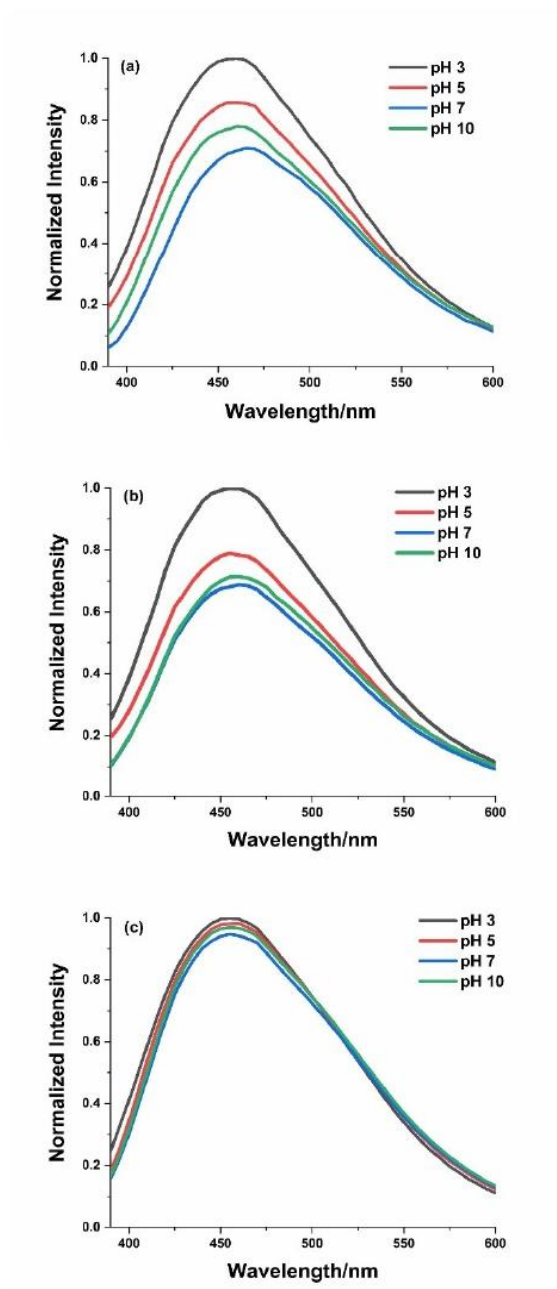


Figure S6. pH-dependence emission spectra for the hydroxide-soluble CDs: (a) CD_{S10.8}, (b) CD_{S1.1} and (c) CD_{S1.2} with a concentration of 100 µg/mL. In water. pH adjust made using 0.1M of HCl and NaOH aqueous solution.

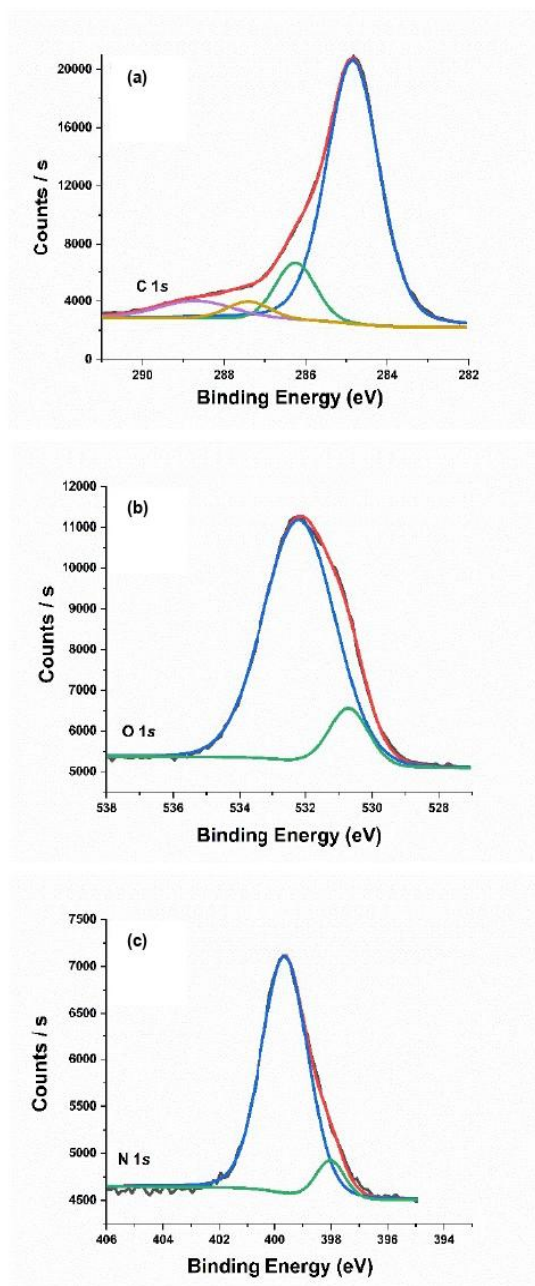


Figure S7. XPS core level spectra of hydroxide-soluble CD_{510.8} (a) C 1s; (b) O 1s and (c) N 1s.

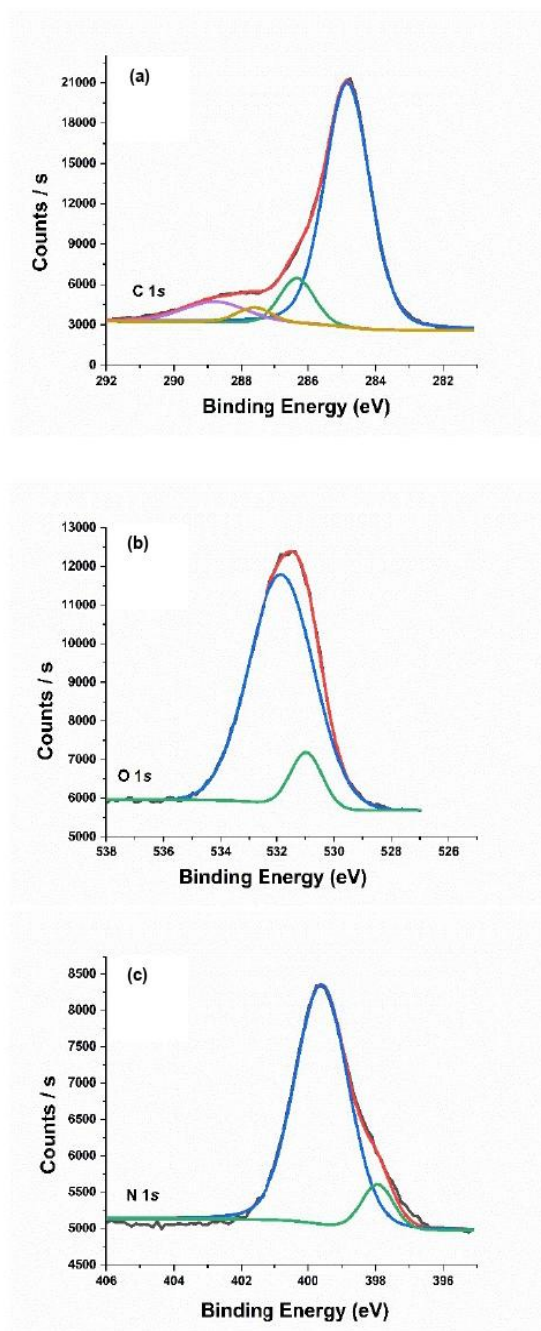


Figure S8. XPS core level spectra of hydroxide-soluble CDs_{1.2} (a) C 1s; (b) O 1s and (c) N 1s.

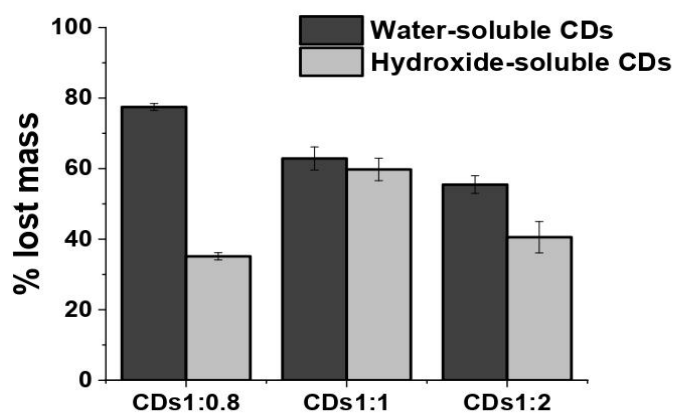


Fig. S9 % lost mass for water- and hydroxide-soluble CDs(a) CDs_{1:0.8}, (b) CDs_{1:1} and (c) CDs_{1:2}. Initial mass of 50 mg. Oven temperature of 400°C / 1 hour.

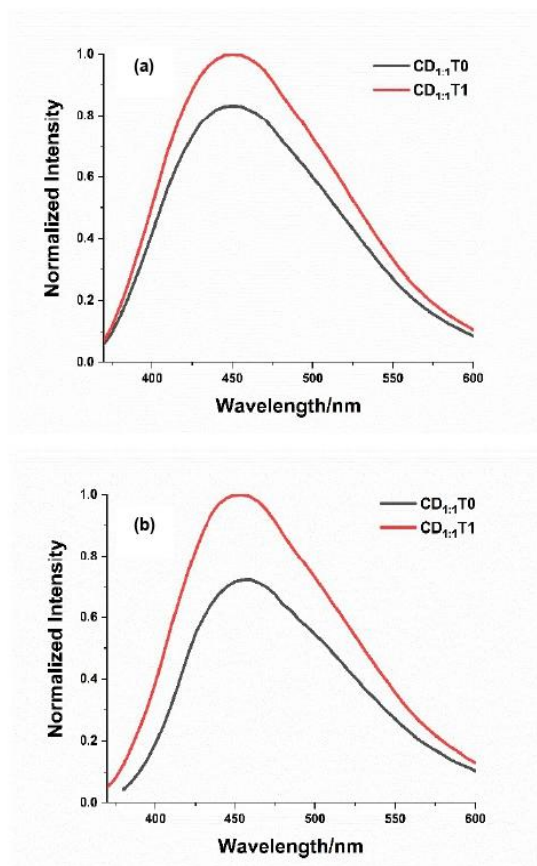


Figure S10. Fluorescent spectra of water-soluble CD_{1:1} (a), and hydroxide-soluble CD_{1:1} (b), at two different times: T0 (after CD preparation) and T1 (60 days after synthesis).

4.3. Conclusions

In this study, we conducted the synthesis and systematic examination of nitrogen-doped carbon dots (N-CDs) fabricated through the dry heating process of solid mixtures comprising glucose as the carbon precursor and urea as the nitrogen dopant. Our investigation unveiled the generation of two distinct types of CDs using these procedures. One type demonstrated ready solubility in water, consistent with findings in many previous studies. The other type consisted of nanoparticles solely soluble under basic pH conditions. Furthermore, the latter type exhibited higher synthesis yields but lower QYFL and thermal stability compared to the former type.

Significantly, our study illuminated the capacity of this bottom-up approach to yield a relatively unexplored variant of CDs, characterized by distinct properties and compositions from the typically encountered water-soluble CDs. Additionally, we explored the potential correlation between N-doping and QYFL by modulating the stoichiometry of the nitrogen dopant. Intriguingly, no observable correlation between urea stoichiometry and QYFL was discerned, suggesting limitations in employing N-doping as an efficient strategy for attaining CDs with high QYFL.

This investigation contributes novel insights pertinent to the methodical advancement of CDs with augmented synthesis yields and QYFL, thus offering valuable information for future endeavors in this domain.

5. Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides

5.1. Application of Fluorescent Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides

Article 4

Application of Fluorescent Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides

Diana M. A. Crista, Joaquim C.G. Esteves da Silva and Luís Pinto da Silva, *Materials*, **2023**, 16, 7620.

5.1.1. Contextualization

Considering the increase in anthropogenic CO₂ emissions, there is an urgency to develop economic incentives for the use of CO₂ capture techniques. The transformation of CO₂ into heterocyclic carbonates presents a promising potential. However, suitable organocatalysts are needed to reach the required efficiency in these reactions. Consequently, there has been a growing emphasis on the development of organocatalytic systems comprising nucleophiles and hydrogen bond donors (HBD) to facilitate CO₂ conversion under ambient conditions.

This study explores the potential of fluorescent CDs as catalytic HBD in the ring-opening reaction of epoxides, which is a rate-limiting step in CO₂ conversion to heterocyclic carbonates. Our findings demonstrate that CDs exert a significant catalytic influence on the model reaction, exhibiting a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$, a reactant conversion rate of 70.8 %, and a rate constant enhancement of 32.2 %. Notably, these results were better than the ones with alternative molecular HBD under investigation. Thus, this investigation demonstrates that CDs have the potential to be used as HBD in organocatalyzed CO₂ conversion processes aimed at generating value-added products.

5.1.2. Contributions to this work

All synthesis of CDs, as well as their characterization, was performed by me (Diana Crista) in the previous work under the supervision of my supervisors. All the catalytic studies and the writing of the original draft was performed by me.

Article

Application of Fluorescent Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides

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Abstract: Considering the increased anthropogenic emissions of CO₂ into the atmosphere, it is important to develop economic incentives for the use of CO₂ capture methodologies. The conversion of CO₂ into heterocyclic carbonates shows significant potential. However, there is a need for suitable organocatalysts to reach the required efficiency for these reactions. Given this, there has been an increasing focus on the development of organocatalytic systems consisting of a nucleophile and a hydrogen bond donor (HBD) so that CO₂ conversion can occur in ambient conditions. In this work, we evaluated the potential of fluorescent carbon dots (CDs) as catalytic HBDs in the ring-opening reaction of epoxides, which is typically the rate-limiting step of CO₂ conversion reactions into heterocyclic carbonates. The obtained results demonstrated that the CDs had a relevant catalytic effect on the studied model reaction, with a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$, a percentage of reactant conversion of 70.8%, and a rate constant enhancement of 32.2%. These results were better than the studied alternative molecular HBDs. Thus, this study demonstrated that CDs have the potential to be used as HBDs and employed in organocatalyzed CO₂ conversion into value-added products.

Keywords: carbon dots; organocatalysts; hydrogen bond donor; epoxides; CO₂ conversion



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1. Introduction

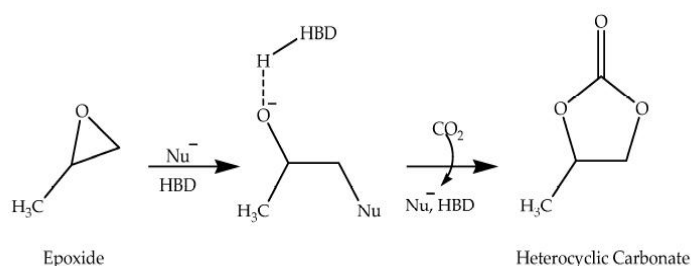
The development of the world economy and industry has led to an unprecedented increase in anthropogenic carbon dioxide (CO₂) emissions into the atmosphere. This had a significant impact on the ecological environment of the earth, with consequences such as glacier melting, the rise of the sea level, and Earth's average surface temperature being correlated to the increased CO₂ levels [1–3]. Despite its harmful consequences when mismanaged, this gas can also be regarded as an environmentally friendly, abundant, and low-cost C1 source [4–8]. Given this, the utilization of excess CO₂ as a carbon feedstock in organic synthesis has become an important goal for researchers. More specifically, it may provide economic incentives for the development and employment of carbon capture strategies.

The development of novel catalytic approaches and innovative pathways for CO₂ fixation are being developed continuously [9]. One of the routes for CO₂ conversion into value-added products occurs via the combination of CO₂ with epoxides to produce cyclic organic carbonates [10–13]. The advantage of this pathway is that the production of heterocyclic carbonates through CO₂ epoxide coupling is an environmentally friendly and toxicological alternative to conventional methods involving diols and toxic phosgenes [14–16]. Heterocyclic carbonates are an important and versatile class of chemicals that have several applications, such as electrolytes for lithium-ion batteries [17], in the synthesis of polymers (as polycarbonates) [18], pharmaceutical manufacturing [19], and polar aprotic solvents [20–22].

However, due to the thermodynamic stability and kinetic inertia of CO₂, there are restrictions in terms of the efficiency of the CO₂ fixation/conversion processes. Thus, there is a need for novel and effective catalytic systems that can overcome these limitations and improve the efficiency of CO₂ conversion processes [23].

Over the years, the focus of the scientific community in terms of CO₂ conversion into heterocyclic carbonates has been the development of both transition metal-based and organo-based approaches [24,25]. Transition metal-based catalysts have shown promising results by allowing the CO₂ conversion reactions to proceed with good yields at ambient temperatures. However, they also show some relevant disadvantages, such as requiring harsh preparation conditions, presenting low thermal stability, and higher prices. On the other hand, organocatalysts are generally low cost, nontoxic, and stable compounds that show inertness toward moisture and air. In some cases, they can be obtained from renewable feedstocks, benefiting these processes in terms of environmental sustainability. So, it is not surprising that different researchers have been interested in using organocatalysts in CO₂ conversion reactions [9,26–29]. Nevertheless, organocatalytic systems also present some limitations, such as requiring higher temperatures and CO₂ pressures [9]. To overcome these issues, researchers have been focused on the development of two-component organocatalytic systems, which are composed of a nucleophile and a hydrogen bond donor (HBD), in which the limitations of both transition metal-based catalysts and organocatalysts can be bridged [30–33].

In this type of system, with the goal of facilitating the epoxide's ring opening by nucleophilic attack, the HBDs interact with the O-donor of the epoxide via hydrogen bonding in a process involving a suitable nucleophile (Scheme 1). Furthermore, the HBDs are expected to stabilize the oxyanion resulting from the ring-opening reaction and via hydrogen bonding. These synergistic effects should make the ring-opening reaction of the epoxide more efficient, allowing the production of heterocyclic carbonates from CO₂ at lower temperatures and CO₂ pressures [13,18,34–36]. It should be noted that in this type of catalytic scheme, the ring opening of the epoxide through nucleophile attack typically represents the rate-limiting step of the process [13,14,17,37,38]. So, the addition of an HBD is made with the expectancy of decreasing the activation barrier for this step, thus increasing efficiency [39]. Considering this, there is a growing interest in the development and identification of new HBDs with the potential to be used in CO₂ conversion reactions.



Scheme 1. Reaction scheme for CO₂ conversion in the presence of a nucleophile (Nu⁻) and a hydrogen bond donor (HBD).

Carbon dots (CDs) are a class of fluorescent carbon-based nanoparticles with a quasi-spherical shape and a size usually below 10 nm [40–42]. These nanoparticles possess remarkable properties such as high photoluminescence [43–46], low toxicity, biocompatibility [47,48], high photostability and chemical stability, and good water solubility [40,43]. Thus, CDs have gained relevance in various fields, such as sensing, bioimaging, drug delivery, photodynamic therapy, and photocatalysis. Depending on the used precursors and synthesis conditions, CDs may present distinct properties, compositions, and potential applications. They are often synthesized from carbon-rich precursors and heteroatom-doping molecules, which can include organic compounds containing oxygen, nitrogen, and other

heteroatoms [40–42,49]. Considering that hydroxyl and amino groups are commonly found on the surface of CDs and that they can participate in hydrogen bonding with acceptor molecules, the presence of these oxygen- and nitrogen-based groups on the CD surface allows them to act as HBDs [50]. As so, CDs could potentially be employed as an HBD for the synthesis of cyclic organic carbonates from CO₂ and epoxides.

In this work, we will evaluate the potential application of CDs, a low-cost and easy-to-prepare nanomaterial, as HBDs in two-component organocatalytic systems for CO₂ fixation at room temperature, without the need for high temperatures and pressure conditions, which should be an advantage when compared with current organocatalysts. These CDs result from the dry heating of solid mixtures of glucose (carbon precursor) and urea (N-dopant). Here, we will specifically focus on the rate-limiting step of the process, namely the epoxide's ring-opening reaction in which the HBD should act. To this end, we tested the use of CDs as HBDs in a model reaction using propylene oxide as the epoxide and pyridine as a nucleophile. Pyridine was chosen due to the fact that after catalyzing the ring opening, it traps the intermediate species of the reaction, allowing a better study of this reaction step [39]. To ascertain the benefits of our method, the results obtained with the CDs were compared with other potential HBDs. More specifically, given that the CDs were prepared from glucose, we compared them with glucose itself and two other saccharides (fructose and sucrose). The CDs showed superior catalytic activity, thereby demonstrating both the advantages of converting glucose into CDs for this application and the better activity of CDs compared with alternative molecular HBDs.

2. Materials and Methods

2.1. Reagents

The following reagents were used as received: propylene oxide 99% (Sigma, St. Louis, MO, USA), pyridine ACS Reag. Ph Eur (Merck, Darmstadt, Germany), D-(+)-glucose $\geq 96\%$ (Sigma, St. Louis, MO, USA), D(–)-fructose $\geq 98\%$ (Panreac, Darmstadt, Germany), sucrose (Sigma, St. Louis, MO, USA), and urea (Sigma, St. Louis, MO, USA).

2.2. Experimental Details

2.2.1. Synthesis of CDs

The CDs were synthesized following a methodology previously developed by us [50]. Briefly, CDs were prepared via a one-pot solvent-free dry-heating method, using glucose and urea as precursors in a molar ratio of 1:1. The solid precursors were placed in a Teflon-lined reactor encased in a stainless-steel shell and heated in an oven for 2 h at 200 °C. The synthesized nanoparticles were purified by centrifugation (10 min/12,000 rpm) and dialysis (Float-A-Lyzer® G2 Dialysis Device, Sigma, St. Louis, MO, USA, MWCO:500 Da, 24 h). The CDs were characterized in our previous work by high-resolution transmission electron microscopy (HR-TEM), X-ray photoelectron (XPS), fluorescence, and UV-Vis spectroscopy, as well as by assessing the thermal stability of the nanoparticles [50].

2.2.2. Monitoring of the Ring-Opening Reaction

The reaction mixture was prepared by adding propylene oxide (2 mmol) and pyridine (10% mmol) in 5 mL of deionized water. The HBD was added to the reaction mixture in a concentration ranging between 5 and 100 µg/mL [34]. After preparation, the mixtures were placed in a water bath (25 °C, stirring rate of 500 rpm). Kinetic studies were performed by measuring the pyridine peak area via HPLC-DAD for reaction times of 0, 0.5, 1, 2, 3, 4, and 5 h. Each sample was made in triplicate and diluted 200 times in the correspondent mobile phase.

2.2.3. Chromatographic Conditions

The chromatographic system consisted of an isocratic pump (Hewlett-Packard 1100 Series, Boeblingen, Germany), a manual sample injection valve with a 20 µL loop (Rheodyne 7725i, Rohnert Park, CA, USA), a silica-based C18 reversed-phase column (Thermo-

scientific Acclaim™ 120 column 4.6 × 150 mm, particle size 3 µm, pore diameter 120 Å), and a photodiode array detector (UV 6000LP, Thermo Scientific, San Jose, CA, USA). The mobile phase was composed of methanol and water (80:20% *v/v*). Chromatographic assays were performed at a constant flow rate (0.5 mL min^{−1}) under isocratic conditions. Absorbance was monitored in a total scan mode from 210 to 600 nm. The system was controlled by Xcalibur version 1.4 SR software.

2.3. Fluorescence Measurement

Fluorescence analysis was performed using a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer (Madrid, Spain) and standard 10 nm fluorescence quartz cells. Fluorescence spectra were obtained with a 1 nm interval and 5 nm slit widths. The samples were analyzed in an aqueous solution with 2 mmol of propylene oxide, 10% mmol of pyridine, and 12.5 µg/mL of CDs for reaction times of 0, 0.5, 1, 2, 3, 4, and 5 h.

2.4. Data Analysis

Monitoring the conversion of pyridine as a function of the time of reaction was carried out via HPLC analysis. The kinetic curves gave rise to rate constants (*k*, in h^{−1}). The percentage of pyridine conversion (%PC), the *k*^{NC} without a catalyst and *k*^C with a catalyst, and the percentage of rate constant enhancement when the HBD was present (%Inc) were calculated using Equations (1)–(3) [51], respectively:

$$\%PC = 100 \times (A_0 - A_{5h})/A_0, \quad (1)$$

$$\ln(A_t/A_0) = -kt, \quad (2)$$

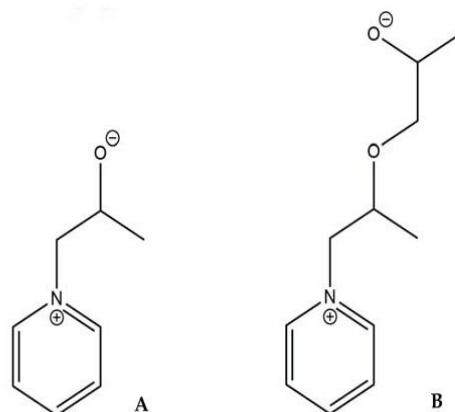
$$\%Inc = 100 \times (k^C - k^{NC})/k^{NC}, \quad (3)$$

where *A*₀ and *A*_{*t*} are the pyridine peak integrated area at the start and *t* time of the reaction, respectively. The pyridine peak was measured since the HPLC method was defined based on this compound.

3. Results and Discussion

In this study, we aimed to evaluate the potential of CDs as an HBD in a two-component organocatalytic system for the ring-opening reaction of a model epoxide, propylene oxide. This is typically considered the rate-limiting step in the CO₂ conversion into value-added heterocyclic carbonates. Thus, an enhanced catalytic activity in this reaction step is required for the organocatalyzed CO₂ conversion to occur at lower temperatures.

The herein-studied CDs were synthesized and characterized in a previous work by our team [50]. Briefly, they were fabricated via the dry heating of solid mixtures of glucose (as carbon precursor) and urea (as the N-dopant) in a 1:1 molar ratio. The CDs were then incorporated as HBDs in a catalytic system in conjugation with pyridine in a two-component organocatalytic system. We used pyridine as a nucleophile due to previously observing that pyridine forms two types of stable complexes with ring-opened epoxides, allowing us to trap the resulting oxyanion intermediate and monitor its formation [39]. A single pyridine molecule can be involved in the ring-opening reaction of up to two molecules of propylene oxide (Scheme 2). More specifically, a complex formed by one pyridine and one propylene oxide is formed via a nucleophilic attack by the former, originating the expected oxyanion. Subsequently, the deprotonated oxygen heteroatom can attack another epoxide molecule, with the formation of an ether bond. The second complex is expected to be formed in a higher ratio [39]. The formation of these complexes was previously investigated by UV-Vis spectroscopy and mass spectrometry, with density functional theory (DFT)-based calculations assisting in the characterization of the postulated reaction mechanism [39].



Scheme 2. Possible complexes formed between pyridine and propylene oxide. Complex A is formed between one pyridine and one propylene oxide molecule. Complex (B) is formed between Complex (A) and another propylene oxide molecule [39].

It is known that water can act as an HBD, with similar results to other molecules [39]. In order to test if using CDs as HBDs instead of water results in any relative enhancement of the catalytic activity, the catalytic system will be tested in an aqueous solution.

The ring-opening reaction of propylene oxide was evaluated using HPLC-DAD via monitoring the consumption of pyridine for the formation of the oxyanion complexes. We started by measuring this ring-opening reaction using a mixture of pyridine and propylene oxide in an aqueous solution and in the absence of CDs (Figure 1). We can see that before the reaction (t_0), there is a peak with a retention time of 2.8 min, which is ascribed to pyridine. This is confirmed by the corresponding UV-Vis spectrum, which is presented in Figure 1c and matches the absorption profile of pyridine. Figure 1b presents the chromatogram obtained after five hours of reaction (t_{5h}). Here, we can observe the formation of a new peak with a retention time of 9.2 min and a corresponding decrease in the peak associated with pyridine. The appearance of a new product with the consumption of pyridine is expected when we consider the use of the latter as a nucleophile in the ring-opening reaction of epoxides [39]. Given this, we attributed the new peak to product complexes formed between pyridine and propylene oxide. It should be noted that this new peak is not so well defined, which might be explained by the fact that these chromatographic conditions were optimized for pyridine, and so it might not be able to properly elute or separate the two potential product complexes. Nevertheless, subsequent kinetic measurements will be made by considering the pyridine peak alone, which is not affected by the peak ascribed to the products.

The next step was to evaluate the potential HBD properties of the CDs in this reaction. To that end, we analyzed the reaction mixture with increasing concentrations of CDs: 5 $\mu\text{g/mL}$, 12.5 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 100 $\mu\text{g/mL}$.

Figure 2a presents the chromatogram obtained at t_0 for the reaction in the presence of CDs (12.5 $\mu\text{g/mL}$), while Figure 2b corresponds to the chromatogram obtained at t_{5h} . These results indicate that there are no qualitative differences between the reactions with and without CDs. We still observe a pyridine peak at 2.87 min and a peak attributed to the pyridine–propylene oxide complex at 9.16 min. There is also no difference regarding the UV-Vis spectrum corresponding to the pyridine peak (Figure 1c). However, with the addition of CDs, when compared to the reaction in their absence, we observed a decrease in the area of the pyridine's peak with a corresponding increase in the area ascribed to the products' peak. Thus, the addition of CDs led to quantitative differences.

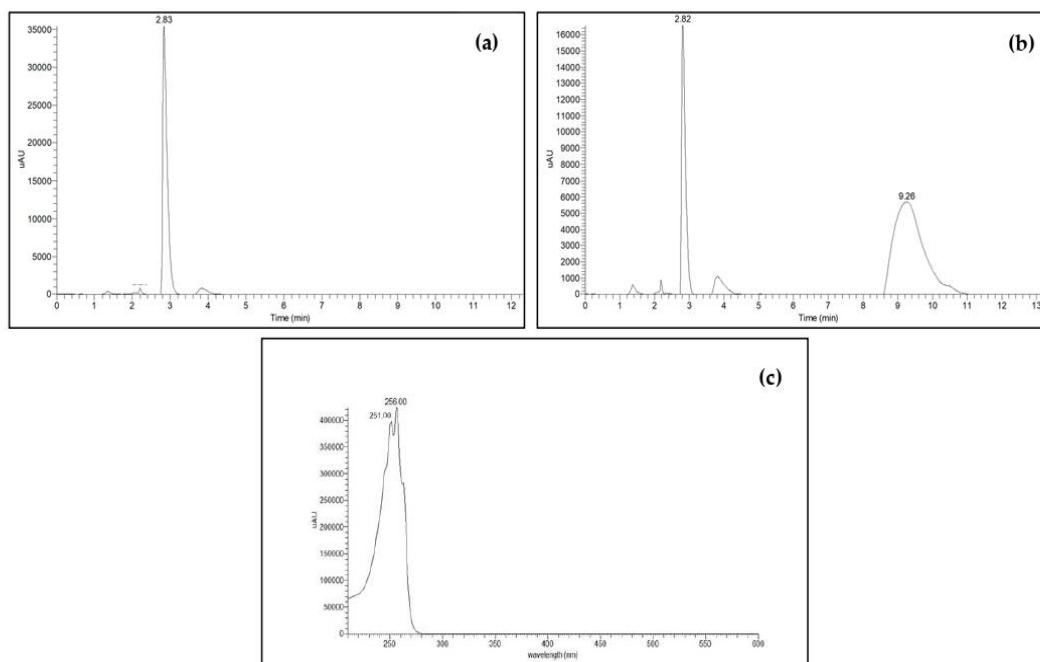


Figure 1. Chromatogram for (a) T_0 and (b) T_{5h} for the reaction in the absence of CDs and (c) UV-Vis spectrum for a peak at ~ 2.8 min at T_0 .

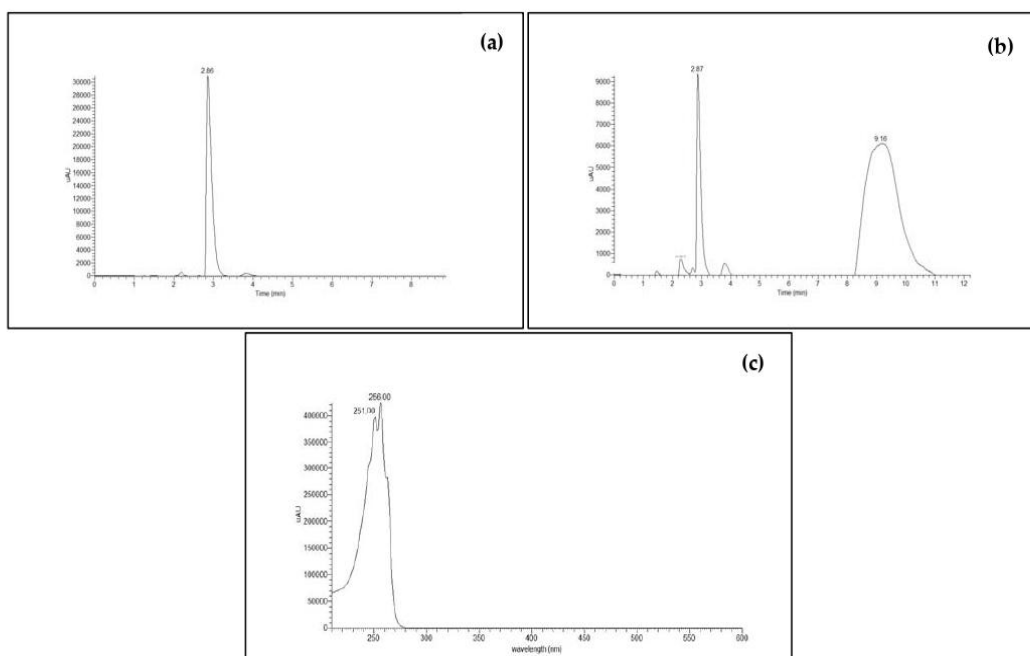


Figure 2. Chromatogram for (a) T_0 and (b) T_{5h} for the reaction with CDs and (c) UV-Vis spectrum for a peak at ~ 2.8 min at T_0 .

The kinetics of these reactions, with increasing concentrations of CDs, were determined by correlating the absolute area of the pyridine's peak in the HPLC chromatogram with the different reaction times. Rate constants (k , in h^{-1}) were obtained for each concentration of CDs ($\mu\text{g/mL}$). These values resulted from a linear fitting. Figure 3 represents the linear fitting for all the analyzed concentrations.

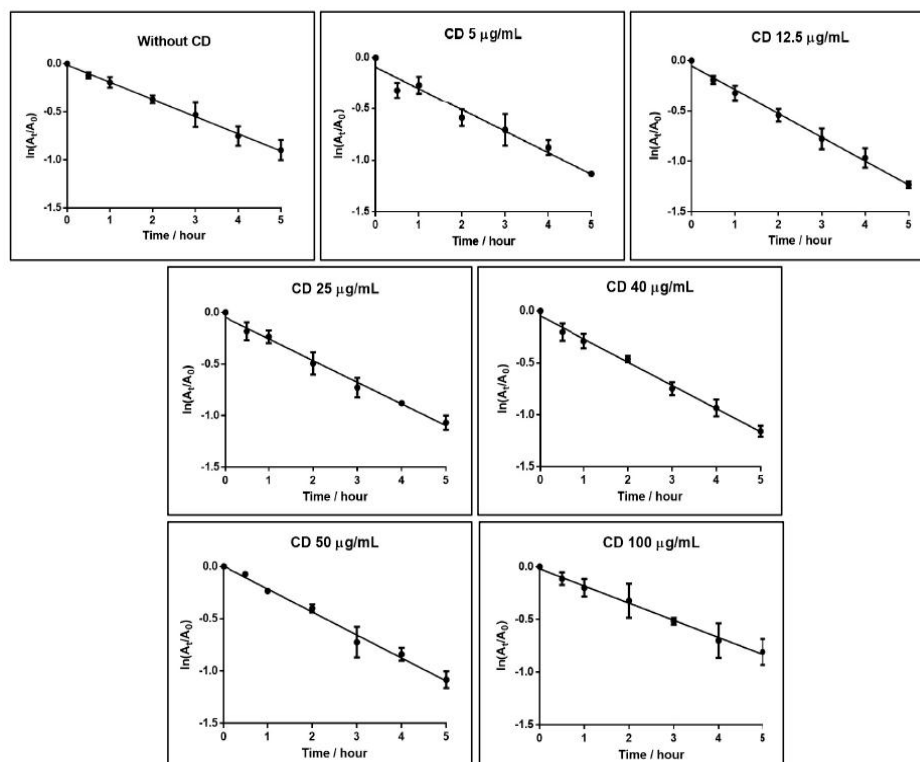


Figure 3. Linear fitting of $\ln(A_t/A_0)$ as a function of time (in hours) for the reaction mixtures composed of propylene oxide, pyridine, and CDs in aqueous solution.

The rate constant for each CD concentration is presented in Table 1, as well as the %Inc relative to the reaction without CDs, and %PC. When comparing the results in the presence and absence of the CDs, we can observe that adding this type of nanomaterial led to an overall increase in the reaction rate constant. The exception is for the highest concentration of CDs, which can potentially be justified by taking into account their typical aggregation behavior (associated with the typical surface composition of these materials [52,53]). Namely, by the presence of typical carboxyl, amine, and carbonyl groups, with the subsequent formation of electrostatic interactions among nanoparticles, as well as hydrogen bonding. Thus, it is possible that at higher concentrations, this aggregation occurs in a way that might limit the ability of CDs to act as HBDs in this reaction, resulting in lower catalytic activity. On the other hand, a $5 \mu\text{g/mL}$ concentration of CDs resulted in a catalytic activity lower than that obtained with $12.5 \mu\text{g/mL}$ of CDs. This could simply be because the lower concentration of CDs is not enough to yield a good enough catalytic activity. Finally, the correlation between the CD concentration and the increased efficiency is not linear. In fact, the concentration of $12.5 \mu\text{g/mL}$ yielded the best results, presenting a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$, a %PC of 70.8%, and a %Inc of 32.2%, whereas the second-best results were not obtained with either the first or third lowest concentration, as would be expected if the correlation was linear.

Table 1. Rate constant (k , h^{−1}), %Inc, and %PC for the reactions in the presence of different concentrations of CDs. All the samples were analyzed in triplicate. The fitting of catalytic reactions resulted in $R^2 > 0.98$.

	k (h ^{−1})	%Inc	%PC
Without CD	0.1786 ± 0.009		59.1
CDs 5 µg/mL	0.2093 ± 0.014	17.2	67.8
CDs 12.5 µg/mL	0.2361 ± 0.008	32.2	70.8
CDs 25 µg/mL	0.2101 ± 0.010	17.6	65.6
CDs 40 µg/mL	0.2233 ± 0.008	25.0	68.6
CDs 50 µg/mL	0.2201 ± 0.010	22.3	66.1
CDs 100 µg/mL	0.1629 ± 0.012	−8.8	55.2

Based on these results, it is clear that the CDs can act as an HBD in this type of reaction and provide better results over water molecules than HBDs. These results are in line with our previous work [50], since by XPS analysis it was found that the surface of CDs contained functional groups, such as hydroxyl and amine ones, which can act as HBDs. Considering this, we have decided to compare the enhancement effect obtained with the CDs with what could be achieved using other potential HBDs. To that end, we compared the catalytic activity of the CDs with three saccharides: glucose, fructose, and sucrose. These HBDs were chosen due to representing the type of carbon source used in the CDs and presenting functional groups capable of hydrogen bonding. In fact, saccharides were already used as catalysts in the cycloaddition of CO₂ to epoxides [22,32,39]. With this comparison, we aim to understand if the conversion of glucose in CDs results in a better catalytic activity than the direct use of glucose (and the other saccharides) as HBDs. Figure 4 presents the linear fitting of $\ln(A_t/A_0)$ as a function of time in the presence of the three saccharides and the optimal tested concentration of CDs (12.5 µg/mL).

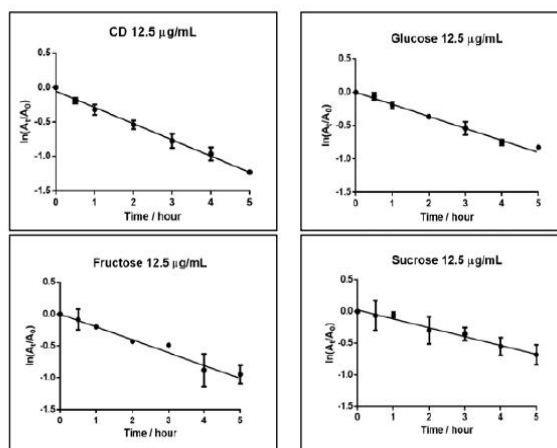


Figure 4. Linear fitting of $\ln(A_t/A_0)$ as a function of time (in hours) for the reaction mixtures composed of propylene oxide, pyridine, and HBD (CDs, glucose, fructose, and sucrose) in aqueous solution.

The rate constant, the %Inc, and %PC obtained for each fitting are present in Table 2.

The %Inc was calculated using the results obtained in the absence of CDs as the reference. The results indicate that the use of CDs promotes a better catalytic activity than the use of the saccharides by themselves. Using fructose, the best-performing saccharide, we obtained a %Inc of 13.0 and a %PC of 66.9, which are worse results than what was obtained using CDs but better than those obtained when just using water as an HBD. For glucose, the %Inc was 1.0, which is almost the same result obtained in the absence of CDs.

Finally, for sucrose, the %Inc was -21.7 and the %PC was 49.1 , indicating that using this saccharide instead of using only water actively worsens the catalytic performance. From these results, we can conclude that the conversion of glucose into CDs provides benefits in terms of catalytic activity, with the CDs presenting an enhanced catalytic activity compared to the use of other potential HBDs.

Table 2. Rate constant (k , h^{-1}), %Inc, and %PC, for the reactions with HBD (CDs, glucose, fructose, and sucrose). All the samples were analyzed in triplicate. The fitting of catalytic reactions resulted in $R^2 > 0.98$.

	$-k$ (h^{-1})	%INC	%PC
CD $12.5 \mu\text{g/mL}$	0.2361 ± 0.008	32.2	70.8
Glucose	0.1804 ± 0.008	1.0	56.2
Fructose	0.2019 ± 0.018	13.0	66.9
Sucrose	0.1399 ± 0.016	-21.7	49.1

Finally, we measured how the fluorescence of the CDs is affected by the ring-opening reaction of propylene oxide in aqueous solution. All the emission spectra were measured using an excitation wavelength of 290 nm . The fluorescence intensity was analyzed in four different scenarios: CDs in aqueous solution; CDs in aqueous solution with propylene oxide; CDs in aqueous solution with pyridine; CDs in aqueous solution with propylene oxide and pyridine (ring-opening reaction). The results are presented in Figure 5. As we can observe, the different components of the ring-opening reaction mixture do not affect the emission wavelength maximum ($\sim 460 \text{ nm}$) of the CDs over the course of the 5 h reaction period. Interestingly, in the presence of each individual component, the variation of the CD fluorescence intensity is quite limited. However, when in the presence of all of the components, we can observe a significant quenching of the fluorescence, indicating that the CDs interact with compounds generated during the ring-opening reaction. These further cements the participation of the CDs in the reaction to the role of an HBD.

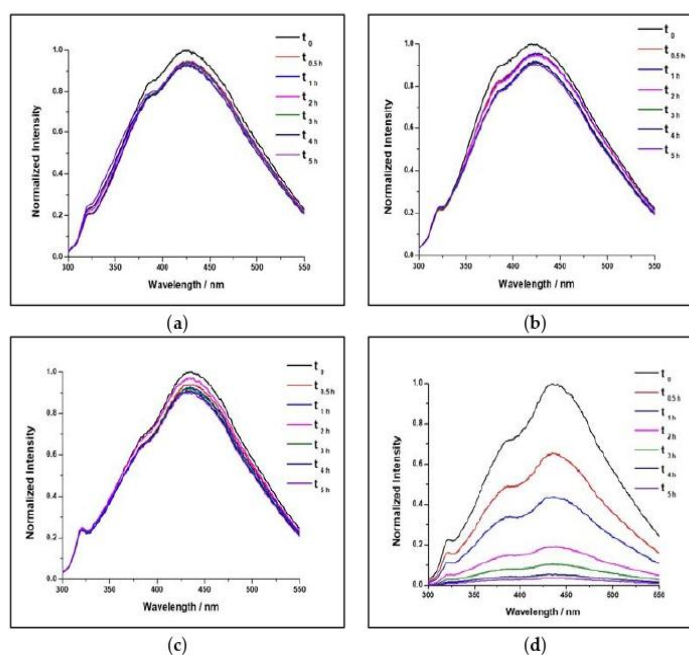


Figure 5. Emission spectra of (a) CDs in water; (b) CDs in water with 2 mmol of propylene oxide; (c) CDs in water with 10% mmol of pyridine; (d) complete reaction mixture during a 5 h reaction.

4. Conclusions

In this work, we have evaluated the potential of CDs to act as HBDs in two-component organocatalytic systems for the ring-opening reaction of epoxides (which can be considered a rate-limiting step in CO₂ conversion into heterocyclic carbonates). The CDs were prepared via dry heating of solid mixtures of glucose and urea, as a carbon precursor and an N-dopant (respectively). The CDs were tested using a model system composed of propylene oxide (used as a model epoxide) and pyridine (used as the nucleophile). The reactions that employed CDs, a low-cost and easy-to-prepare nanomaterial, took place in aqueous solution at room temperature. Pyridine was selected as a nucleophile, as its use generates a ring-opened epoxide-nucleophile structure that is stable enough for this ring-opening reaction to be properly monitored.

Our results demonstrated that CDs possess a significant catalytic activity that results in a substantial increase in reaction efficiency compared to the use of water and other molecules (e.g., saccharides) for the same purpose. More specifically, CDs showed a relevant catalytic performance, with a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$, a percentage of reactant conversion of 70.8%, and a rate constant enhancement of 32.2%. A comparison with alternative molecular HBDs (glucose, fructose, and sucrose) showed the superior performance of the studied nanomaterials and the benefits of converting glucose (carbon precursor) into CDs. This work demonstrated that CDs possess catalytic activity in the ring-opening reaction of epoxides, which shows their potential to be used as HBDs in the catalytic conversion of CO₂ into heterocyclic carbonates. Therefore, this work provides a basis on which future works concerning the incorporation of CO₂ into value-added products can be built.

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References

1. Solangi, N.H.; Anjum, A.; Tanjung, F.A.; Mazari, S.A.; Mubarak, N.M. A Review of Recent Trends and Emerging Perspectives of Ionic Liquid Membranes for CO₂ Separation. *J. Environ. Chem. Eng.* **2021**, *9*, 105860. [\[CrossRef\]](#)
2. de Kleijne, K.; Hanssen, S.V.; van Dinteren, L.; Huijbregts, M.A.J.; van Zelm, R.; de Coninck, H. Limits to Paris Compatibility of CO₂ Capture and Utilization. *One Earth* **2022**, *5*, 168–185. [\[CrossRef\]](#)
3. Yan, T.; Liu, H.; Zeng, Z.X.; Pan, W.G. Recent Progress of Catalysts for Synthesis of Cyclic Carbonates from CO₂ and Epoxides. *J. CO₂ Util.* **2023**, *68*, 102355. [\[CrossRef\]](#)
4. Liu, Q.; Wu, L.; Jackstell, R.; Beller, M. Using Carbon Dioxide as a Building Block in Organic Synthesis. *Nat. Commun.* **2015**, *6*, 5933. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Kumatabara, Y.; Okada, M.; Shirakawa, S. Triethylamine Hydroiodide as a Simple Yet Effective Bifunctional Catalyst for CO₂ Fixation Reactions with Epoxides under Mild Conditions. *ACS Sustain. Chem. Eng.* **2017**, *5*, 7295–7301. [\[CrossRef\]](#)
6. Kondratenko, E.V.; Mul, G.; Baltrusaitis, J.; Larrazábal, G.O.; Pérez-Ramírez, J. Status and Perspectives of CO₂ Conversion into Fuels and Chemicals by Catalytic, Photocatalytic and Electrocatalytic Processes. *Energy Environ. Sci.* **2013**, *6*, 3112–3135. [\[CrossRef\]](#)
7. Aresta, M.; Dibenedetto, A.; Angelini, A. Catalysis for the Valorization of Exhaust Carbon: From CO₂ to Chemicals, Materials, and Fuels. Technological Use of CO₂. *Chem. Rev.* **2014**, *114*, 1709–1742. [\[CrossRef\]](#)
8. Norouzi, F.; Abdolmaleki, A. Acidic Pyridinium Ionic Liquid: An Efficient Bifunctional Organocatalyst to Synthesis Carbonate from Atmospheric CO₂ and Epoxide. *Mol. Catal.* **2023**, *538*, 112988. [\[CrossRef\]](#)

9. Song, Q.-W.; Ma, R.; Liu, P.; Zhang, K.; He, L.-N. Recent Progress in CO₂ Conversion into Organic Chemicals by Molecular Catalysis. *Green Chem.* **2023**, *25*, 6538–6560. [\[CrossRef\]](#)
10. Toda, Y.; Komiyama, Y.; Kikuchi, A.; Suga, H. Tetraarylphosphonium Salt-Catalyzed Carbon Dioxide Fixation at Atmospheric Pressure for the Synthesis of Cyclic Carbonates. *ACS Catal.* **2016**, *6*, 6906–6910. [\[CrossRef\]](#)
11. Martin, C.; Fiorani, G.; Kleij, A.W. Recent Advances in the Catalytic Preparation of Cyclic Organic Carbonates. *ACS Catal.* **2015**, *5*, 1353–1370. [\[CrossRef\]](#)
12. Song, Q.-W.; Zhou, Z.-H.; He, L.-N. Efficient, Selective and Sustainable Catalysis of Carbon Dioxide. *Green Chem.* **2017**, *19*, 3707–3728. [\[CrossRef\]](#)
13. Sun, Q.; Jin, Y.; Aguila, B.; Meng, X.; Ma, S.; Xiao, F.-S. Porous Ionic Polymers as a Robust and Efficient Platform for Capture and Chemical Fixation of Atmospheric CO₂. *ChemSusChem* **2017**, *10*, 1160–1165. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Büttner, H.; Steinbauer, J.; Wulf, C.; Dindaroglu, M.; Schmalz, H.-G.; Werner, T. Organocatalyzed Synthesis of Oleochemical Carbonates from CO₂ and Renewables. *ChemSusChem* **2017**, *10*, 1076–1079. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Markewitz, P.; Kuckshinrichs, W.; Leitner, W.; Linssen, J.; Zapp, P.; Bongartz, R.; Schreiber, A.; Müller, T.E. Worldwide Innovations in the Development of Carbon Capture Technologies and the Utilization of CO₂. *Energy Environ. Sci.* **2012**, *5*, 7281–7305. [\[CrossRef\]](#)
16. Peters, M.; Köhler, B.; Kuckshinrichs, W.; Leitner, W.; Markewitz, P.; Müller, T.E. Chemical Technologies for Exploiting and Recycling Carbon Dioxide into the Value Chain. *ChemSusChem* **2011**, *4*, 1216–1240. [\[CrossRef\]](#)
17. Crowther, O.; Keeney, D.; Moureau, D.M.; Meyer, B.; Salomon, M.; Hendrickson, M. Electrolyte Optimization for the Primary Lithium Metal Air Battery Using an Oxygen Selective Membrane. *J. Power Sources* **2012**, *202*, 347–351. [\[CrossRef\]](#)
18. Sakakura, T.; Kohno, K. The Synthesis of Organic Carbonates from Carbon Dioxide. *Chem. Commun.* **2009**, *11*, 1312–1330. [\[CrossRef\]](#)
19. Dai, W.-L.; Luo, S.-L.; Yin, S.-F.; Au, C.-T. The Direct Transformation of Carbon Dioxide to Organic Carbonates over Heterogeneous Catalysts. *Appl. Catal. Gen.* **2009**, *366*, 2–12. [\[CrossRef\]](#)
20. Schäffner, B.; Schäffner, F.; Verevkin, S.P.; Börner, A. Organic Carbonates as Solvents in Synthesis and Catalysis. *Chem. Rev.* **2010**, *110*, 4554–4581. [\[CrossRef\]](#)
21. Sathish, M.; Sreeram, K.J.; Raghava Rao, J.; Unni Nair, B. Cyclic Carbonate: A Recyclable Medium for Zero Discharge Tanning. *ACS Sustain. Chem. Eng.* **2016**, *4*, 1032–1040. [\[CrossRef\]](#)
22. Arayachukiat, S.; Kongtes, C.; Barthel, A.; Vummaleti, S.V.C.; Poater, A.; Wannakao, S.; Cavallo, L.; D'Elia, V. Ascorbic Acid as a Bifunctional Hydrogen Bond Donor for the Synthesis of Cyclic Carbonates from CO₂ under Ambient Conditions. *ACS Sustain. Chem. Eng.* **2017**, *5*, 6392–6397. [\[CrossRef\]](#)
23. Schulz, M.; Kriegl, R.; Kämpfer, A. Assessment of CO₂ Stability and Oxygen Flux of Oxygen Permeable Membranes. *J. Membr. Sci.* **2011**, *378*, 10–17. [\[CrossRef\]](#)
24. Li, Z.-J.; Sun, J.-F.; Xu, Q.-Q.; Yin, J.-Z. Homogeneous and Heterogeneous Ionic Liquid System: Promising “Ideal Catalysts” for the Fixation of CO₂ into Cyclic Carbonates. *ChemCatChem* **2021**, *13*, 1848–1866. [\[CrossRef\]](#)
25. Keshri, K.S.; Bhattacharjee, S.; Singha, A.; Bhaumik, A.; Chowdhury, B. Synthesis of Cyclic Carbonates of Different Epoxides Using CO₂ as a C1 Building Block over Ag/TUD-1 Mesoporous Silica Catalyst: A Solvent Free Approach. *Mol. Catal.* **2022**, *522*, 112234. [\[CrossRef\]](#)
26. Fiorani, G.; Guo, W.; Kleij, A.W. Sustainable Conversion of Carbon Dioxide: The Advent of Organocatalysis. *Green Chem.* **2015**, *17*, 1375–1389. [\[CrossRef\]](#)
27. Cokoja, M.; Wilhelm, M.E.; Anthofer, M.H.; Herrmann, W.A.; Kühn, F.E. Synthesis of Cyclic Carbonates from Epoxides and Carbon Dioxide by Using Organocatalysts. *ChemSusChem* **2015**, *8*, 2436–2454. [\[CrossRef\]](#)
28. Stephan, D.W.; Erker, G. Frustrated Lewis Pair Chemistry: Development and Perspectives. *Angew. Chem. Int. Ed.* **2015**, *54*, 6400–6441. [\[CrossRef\]](#)
29. Bontemps, S. Boron-Mediated Activation of Carbon Dioxide. *Coord. Chem. Rev.* **2016**, *308*, 117–130. [\[CrossRef\]](#)
30. Song, J.; Zhang, Z.; Han, B.; Hu, S.; Li, W.; Xie, Y. Synthesis of Cyclic Carbonates from Epoxides and CO₂ Catalyzed by Potassium Halide in the Presence of β -Cyclodextrin. *Green Chem.* **2008**, *10*, 1337–1341. [\[CrossRef\]](#)
31. Wang, J.-Q.; Sun, J.; Shi, C.-Y.; Cheng, W.-G.; Zhang, X.-P.; Zhang, S.-J. Synthesis of Dimethyl Carbonate from CO₂ and Ethylene Oxide Catalyzed by K₂CO₃-Based Binary Salts in the Presence of H₂O. *Green Chem.* **2011**, *13*, 3213–3217. [\[CrossRef\]](#)
32. Liang, S.; Liu, H.; Jiang, T.; Song, J.; Yang, G.; Han, B. Highly Efficient Synthesis of Cyclic Carbonates from CO₂ and Epoxides over Cellulose/KI. *Chem. Commun.* **2011**, *47*, 2131–2133. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Takahashi, T.; Watahiki, T.; Kitazume, S.; Yasuda, H.; Sakakura, T. Synergistic Hybrid Catalyst for Cyclic Carbonate Synthesis: Remarkable Acceleration Caused by Immobilization of Homogeneous Catalyst on Silica. *Chem. Commun.* **2006**, *15*, 1664–1666. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Pinto da Silva, L. Theoretical Study of the Ring-Opening of Epoxides Catalyzed by Boronic Acids and Pyridinic Bases. *J. Phys. Chem. C* **2017**, *121*, 16300–16307. [\[CrossRef\]](#)
35. Alves, M.; Grignard, B.; Gennen, S.; Mereau, R.; Detrembleur, C.; Jerome, C.; Tassaing, T. Organocatalytic Promoted Coupling of Carbon Dioxide with Epoxides: A Rational Investigation of the Cocatalytic Activity of Various Hydrogen Bond Donors. *Catal. Sci. Technol.* **2015**, *5*, 4636–4643. [\[CrossRef\]](#)

36. Wang, L.; Zhang, G.; Kodama, K.; Hirose, T. An Efficient Metal- and Solvent-Free Organocatalytic System for Chemical Fixation of CO₂ into Cyclic Carbonates under Mild Conditions. *Green Chem.* **2016**, *18*, 1229–1233. [\[CrossRef\]](#)
37. Orr, F.M., Jr. CO₂ Capture and Storage: Are We Ready? *Energy Environ. Sci.* **2009**, *2*, 449–458. [\[CrossRef\]](#)
38. Wang, J.; Zhang, Y. Boronic Acids as Hydrogen Bond Donor Catalysts for Efficient Conversion of CO₂ into Organic Carbonate in Water. *ACS Catal.* **2016**, *6*, 4871–4876. [\[CrossRef\]](#)
39. Pinto da Silva, L. Mechanistic Study of the Role of Hydrogen Bond Donors in the Two-Component Organocatalysis of the Ring-Opening Reaction of Epoxides. *Mol. Catal.* **2019**, *474*, 110425. [\[CrossRef\]](#)
40. Lim, S.Y.; Shen, W.; Gao, Z. Carbon Quantum Dots and Their Applications. *Chem. Soc. Rev.* **2014**, *44*, 362–381. [\[CrossRef\]](#)
41. Crista, D.M.A.; El Mragui, A.; Algarra, M.; Esteves da Silva, J.C.G.; Luque, R.; Pinto da Silva, L. Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots. *Nanomaterials* **2020**, *10*, 1209. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Kang, Z.; Lee, S.-T. Carbon Dots: Advances in Nanocarbon Applications. *Nanoscale* **2019**, *11*, 19214–19224. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Esteves da Silva, J.C.G.; Gonçalves, H.M.R. Analytical and Bioanalytical Applications of Carbon Dots. *TrAC Trends Anal. Chem.* **2011**, *30*, 1327–1336. [\[CrossRef\]](#)
44. Xiong, Y.; Schneider, J.; Ushakova, E.V.; Rogach, A.L. Influence of Molecular Fluorophores on the Research Field of Chemically Synthesized Carbon Dots. *Nano Today* **2018**, *23*, 124–139. [\[CrossRef\]](#)
45. Wang, R.; Lu, K.-Q.; Tang, Z.-R.; Xu, Y.-J. Recent Progress in Carbon Quantum Dots: Synthesis, Properties and Applications in Photocatalysis. *J. Mater. Chem. A* **2017**, *5*, 3717–3734. [\[CrossRef\]](#)
46. Barman, M.K.; Patra, A. Current Status and Prospects on Chemical Structure Driven Photoluminescence Behaviour of Carbon Dots. *J. Photochem. Photobiol. C Photochem. Rev.* **2018**, *37*, 1–22. [\[CrossRef\]](#)
47. Baker, S.N.; Baker, G.A. Luminescent Carbon Nanodots: Emergent Nanolights. *Angew. Chem. Int. Ed.* **2010**, *49*, 6726–6744. [\[CrossRef\]](#)
48. Sun, Y.-P.; Zhou, B.; Lin, Y.; Wang, W.; Fernando, K.A.S.; Pathak, P.; Meziani, M.J.; Harruff, B.A.; Wang, X.; Wang, H.; et al. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence. *J. Am. Chem. Soc.* **2006**, *128*, 7756–7757. [\[CrossRef\]](#)
49. Crista, D.M.A.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Evaluation of Different Bottom-up Routes for the Fabrication of Carbon Dots. *Nanomaterials* **2020**, *10*, 1316. [\[CrossRef\]](#)
50. Crista, D.; Algarra, M.; Martínez De Yuso, M.V.; Esteves Da Silva, J.C.G.; Pinto Da Silva, L. Investigation of the Role of pH and the Stoichiometry of the N-Dopant in the Luminescence, Composition and Synthesis Yield of Carbon Dots. *J. Mater. Chem. B* **2023**, *11*, 1131–1143. [\[CrossRef\]](#)
51. Cardoso, R.M.F.; Cardoso, I.M.F.; da Silva, L.P.; Esteves da Silva, J.C.G. Copper(II)-Doped Carbon Dots as Catalyst for Ozone Degradation of Textile Dyes. *Nanomaterials* **2022**, *12*, 1211. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Algarra, M.; Cuevas, A.L.; de Yuso, M.V.M.; Benavente, J. Insights into the Formation of an Emissive CdTe-Quantum-Dots/Cellulose Hybrid Film. *J. Colloid Interface Sci.* **2020**, *579*, 714–722. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Cuevas, A.; Campos, B.B.; Romero, R.; Algarra, M.; Vázquez, M.I.; Benavente, J. Eco-Friendly Modification of a Regenerated Cellulose Based Film by Silicon, Carbon and N-Doped Carbon Quantum Dots. *Carbohydr. Polym.* **2019**, *206*, 238–244. [\[CrossRef\]](#) [\[PubMed\]](#)

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5.3. Conclusions

In this study, we investigated the potential of CDs to serve as HBD in two-component organocatalytic systems for the ring-opening reaction of epoxides, a limited step in the conversion of carbon dioxide into heterocyclic carbonates. CDs were synthesized via the dry heating method using solid mixtures of glucose and urea as the carbon precursor and nitrogen dopant, respectively. The catalytic performance of CDs was assessed using propylene oxide as a model epoxide and pyridine as the nucleophile in an aqueous solution at room temperature. Pyridine was chosen due to its ability to form a stable ring-opened epoxide-nucleophile structure, facilitating proper monitoring of the reaction.

Our findings revealed that CDs exhibit significant catalytic activity, leading to a marked improvement in reaction efficiency compared to water and other molecules such as saccharides. Specifically, CDs demonstrated notable catalytic performance with a rate constant of $0.2361 \pm 0.008 \text{ h}^{-1}$, a reactant conversion percentage of 70.8 %, and a rate constant enhancement of 32.2 %. Comparative analysis with alternative molecular HBD (glucose, fructose, and sucrose) underscored the superior performance of CDs, highlighting the advantages of converting glucose into CDs.

This study underscores the catalytic activity of CDs in the ring-opening reaction of epoxides, suggesting their potential as HBD in the catalytic conversion of CO₂ into heterocyclic carbonates. Consequently, our findings lay the groundwork for future research endeavors aimed at integrating CO₂ into high-value products.

6. Conclusion and Future Perspectives

6.1. Conclusions

CO₂ emissions have become a focal point in climate change and environmental sustainability discussions. With rapid industrialization and increased reliance on fossil fuels, human activities have led to a substantial rise in atmospheric CO₂ levels. This influx of CO₂ contributes significantly to the greenhouse effect, trapping heat within the Earth's atmosphere and leading to global warming and associated environmental consequences such as rising sea levels, extreme weather events, and habitat degradation.

Amidst growing concerns about CO₂ emissions and their impacts, there has been a burgeoning interest in developing novel technologies to mitigate carbon emissions and utilize CO₂ as a valuable resource. One such innovation lies in the realm of CDs, which are emerging as promising nanomaterials with diverse applications spanning environmental, biomedical, and catalytic domains.

Carbon dots are nanoscale carbon-based materials with unique optical properties, including strong fluorescence and excellent photostability. These properties make CDs highly attractive for various applications, including bioimaging, sensing, drug delivery, and catalysis.

In this context, the exploration of carbon dots represents not only a technological advancement but also a step toward addressing the pressing challenges of climate change and environmental degradation. By harnessing the versatility of carbon dots, researchers aim to develop innovative strategies for carbon capture, renewable energy generation, and sustainable materials production, paving the way toward a greener and more sustainable future.

In summary, in this thesis, we did a comprehensive exploration of various aspects of CDs, offering insights into their synthesis, structural properties, optical characteristics, environmental implications, and functional applications.

Firstly, using different precursors, including SCGs, citric acid, urea, glucose, and combinations thereof, highlighted the versatility of CD fabrication methods and their impact on yield, quantum efficiency, and environmental sustainability.

Secondly, a comparative analysis of different synthesis techniques, including hydrothermal, microwave-assisted, calcination, and dry heating methods, provided valuable insights into their respective efficiencies, synthesis yields, nitrogen-doping capabilities, and environmental impacts. These findings not only elucidated optimal synthesis routes but also underscored the importance of considering both environmental sustainability and functional efficiency in CD production. Despite variations in synthesis routes and precursor materials, the resultant CDs exhibited promising luminescent properties, with potential applications ranging from sensing probes to biomedical imaging agents.

Furthermore, investigations into nitrogen-doped CDs unveiled distinct variants with unique solubility profiles, thermal stabilities, and quantum yields, shedding light on the diverse range of CD properties achievable through controlled synthesis methodologies. The study also explored the potential correlation between nitrogen doping and fluorescence quantum yield, offering valuable guidance for future endeavors in CD optimization.

Lastly, the catalytic activity of CDs in the ring-opening reaction of epoxides highlighted their potential as HBD in organocatalytic systems for converting carbon dioxide into heterocyclic carbonates. This discovery not only demonstrated the practical utility of CDs in sustainable chemical transformations but also laid the groundwork for innovative approaches to carbon capture and utilization.

Overall, this multidisciplinary investigation contributes to the evolving understanding of CDs as versatile nanomaterials with diverse applications across environmental, biomedical, and catalytic domains, emphasizing the importance of holistic approaches to their synthesis, characterization, and utilization in advancing sustainable technologies.

6.2. Future Perspectives

The catalytic activity of CDs in the ring-opening reaction of epoxides highlighted their potential as HBD in organocatalytic systems for converting carbon dioxide into heterocyclic carbonates. Due to that as future work, it's important to explore this catalytic activity in the presence of CO₂ with the identification and quantification of the reaction products using gas chromatography coupled with mass spectroscopy. The reaction mixtures will be composed of a solution containing the CDs, the epoxide, and a nucleophile, to which CO₂ will be added.

It is also of great interest to synthesize CDs that can act not only as HBD but also as nucleophiles.

Also, the CDs that allow the highest yields of CO₂ recycling, in mild conditions, can be subjected to recovery assays. These organocatalysts can be recovered and will then be used in further CO₂ conversion reactions. After this, the selected CDs will be once again recovered and used in new CO₂ conversion reactions

References

1. Eyring, V.; Gillett, N.P.; Achutarao, K.M.; Barimalala, R.; Barreiro Parrillo, M.; Bellouin, N.; Cassou, C.; Durack, P.J.; Kosaka, Y.; McGregor, S.; et al. Human Influence on the Climate System. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, Ö., Yu, R., Zhou, B., Eds.; Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, **2021**; pp. 423–552.
2. Gulev, S.K.; Thorne, P.W.; Ahn, J.; Dentener, F.J.; Domingues, C.M.; Gerland, S.; Gong, D.; Kaufman, D.S.; Nnamchi, H.C.; Quaas, J.; et al. Changing State of the Climate System. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, Ö., Yu, R., Zhou, B., Eds.; Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, **2021**; pp. 287–422.
3. Hansen, J.; Sato, M.; Ruedy, R.; Lo, K.; Lea, D.W.; Medina-Elizade, M. Global Temperature Change. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 14288–14293, doi:10.1073/pnas.0606291103.
4. Trenberth, K.E.; Dai, A.; van der Schrier, G.; Jones, P.D.; Barichivich, J.; Briffa, K.R.; Sheffield, J. Global Warming and Changes in Drought. *Nature Clim Change* **2014**, *4*, 17–22, doi:10.1038/nclimate2067.
5. WG1AR5_Chapter13_FINAL.Pdf.
6. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation — IPCC.
7. Doney, S.C.; Fabry, V.J.; Feely, R.A.; Kleypas, J.A. Ocean Acidification: The Other CO₂ Problem. *Annual Review of Marine Science* **2009**, *1*, 169–192, doi:10.1146/annurev.marine.010908.163834.
8. Thomas, C.D.; Cameron, A.; Green, R.E.; Bakkenes, M.; Beaumont, L.J.; Collingham, Y.C.; Erasmus, B.F.N.; de Siqueira, M.F.; Grainger, A.; Hannah, L.; et al.

Extinction Risk from Climate Change. *Nature* **2004**, 427, 145–148, doi:10.1038/nature02121.

9. Lobell, D.B. Climate Change Adaptation in Crop Production: Beware of Illusions. *Global Food Security* **2014**, 3, 72–76, doi:10.1016/j.gfs.2014.05.002.

10. Haines, A.; McMichael, A.J.; Smith, K.R.; Roberts, I.; Woodcock, J.; Markandya, A.; Armstrong, B.G.; Campbell-Lendrum, D.; Dangour, A.D.; Davies, M.; et al. Public Health Benefits of Strategies to Reduce Greenhouse-Gas Emissions: Overview and Implications for Policy Makers. *Lancet* **2009**, 374, 2104–2114, doi:10.1016/S0140-6736(09)61759-1.

11. Solomon, S.; Plattner, G.-K.; Knutti, R.; Friedlingstein, P. Irreversible Climate Change Due to Carbon Dioxide Emissions. *Proceedings of the National Academy of Sciences* **2009**, 106, 1704–1709, doi:10.1073/pnas.0812721106.

12. World Energy Outlook 2022 – Analysis - IEA Available online: <https://www.iea.org/reports/world-energy-outlook-2022> (accessed on 22 May 2024).

13. Figueres, C.; Le Quéré, C.; Mahindra, A.; Bäte, O.; Whiteman, G.; Peters, G.; Guan, D. Emissions Are Still Rising: Ramp up the Cuts. *Nature* **2018**, 564, 27–30, doi:10.1038/d41586-018-07585-6.

14. Global Status of CCS Report: 2018 Available online: <https://www.globalccsinstitute.com/resources/publications-reports-research/global-status-of-ccs-report-2018/> (accessed on 21 May 2024).

15. BP Energy Outlook – 2018 Edition. **2018**.

16. NOC Contributes to Latest IPCC Report Assessing Impacts of Climate Change | National Oceanography Centre Available online: https://noc.ac.uk/news/noc-contributes-latest-ipcc-report-assessing-impacts-climate-change?gad_source=1&gclid=Cj0KCQjwjLGyBhCYARIsAPqTz19L2O5Q7LocsqepqLZn-JcahfhtkC4VTIKdNfrGinjh0YihtzISUaAiG9EALw_wcB (accessed on 21 May 2024).

17. Crista, D.M.A.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Application of Fluorescent Carbon Dots as Catalysts for the Ring-Opening Reaction of Epoxides. *Materials* **2023**, 16, 7620, doi:10.3390/ma16247620.

18. Yan, J.; Zhang, Z. Carbon Capture, Utilization and Storage (CCUS). *Applied Energy* **2019**, 235, 1289–1299, doi:10.1016/j.apenergy.2018.11.019.

19. Vega, F.; Baena-Moreno, F.M.; Gallego Fernández, L.M.; Portillo, E.; Navarrete, B.; Zhang, Z. Current Status of CO₂ Chemical Absorption Research Applied to CCS: Towards Full Deployment at Industrial Scale. *Applied Energy* **2020**, *260*, 114313, doi:10.1016/j.apenergy.2019.114313.
20. Second State of the Carbon Cycle Report Available online: <https://carbon2018.globalchange.gov/> (accessed on 21 May 2024).
21. Special Report on the Ocean and Cryosphere in a Changing Climate —.
22. Zhang, Z.; Pan, S.-Y.; Li, H.; Cai, J.; Olabi, A.G.; Anthony, E.J.; Manovic, V. Recent Advances in Carbon Dioxide Utilization. *Renewable and Sustainable Energy Reviews* **2020**, *125*, 109799, doi:10.1016/j.rser.2020.109799.
23. Putting CO₂ to Use – Analysis Available online: <https://www.iea.org/reports/putting-co2-to-use> (accessed on 22 May 2024).
24. Perspectives on Advancing Sustainable CO₂ Conversion Processes: Trinomial Technology, Environment, and Economy | ACS Sustainable Chemistry & Engineering Available online: <https://pubs.acs.org/doi/10.1021/acssuschemeng.3c07133?ref=PDF> (accessed on 22 May 2024).
25. Driving CO₂ Emissions to Zero (and beyond) with Carbon Capture, Use, and Storage | McKinsey Available online: <https://www.mckinsey.com/capabilities/sustainability/our-insights/driving-co2-emissions-to-zero-and-beyond-with-carbon-capture-use-and-storage#/> (accessed on 23 May 2024).
26. National Algal Biofuels Technology Roadmap.
27. Liang, Y. Producing Liquid Transportation Fuels from Heterotrophic Microalgae. *Applied Energy* **2013**, *104*, 860–868, doi:10.1016/j.apenergy.2012.10.067.
28. Naraharisetti, P.K.; Yeo, T.Y.; Bu, J. New Classification of CO₂ Mineralization Processes and Economic Evaluation. *Renewable and Sustainable Energy Reviews* **2019**, *99*, 220–233.
29. Ding, J.; Ye, R.; Fu, Y.; He, Y.; Wu, Y.; Zhang, Y.; Zhong, Q.; Kung, H.H.; Fan, M. Direct Synthesis of Urea from Carbon Dioxide and Ammonia. *Nat Commun* **2023**, *14*, 4586, doi:10.1038/s41467-023-40351-5.
30. El Sibai, A.; Rihko-Struckmann, L.; Sundmacher, K. Synthetic Methane from CO₂: Dynamic Optimization of the Sabatier Process for Power-to-Gas Applications. In

Computer Aided Chemical Engineering; Gernaey, K.V., Huusom, J.K., Gani, R., Eds.; 12 International Symposium on Process Systems Engineering and 25 European Symposium on Computer Aided Process Engineering; Elsevier, 2015; Vol. 37, pp. 1157–1162.

31. Lin, X.; Foo, J.; Ong, W.-J. Unveiling Environmental Impacts of Methanol Production via Electrocatalysis against Conventional and Thermochemical Routes by Life Cycle Assessment. *Sustainable Materials and Technologies* **2023**, 37, e00663, doi:10.1016/j.susmat.2023.e00663.

32. CO₂ Conversion via Reverse Water Gas Shift Reaction Using Fully Selective Mo–P Multicomponent Catalysts | Industrial & Engineering Chemistry Research Available online: <https://pubs.acs.org/doi/10.1021/acs.iecr.2c00305> (accessed on 23 May 2024).

33. Kirsch, H.; Sommer, U.; Pfeifer, P.; Dittmeyer, R. Power-to-Fuel Conversion Based on Reverse Water-Gas-Shift, Fischer-Tropsch Synthesis and Hydrocracking: Mathematical Modeling and Simulation in Matlab/Simulink. *Chemical Engineering Science* **2020**, 227, 115930, doi:10.1016/j.ces.2020.115930.

34. Zha, B.; Li, C.; Li, J. Efficient Electrochemical Reduction of CO₂ into Formate and Acetate in Polyoxometalate Catholyte with Indium Catalyst. *Journal of Catalysis* **2020**, 382, 69–76, doi:10.1016/j.jcat.2019.12.010.

35. Yaashikaa, P.R.; Kumar, P.S.; Varjani, S.J.; Saravanan, A. A Review on Photochemical, Biochemical and Electrochemical Transformation of CO₂ into Value-Added Products. *Journal of CO₂ Utilization* **2019**, 33, 131–147, doi:10.1016/j.jcou.2019.05.017.

36. Grim, R.G.; Iii, J.F.; Huang, Z.; Tao, L.; Resch, M. The Feasibility of Direct CO₂ Conversion Technologies on Impacting Mid-Century Climate Goals. *Joule* **2023**, 7, 1684–1699, doi:10.1016/j.joule.2023.07.008.

37. Karatok, M.; Ngan, H.T.; Jia, X.; O'Connor, C.R.; Boscoboinik, J.A.; Stacchiola, D.J.; Sautet, P.; Madix, R.J. Achieving Ultra-High Selectivity to Hydrogen Production from Formic Acid on Pd-Ag Alloys. *J Am Chem Soc* **2023**, 145, 5114–5124, doi:10.1021/jacs.2c11323.

38. Chen, Y.; Xu, C.; Vaidyanathan, S. Influence of Gas Management on Biochemical Conversion of CO₂ by Microalgae for Biofuel Production. *Applied Energy* **2020**, 261, 114420, doi:10.1016/j.apenergy.2019.114420.

39. Goli, A.; Shamiri, A.; Talaiekhosani, A.; Eshtiaghi, N.; Aghamohammadi, N.; Aroua, M.K. An Overview of Biological Processes and Their Potential for CO₂ Capture. *J Environ Manage* **2016**, *183*, 41–58, doi:10.1016/j.jenvman.2016.08.054.
40. Gupta, R.; Mishra, A.; Thirupathaiah, Y.; Chandel, A. Biochemical Conversion of CO₂ in Fuels and Chemicals: Status, Innovation, and Industrial Aspects. *Biomass Conversion and Biorefinery* **2022**, *14*, doi:10.1007/s13399-022-02552-8.
41. Appel, A.M.; Bercaw, J.E.; Bocarsly, A.B.; Dobbek, H.; DuBois, D.L.; Dupuis, M.; Ferry, J.G.; Fujita, E.; Hille, R.; Kenis, P.J.A.; et al. Frontiers, Opportunities, and Challenges in Biochemical and Chemical Catalysis of CO₂ Fixation. *Chem Rev* **2013**, *113*, 6621–6658, doi:10.1021/cr300463y.
42. Xu, L.; Weathers, P.; Xiong, X.-R.; Liu, C.-Z. Microalgal Bioreactors: Challenges and Opportunities. *Engineering in Life Sciences* **2009**, *9*, 178–189, doi:10.1002/elsc.200800111.
43. Yuan, J.; Hao, C. Solar-Driven Photoelectrochemical Reduction of Carbon Dioxide to Methanol at CuInS₂ Thin Film Photocathode. *Solar Energy Materials and Solar Cells* **2013**, *108*, 170–174, doi:10.1016/j.solmat.2012.09.024.
44. Kočí, K.; Obalová, L.; Matějová, L.; Plachá, D.; Lacný, Z.; Jirkovský, J.; Šolcová, O. Effect of TiO₂ Particle Size on the Photocatalytic Reduction of CO₂. *Applied Catalysis B: Environmental* **2009**, *89*, 494–502, doi:10.1016/j.apcatb.2009.01.010.
45. Rasheed, T.; Shafi, S.; Anwar, M.T.; Rizwan, K.; Ahmad, T.; Bilal, M. Revisiting Photo and Electro-Catalytic Modalities for Sustainable Conversion of CO₂. *Applied Catalysis A: General* **2021**, *623*, 118248, doi:10.1016/j.apcata.2021.118248.
46. Wang, J.; Hao, C.; Zhang, Q.; Meng, Q.; Liu, H. Research Advances on Photo-Assisted CO₂ Conversion to Methanol. *Applied Catalysis A: General* **2022**, *643*, 118738, doi:10.1016/j.apcata.2022.118738.
47. Yap, D.; Tatibouët, J.-M.; Batiot-Dupeyrat, C. Carbon Dioxide Dissociation to Carbon Monoxide by Non-Thermal Plasma. *Journal of CO₂ Utilization* **2015**, *12*, 54–61, doi:10.1016/j.jcou.2015.07.002.
48. Bacariza, M.C.; Biset-Peiró, M.; Graça, I.; Guilera, J.; Morante, J.; Lopes, J.M.; Andreu, T.; Henriques, C. DBD Plasma-Assisted CO₂ Methanation Using Zeolite-Based Catalysts: Structure Composition-Reactivity Approach and Effect of Ce as Promoter. *Journal of CO₂ Utilization* **2018**, *26*, 202–211, doi:10.1016/j.jcou.2018.05.013.

49. Snoeckx, R.; Bogaerts, A. Plasma Technology – a Novel Solution for CO₂ Conversion? *Chem. Soc. Rev.* **2017**, *46*, 5805–5863, doi:10.1039/C6CS00066E.
50. Bogaerts, A.; Centi, G. Plasma Technology for CO₂ Conversion: A Personal Perspective on Prospects and Gaps. *Front. Energy Res.* **2020**, *8*, doi:10.3389/fenrg.2020.00111.
51. Austin, N.; Butina, B.; Mpourmpakis, G. CO₂ Activation on Bimetallic CuNi Nanoparticles. *Progress in Natural Science: Materials International* **2016**, *26*, 487–492, doi:10.1016/j.pnsc.2016.08.007.
52. Masel, R.I.; Liu, Z.; Yang, H.; Kaczur, J.J.; Carrillo, D.; Ren, S.; Salvatore, D.; Berlinguette, C.P. An Industrial Perspective on Catalysts for Low-Temperature CO₂ Electrolysis. *Nat. Nanotechnol.* **2021**, *16*, 118–128, doi:10.1038/s41565-020-00823-x.
53. Das, S.; Pérez-Ramírez, J.; Gong, J.; Dewangan, N.; Hidajat, K.; Gates, B.C.; Kawi, S. Core–Shell Structured Catalysts for Thermocatalytic, Photocatalytic, and Electrocatalytic Conversion of CO₂. *Chem. Soc. Rev.* **2020**, *49*, 2937–3004, doi:10.1039/C9CS00713J.
54. Singh, G.; Lee, J.; Karakoti, A.; Bahadur, R.; Yi, J.; Zhao, D.; AlBahily, K.; Vinu, A. Emerging Trends in Porous Materials for CO₂ Capture and Conversion. *Chem. Soc. Rev.* **2020**, *49*, 4360–4404, doi:10.1039/D0CS00075B.
55. Wang, D.; Bi, Q.; Yin, G.; Wang, P.; Huang, F.; Xie, X.; Jiang, M. Photochemical Preparation of Anatase Titania Supported Gold Catalyst for Ethanol Synthesis from CO₂ Hydrogenation. *Catal Lett* **2018**, *148*, 11–22, doi:10.1007/s10562-017-2192-4.
56. Wang, J.; Han, B.; Nie, R.; Xu, Y.; Yu, X.; Dong, Y.; Wang, J.; Jing, H. Photoelectrocatalytic Reduction of CO₂ to Chemicals via ZnO@Nickel Foam: Controlling C–C Coupling by Ligand or Morphology. *Top Catal* **2018**, *61*, 1563–1573, doi:10.1007/s11244-018-1018-y.
57. Dong, W.-H.; Wu, D.-D.; Luo, J.-M.; Xing, Q.-J.; Liu, H.; Zou, J.-P.; Luo, X.-B.; Min, X.-B.; Liu, H.-L.; Luo, S.-L.; et al. Coupling of Photodegradation of RhB with Photoreduction of CO₂ over rGO/SrTi_{0.95}Fe_{0.05}O_{3-δ} Catalyst: A Strategy for One-Pot Conversion of Organic Pollutants to Methanol and Ethanol. *Journal of Catalysis* **2017**, *349*, 218–225, doi:10.1016/j.jcat.2017.02.004.
58. Zhang, W.; Mohamed, A.R.; Ong, W.-J. Z-Scheme Photocatalytic Systems for Carbon Dioxide Reduction: Where Are We Now? *Angewandte Chemie International Edition* **2020**, *59*, 22894–22915, doi:10.1002/anie.201914925.

59. Wang, Z.; Song, H.; Liu, H.; Ye, J. Coupling of Solar Energy and Thermal Energy for Carbon Dioxide Reduction: Status and Prospects. *Angewandte Chemie International Edition* **2020**, *59*, 8016–8035, doi:10.1002/anie.201907443.
60. Jeyalakshmi, V.; Tamilmani, S.; Mahalakshmy, R.; Bhyrappa, P.; Krishnamurthy, K.R.; Viswanathan, B. Sensitization of La Modified NaTaO₃ with Cobalt Tetra Phenyl Porphyrin for Photo Catalytic Reduction of CO₂ by Water with UV–Visible Light. *Journal of Molecular Catalysis A: Chemical* **2016**, *420*, 200–207, doi:10.1016/j.molcata.2016.04.027.
61. Liu, H.; Meng, X.; Dao, T.D.; Zhang, H.; Li, P.; Chang, K.; Wang, T.; Li, M.; Nagao, T.; Ye, J. Conversion of Carbon Dioxide by Methane Reforming under Visible-Light Irradiation: Surface-Plasmon-Mediated Nonpolar Molecule Activation. *Angewandte Chemie* **2015**, *127*, 11707–11711, doi:10.1002/ange.201504933.
62. Liu, H.; Li, M.; Dao, T.D.; Liu, Y.; Zhou, W.; Liu, L.; Meng, X.; Nagao, T.; Ye, J. Design of PdAu Alloy Plasmonic Nanoparticles for Improved Catalytic Performance in CO₂ Reduction with Visible Light Irradiation. *Nano Energy* **2016**, *26*, 398–404, doi:10.1016/j.nanoen.2016.05.045.
63. Liu, H.; Dao, T.D.; Liu, L.; Meng, X.; Nagao, T.; Ye, J. Light Assisted CO₂ Reduction with Methane over Group VIII Metals: Universality of Metal Localized Surface Plasmon Resonance in Reactant Activation. *Applied Catalysis B: Environmental* **2017**, *209*, 183–189, doi:10.1016/j.apcatb.2017.02.080.
64. Zhao, Y.; Wei, Y.; Wu, X.; Zheng, H.; Zhao, Z.; Liu, J.; Li, J. Graphene-Wrapped Pt/TiO₂ Photocatalysts with Enhanced Photogenerated Charges Separation and Reactant Adsorption for High Selective Photoreduction of CO₂ to CH₄. *Applied Catalysis B: Environmental* **2018**, *226*, 360–372, doi:10.1016/j.apcatb.2017.12.071.
65. Li, X.; Sun, Y.; Xu, J.; Shao, Y.; Wu, J.; Xu, X.; Pan, Y.; Ju, H.; Zhu, J.; Xie, Y. Selective Visible-Light-Driven Photocatalytic CO₂ Reduction to CH₄ Mediated by Atomically Thin CuIn₅S₈ Layers. *Nat Energy* **2019**, *4*, 690–699, doi:10.1038/s41560-019-0431-1.
66. Wu, Y.A.; McNulty, I.; Liu, C.; Lau, K.C.; Liu, Q.; Paulikas, A.P.; Sun, C.-J.; Cai, Z.; Guest, J.R.; Ren, Y.; et al. Facet-Dependent Active Sites of a Single Cu₂O Particle Photocatalyst for CO₂ Reduction to Methanol. *Nat Energy* **2019**, *4*, 957–968, doi:10.1038/s41560-019-0490-3.

67. Chen, G.; Waterhouse, G.I.N.; Shi, R.; Zhao, J.; Li, Z.; Wu, L.-Z.; Tung, C.-H.; Zhang, T. From Solar Energy to Fuels: Recent Advances in Light-Driven C1 Chemistry. *Angewandte Chemie International Edition* **2019**, *58*, 17528–17551, doi:10.1002/anie.201814313.
68. Singh, M.R.; Bell, A.T. Design of an Artificial Photosynthetic System for Production of Alcohols in High Concentration from CO₂. *Energy Environ. Sci.* **2016**, *9*, 193–199, doi:10.1039/C5EE02783G.
69. Sans, J.; Sanz, V.; Turon, P.; Alemán, C. Enhanced CO₂ Conversion into Ethanol by Permanently Polarized Hydroxyapatite through C–C Coupling. *ChemCatChem* **2021**, *13*, 5025–5033, doi:10.1002/cctc.202101157.
70. Usubharatana, P.; McMartin, D.; Veawab, A.; Tontiwachwuthikul, P. Photocatalytic Process for CO₂ Emission Reduction from Industrial Flue Gas Streams. *Ind. Eng. Chem. Res.* **2006**, *45*, 2558–2568, doi:10.1021/ie0505763.
71. Kumaravel, V.; Bartlett, J.; Pillai, S.C. Photoelectrochemical Conversion of Carbon Dioxide (CO₂) into Fuels and Value-Added Products. *ACS Energy Lett.* **2020**, *5*, 486–519, doi:10.1021/acsenerylett.9b02585.
72. Li, D.; Hao, C.; Liu, H.; Zhang, R.; Li, Y.; Guo, J.; Vilancuo, C.C.; Guo, J. Photocatalytic CO₂ Conversion to Ethanol: A Concise Review. *Catalysts* **2022**, *12*, 1549, doi:10.3390/catal12121549.
73. Ping, G.; Wang, C.; Chen, D.; Liu, S.; Huang, X.; Qin, L.; Huang, Y.; Shu, K. Fabrication of Self-Organized TiO₂ Nanotube Arrays for Photocatalytic Reduction of CO₂. *J Solid State Electrochem* **2013**, *17*, 2503–2510, doi:10.1007/s10008-013-2143-y.
74. Ribeiro, C.S.; Lansarin, M.A. Enhanced Photocatalytic Activity of Bi₂WO₆ with PVP Addition for CO₂ Reduction into Ethanol under Visible Light. *Environ Sci Pollut Res* **2021**, *28*, 23667–23674, doi:10.1007/s11356-020-10765-5.
75. Li, F.; Zhang, D.; Xiang, Q. Nanosheet-Assembled Hierarchical Flower-like g-C₃N₄ for Enhanced Photocatalytic CO₂ Reduction Activity. *Chem. Commun.* **2020**, *56*, 2443–2446, doi:10.1039/C9CC08793A.
76. Cheng, M.; Yang, S.; Chen, R.; Zhu, X.; Liao, Q.; Huang, Y. Copper-Decorated TiO₂ Nanorod Thin Films in Optofluidic Planar Reactors for Efficient Photocatalytic Reduction of CO₂. *International Journal of Hydrogen Energy* **2017**, *42*, 9722–9732, doi:10.1016/j.ijhydene.2017.01.126.

77. Li, P.; Liu, L.; An, W.; Wang, H.; Guo, H.; Liang, Y.; Cui, W. Ultrathin Porous G-C₃N₄ Nanosheets Modified with AuCu Alloy Nanoparticles and C-C Coupling Photothermal Catalytic Reduction of CO to Ethanol. *Applied Catalysis B: Environmental* **2020**, *266*, 118618, doi:10.1016/j.apcatb.2020.118618.
78. Zhang, X.; Zhang, G.; Song, C.; Guo, X. Catalytic Conversion of Carbon Dioxide to Methanol: Current Status and Future Perspective. *Front. Energy Res.* **2021**, *8*, doi:10.3389/fenrg.2020.621119.
79. Lam, E.; Corral-Pérez, J.J.; Larmier, K.; Noh, G.; Wolf, P.; Comas-Vives, A.; Urakawa, A.; Copéret, C. CO₂ Hydrogenation on Cu/Al₂O₃: Role of the Metal/Support Interface in Driving Activity and Selectivity of a Bifunctional Catalyst. *Angewandte Chemie International Edition* **2019**, *58*, 13989–13996, doi:10.1002/anie.201908060.
80. Usman, M.; Humayun, M.; Garba, M.D.; Ullah, L.; Zeb, Z.; Helal, A.; Suliman, M.H.; Alfaifi, B.Y.; Iqbal, N.; Abdinejad, M.; et al. Electrochemical Reduction of CO₂: A Review of Cobalt Based Catalysts for Carbon Dioxide Conversion to Fuels. *Nanomaterials* **2021**, *11*, 2029, doi:10.3390/nano11082029.
81. Zhao, Y.; Björk, E.M.; Yan, Y.; Schaaf, P.; Wang, D. Recent Progress in Transition Metal Based Catalysts and Mechanism Analysis for Alcohol Electrooxidation Reactions. *Green Chem.* **2024**, *26*, 4987–5003, doi:10.1039/D4GC00227J.
82. Chen, C.; Li, J.; Tan, X.; Zhang, Y.; Li, Y.; He, C.; Xu, Z.; Zhang, C.; Chen, C. Harnessing Single-Atom Catalysts for CO₂ Electroreduction: A Review of Recent Advances. *EES. Catal.* **2024**, *2*, 71–93, doi:10.1039/D3EY00150D.
83. Fiorani, G.; Guo, W.; Kleij, A.W. Sustainable Conversion of Carbon Dioxide: The Advent of Organocatalysis. *Green Chem.* **2015**, *17*, 1375–1389, doi:10.1039/C4GC01959H.
84. Whiteoak, C.J.; Kielland, N.; Laserna, V.; Escudero-Adán, E.C.; Martin, E.; Kleij, A.W. A Powerful Aluminum Catalyst for the Synthesis of Highly Functional Organic Carbonates. *J. Am. Chem. Soc.* **2013**, *135*, 1228–1231, doi:10.1021/ja311053h.
85. Clegg, W.; Harrington, R.W.; North, M.; Pasquale, R. Cyclic Carbonate Synthesis Catalysed by Bimetallic Aluminium-Salen Complexes. *Chemistry* **2010**, *16*, 6828–6843, doi:10.1002/chem.201000030.
86. He, Q.; O'Brien, J.W.; Kitzelman, K.A.; Tompkins, L.E.; Curtis, G.C.T.; Kerton, F.M. Synthesis of Cyclic Carbonates from CO₂ and Epoxides Using Ionic Liquids and

Related Catalysts Including Choline Chloride–Metal Halide Mixtures. *Catal. Sci. Technol.* **2014**, *4*, 1513–1528, doi:10.1039/C3CY00998J.

87. Monassier, A.; D'Elia, V.; Cokoja, M.; Dong, H.; Pelletier, J.D.A.; Basset, J.-M.; Kühn, F.E. Synthesis of Cyclic Carbonates from Epoxides and CO₂ under Mild Conditions Using a Simple, Highly Efficient Niobium-Based Catalyst. *ChemCatChem* **2013**, *5*, 1321–1324, doi:10.1002/cctc.201200916.

88. Lignin-Based Green Catalyst for the Chemical Fixation of Carbon Dioxide with Epoxides To Form Cyclic Carbonates under Solvent-Free Conditions - Wu - 2013 - ChemCatChem - Wiley Online Library Available online: <https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/cctc.201200894> (accessed on 6 June 2024).

89. Song, Q.-W.; Ma, R.; Liu, P.; Zhang, K.; He, L.-N. Recent Progress in CO₂ Conversion into Organic Chemicals by Molecular Catalysis. *Green Chem.* **2023**, *25*, 6538–6560, doi:10.1039/D3GC01892J.

90. North, M.; Pasquale, R.; Young, C. Synthesis of Cyclic Carbonates from Epoxides and CO₂. *Green Chem.* **2010**, *12*, 1514–1539, doi:10.1039/C0GC00065E.

91. Sakakura, T.; Kohno, K. The Synthesis of Organic Carbonates from Carbon Dioxide. *Chem. Commun.* **2009**, 1312–1330, doi:10.1039/B819997C.

92. Clegg, W.; Harrington, R.W.; North, M.; Pizzato, F.; Villuendas, P. Cyclic Carbonates as Sustainable Solvents for Proline-Catalysed Aldol Reactions. *Tetrahedron: Asymmetry* **2010**, *21*, 1262–1271, doi:10.1016/j.tetasy.2010.03.051.

93. Li, Q.; Chen, J.; Fan, L.; Kong, X.; Lu, Y. Progress in Electrolytes for Rechargeable Li-Based Batteries and Beyond. *Green Energy & Environment* **2016**, *1*, 18–42, doi:10.1016/j.gee.2016.04.006.

94. Calabrese, C.; Giacalone, F.; Aprile, C. Hybrid Catalysts for CO₂ Conversion into Cyclic Carbonates. *Catalysts* **2019**, *9*, 325, doi:10.3390/catal9040325.

95. Alves, M.; Grignard, B.; Mereau, R.; Jerome, C.; Tassaing, T.; Detrembleur, C. Organocatalyzed Coupling of Carbon Dioxide with Epoxides for the Synthesis of Cyclic Carbonates: Catalyst Design and Mechanistic Studies. *Catal. Sci. Technol.* **2017**, *7*, 2651–2684, doi:10.1039/C7CY00438A.

96. Bobbink, F.D.; Dyson, P.J. Synthesis of Carbonates and Related Compounds Incorporating CO₂ Using Ionic Liquid-Type Catalysts: State-of-the-Art and Beyond. *Journal of Catalysis* **2016**, *343*, 52–61, doi:10.1016/j.jcat.2016.02.033.
97. Shaikh, R.R.; Pornpraprom, S.; D'Elia, V. Catalytic Strategies for the Cycloaddition of Pure, Diluted, and Waste CO₂ to Epoxides under Ambient Conditions. *ACS Catal.* **2018**, *8*, 419–450, doi:10.1021/acscatal.7b03580.
98. Xie, J.; Chen, F.; Li, M.; Liu, N. [ONSN]-Type Chromium Complexes Catalyzed Coupling of CO₂ with Epoxides. *Journal of CO₂ Utilization* **2022**, *62*, 102100, doi:10.1016/j.jcou.2022.102100.
99. Alassmy, Y.A.; Pescarmona, P.P. The Role of Water Revisited and Enhanced: A Sustainable Catalytic System for the Conversion of CO₂ into Cyclic Carbonates under Mild Conditions. *ChemSusChem* **2019**, *12*, 3856–3863, doi:10.1002/cssc.201901124.
100. Qin, Y.; Guo, H.; Sheng, X.; Wang, X.; Wang, F. An Aluminum Porphyrin Complex with High Activity and Selectivity for Cyclic Carbonate Synthesis. *Green Chem.* **2015**, *17*, 2853–2858, doi:10.1039/C4GC02310B.
101. Chatelet, B.; Joucla, L.; Dutasta, J.-P.; Martinez, A.; Dufaud, V. Azaphosphatran Organocatalysts in Confined Space: Cage Effect in CO₂ Conversion. *Chemistry – A European Journal* **2014**, *20*, 8571–8574, doi:10.1002/chem.201402058.
102. Aoyagi, N.; Furusho, Y.; Endo, T. Effective Synthesis of Cyclic Carbonates from Carbon Dioxide and Epoxides by Phosphonium Iodides as Catalysts in Alcoholic Solvents. *Tetrahedron Letters* **2013**, *54*, 7031–7034, doi:10.1016/j.tetlet.2013.10.068.
103. Pal, T.K.; De, D.; Bharadwaj, P.K. Metal–Organic Frameworks for the Chemical Fixation of CO₂ into Cyclic Carbonates. *Coordination Chemistry Reviews* **2020**, *408*, 213173, doi:10.1016/j.ccr.2019.213173.
104. Jiang, Y.; Li, D.; Zhao, Y.; Sun, J. Hydrogen Bond Donor Functionalized Poly(Ionic Liquids)@MIL-101 for the CO₂ Capture and Improving the Catalytic CO₂ Conversion with Epoxide. *Journal of Colloid and Interface Science* **2022**, *618*, 22–33, doi:10.1016/j.jcis.2022.03.038.
105. Zhou, F.; Xie, S.-L.; Gao, X.-T.; Zhang, R.; Wang, C.-H.; Yin, G.-Q.; Zhou, J. Activation of (Salen)Co Complex by Phosphorane for Carbon Dioxide Transformation at Ambient Temperature and Pressure. *Green Chem.* **2017**, *19*, 3908–3915, doi:10.1039/C7GC01458A.

106. Motuzova, G.V.; Minkina, T.M.; Karpova, E.A.; Barsova, N.U.; Mandzhieva, S.S. Soil Contamination with Heavy Metals as a Potential and Real Risk to the Environment. *Journal of Geochemical Exploration* **2014**, *144*, 241–246, doi:10.1016/j.gexplo.2014.01.026.
107. Liu, M.; Wang, X.; Jiang, Y.; Sun, J.; Arai, M. Hydrogen Bond Activation Strategy for Cyclic Carbonates Synthesis from Epoxides and CO₂: Current State-of-the Art of Catalyst Development and Reaction Analysis. *Catalysis Reviews* **2019**, *61*, 214–269, doi:10.1080/01614940.2018.1550243.
108. Pinto da Silva, L. Mechanistic Study of the Role of Hydrogen Bond Donors in the Two-Component Organocatalysis of the Ring-Opening Reaction of Epoxides. *Molecular Catalysis* **2019**, *474*, 110425, doi:10.1016/j.mcat.2019.110425.
109. Ascorbic Acid as a Bifunctional Hydrogen Bond Donor for the Synthesis of Cyclic Carbonates from CO₂ under Ambient Conditions | ACS Sustainable Chemistry & Engineering Available online: <https://pubs.acs.org/doi/10.1021/acssuschemeng.7b01650> (accessed on 13 June 2024).
110. Wang, J.; Zhang, Y. Boronic Acids as Hydrogen Bond Donor Catalysts for Efficient Conversion of CO₂ into Organic Carbonate in Water. *ACS Catal.* **2016**, *6*, 4871–4876, doi:10.1021/acscatal.6b01422.
111. Liu, M.; Gao, K.; Liang, L.; Wang, F.; Shi, L.; Sheng, L.; Sun, J. Insights into Hydrogen Bond Donor Promoted Fixation of Carbon Dioxide with Epoxides Catalyzed by Ionic Liquids. *Phys. Chem. Chem. Phys.* **2015**, *17*, 5959–5965, doi:10.1039/C4CP05464D.
112. Wang, L.; Zhang, G.; Kodama, K.; Hirose, T. An Efficient Metal- and Solvent-Free Organocatalytic System for Chemical Fixation of CO₂ into Cyclic Carbonates under Mild Conditions. *Green Chem.* **2016**, *18*, 1229–1233, doi:10.1039/C5GC02697K.
113. Whiteoak, C.J.; Nova, A.; Maseras, F.; Kleij, A.W. Merging Sustainability with Organocatalysis in the Formation of Organic Carbonates by Using CO₂ as a Feedstock. *ChemSusChem* **2012**, *5*, 2032–2038, doi:10.1002/cssc.201200255.
114. Alves, M.; Grignard, B.; Gennen, S.; Mereau, R.; Detrembleur, C.; Jerome, C.; Tassaing, T. Organocatalytic Promoted Coupling of Carbon Dioxide with Epoxides: A Rational Investigation of the Cocatalytic Activity of Various Hydrogen Bond Donors. *Catal. Sci. Technol.* **2015**, *5*, 4636–4643, doi:10.1039/C5CY00891C.

115. Hardman-Baldwin, A.M.; Mattson, A.E. Silanediol-Catalyzed Carbon Dioxide Fixation. *ChemSusChem* **2014**, *7*, 3275–3278, doi:10.1002/cssc.201402783.
116. Claver, C.; Yeamin, M.B.; Reguero, M.; Masdeu-Bultó, A.M. Recent Advances in the Use of Catalysts Based on Natural Products for the Conversion of CO₂ into Cyclic Carbonates. *Green Chem.* **2020**, *22*, 7665–7706, doi:10.1039/D0GC01870H.
117. Büttner, H.; Lau, K.; Spannenberg, A.; Werner, T. Bifunctional One-Component Catalysts for the Addition of Carbon Dioxide to Epoxides. *ChemCatChem* **2015**, *7*, 459–467, doi:10.1002/cctc.201402816.
118. Liu, S.; Suematsu, N.; Maruoka, K.; Shirakawa, S. Design of Bifunctional Quaternary Phosphonium Salt Catalysts for CO₂ Fixation Reaction with Epoxides under Mild Conditions. *Green Chem.* **2016**, *18*, 4611–4615, doi:10.1039/C6GC01630H.
119. Liu, M.; Li, X.; Liang, L.; Sun, J. Protonated Triethanolamine as Multi-Hydrogen Bond Donors Catalyst for Efficient Cycloaddition of CO₂ to Epoxides under Mild and Cocatalyst-Free Conditions. *Journal of CO₂ Utilization* **2016**, *16*, 384–390, doi:10.1016/j.jcou.2016.10.004.
120. Guo, L.; Lamb, K.J.; North, M. Recent Developments in Organocatalysed Transformations of Epoxides and Carbon Dioxide into Cyclic Carbonates. *Green Chem.* **2021**, *23*, 77–118, doi:10.1039/D0GC03465G.
121. Kamphuis, A.J.; Picchioni, F.; Pescarmona, P.P. CO₂-Fixation into Cyclic and Polymeric Carbonates: Principles and Applications. *Green Chem.* **2019**, *21*, 406–448, doi:10.1039/C8GC03086C.
122. Biswas, T.; Mahalingam, V. Efficient CO₂ Fixation under Ambient Pressure Using Poly(Ionic Liquid)-Based Heterogeneous Catalysts. *Sustainable Energy Fuels* **2019**, *3*, 935–941, doi:10.1039/C8SE00401C.
123. Dai, W.-L.; Luo, S.-L.; Yin, S.-F.; Au, C.-T. The Direct Transformation of Carbon Dioxide to Organic Carbonates over Heterogeneous Catalysts. *Applied Catalysis A: General* **2009**, *366*, 2–12, doi:10.1016/j.apcata.2009.06.045.
124. Xie, Y.; Zhang, Z.; Jiang, T.; He, J.; Han, B.; Wu, T.; Ding, K. CO₂ Cycloaddition Reactions Catalyzed by an Ionic Liquid Grafted onto a Highly Cross-Linked Polymer Matrix. *Angewandte Chemie International Edition* **2007**, *46*, 7255–7258, doi:10.1002/anie.200701467.

125. Raju, N.A.; Prasad, D.; Srinivasappa, P.M.; Biradar, A.V.; Gholap, S.S.; Samal, A.K.; Nagaraja, B.M.; Jadhav, A.H. Recent Developments in State-of-the-Art Silica-Modified Catalysts for the Fixation of CO₂ in Epoxides to Form Organic Carbonates. *Sustainable Energy Fuels* **2022**, *6*, 1198–1248, doi:10.1039/D1SE01916C.
126. Chen, J.; Chiarioni, G.; Euverink, G.-J.W.; Pescarmona, P.P. Dyes as Efficient and Reusable Organocatalysts for the Synthesis of Cyclic Carbonates from Epoxides and CO₂. *Green Chem.* **2023**, *25*, 9744–9759, doi:10.1039/D3GC02161K.
127. Lim, S.Y.; Shen, W.; Gao, Z. Carbon Quantum Dots and Their Applications. *Chem. Soc. Rev.* **2014**, *44*, 362–381, doi:10.1039/C4CS00269E.
128. Sun, Y.-P.; Zhou, B.; Lin, Y.; Wang, W.; Fernando, K.A.S.; Pathak, P.; Mezziani, M.J.; Harruff, B.A.; Wang, X.; Wang, H.; et al. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence. *J. Am. Chem. Soc.* **2006**, *128*, 7756–7757, doi:10.1021/ja062677d.
129. Jeong, S.; Pinals, R.L.; Dharmadhikari, B.; Song, H.; Kalluri, A.; Debnath, D.; Wu, Q.; Ham, M.-H.; Patra, P.; Landry, M.P. Graphene Quantum Dot Oxidation Governs Noncovalent Biopolymer Adsorption. *Sci Rep* **2020**, *10*, 7074, doi:10.1038/s41598-020-63769-z.
130. Wang, Y.; Yang, P.; Zheng, L.; Shi, X.; Zheng, H. Carbon Nanomaterials with *Sp*² or/and *Sp* Hybridization in Energy Conversion and Storage Applications: A Review. *Energy Storage Materials* **2020**, *26*, 349–370, doi:10.1016/j.ensm.2019.11.006.
131. Liang, W.; Bunker, C.E.; Sun, Y.-P. Carbon Dots: Zero-Dimensional Carbon Allotrope with Unique Photoinduced Redox Characteristics. *ACS Omega* **2020**, *5*, 965–971, doi:10.1021/acsomega.9b03669.
132. Esteves da Silva, J.C.G.; Gonçalves, H.M.R. Analytical and Bioanalytical Applications of Carbon Dots. *TrAC Trends in Analytical Chemistry* **2011**, *30*, 1327–1336, doi:10.1016/j.trac.2011.04.009.
133. Xiong, Y.; Schneider, J.; Ushakova, E.V.; Rogach, A.L. Influence of Molecular Fluorophores on the Research Field of Chemically Synthesized Carbon Dots. *Nano Today* **2018**, *23*, 124–139, doi:10.1016/j.nantod.2018.10.010.
134. Wang, R.; Lu, K.-Q.; Tang, Z.-R.; Xu, Y.-J. Recent Progress in Carbon Quantum Dots: Synthesis, Properties and Applications in Photocatalysis. *J. Mater. Chem. A* **2017**, *5*, 3717–3734, doi:10.1039/C6TA08660H.

135. Wang, K.; Gao, Z.; Gao, G.; Wo, Y.; Wang, Y.; Shen, G.; Cui, D. Systematic Safety Evaluation on Photoluminescent Carbon Dots. *Nanoscale Res Lett* **2013**, *8*, 122, doi:10.1186/1556-276X-8-122.
136. Luminescent Carbon Nanodots: Emergent Nanolights - Baker - 2010 - Angewandte Chemie International Edition - Wiley Online Library Available online: <https://onlinelibrary.wiley.com/doi/10.1002/anie.200906623> (accessed on 10 May 2024).
137. Kozák, O.; Datta, K.K.R.; Greplová, M.; Ranc, V.; Kašlík, J.; Zbořil, R. Surfactant-Derived Amphiphilic Carbon Dots with Tunable Photoluminescence. *J. Phys. Chem. C* **2013**, *117*, 24991–24996, doi:10.1021/jp4040166.
138. Sendão, R.M.S.; Crista, D.M.A.; Afonso, A.C.P.; Yuso, M. del V.M. de; Algarra, M.; Silva, J.C.G.E. da; Silva, L.P. da Insight into the Hybrid Luminescence Showed by Carbon Dots and Molecular Fluorophores in Solution. *Phys. Chem. Chem. Phys.* **2019**, *21*, 20919–20926, doi:10.1039/C9CP03730F.
139. Shen, C.-L.; Su, L.-X.; Zang, J.-H.; Li, X.-J.; Lou, Q.; Shan, C.-X. Carbon Nanodots as Dual-Mode Nanosensors for Selective Detection of Hydrogen Peroxide. *Nanoscale Res Lett* **2017**, *12*, 447, doi:10.1186/s11671-017-2214-6.
140. Simões, E.F.C.; Leitão, J.M.M.; da Silva, J.C.G.E. Carbon Dots Prepared from Citric Acid and Urea as Fluorescent Probes for Hypochlorite and Peroxynitrite. *Microchim Acta* **2016**, *183*, 1769–1777, doi:10.1007/s00604-016-1807-6.
141. Simões, E.F.C.; da Silva, L.P.; da Silva, J.C.G.E.; Leitão, J.M.M. Hypochlorite Fluorescence Sensing by Phenylboronic Acid-Alizarin Adduct Based Carbon Dots. *Talanta* **2020**, *208*, 120447, doi:10.1016/j.talanta.2019.120447.
142. Kang, Y.-F.; Li, Y.-H.; Fang, Y.-W.; Xu, Y.; Wei, X.-M.; Yin, X.-B. Carbon Quantum Dots for Zebrafish Fluorescence Imaging. *Sci Rep* **2015**, *5*, 11835, doi:10.1038/srep11835.
143. Hettiarachchi, S.D.; Graham, R.M.; Mintz, K.J.; Zhou, Y.; Vanni, S.; Peng, Z.; Leblanc, R.M. Triple Conjugated Carbon Dots as a Nano-Drug Delivery Model for Glioblastoma Brain Tumors. *Nanoscale* **2019**, *11*, 6192–6205, doi:10.1039/C8NR08970A.
144. Yan, L.; Yang, Y.; Ma, C.-Q.; Liu, X.; Wang, H.; Xu, B. Synthesis of Carbon Quantum Dots by Chemical Vapor Deposition Approach for Use in Polymer Solar Cell

as the Electrode Buffer Layer. *Carbon* **2016**, *109*, 598–607, doi:10.1016/j.carbon.2016.08.058.

145. He, H.; Zheng, X.; Liu, S.; Zheng, M.; Xie, Z.; Wang, Y.; Yu, M.; Shuai, X. Diketopyrrolopyrrole-Based Carbon Dots for Photodynamic Therapy. *Nanoscale* **2018**, *10*, 10991–10998, doi:10.1039/C8NR02643B.

146. Li, H.; Liu, R.; Kong, W.; Liu, J.; Liu, Y.; Zhou, L.; Zhang, X.; Lee, S.-T.; Kang, Z. Carbon Quantum Dots with Photo-Generated Proton Property as Efficient Visible Light Controlled Acid Catalyst. *Nanoscale* **2013**, *6*, 867–873, doi:10.1039/C3NR03996J.

147. Medeiros, T.V. de; Manioudakis, J.; Noun, F.; Macairan, J.-R.; Victoria, F.; Naccache, R. Microwave-Assisted Synthesis of Carbon Dots and Their Applications. *J. Mater. Chem. C* **2019**, *7*, 7175–7195, doi:10.1039/C9TC01640F.

148. Sendão, Ricardo.; Yuso, M. del V.M. de; Algarra, M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Comparative Life Cycle Assessment of Bottom-up Synthesis Routes for Carbon Dots Derived from Citric Acid and Urea. *Journal of Cleaner Production* **2020**, *254*, 120080, doi:10.1016/j.jclepro.2020.120080.

149. Choi, Y.; Choi, Y.; Kwon, O.-H.; Kim, B.-S. Carbon Dots: Bottom-Up Syntheses, Properties, and Light-Harvesting Applications. *Chemistry – An Asian Journal* **2018**, *13*, 586–598, doi:10.1002/asia.201701736.

150. Chen, Y.; Wang, C.; Xu, Y.; Ran, G.; Song, Q. Red Emissive Carbon Dots Obtained from Direct Calcination of 1,2,4-Triaminobenzene for Dual-Mode pH Sensing in Living Cells. *New J. Chem.* **2020**, *44*, 7210–7217, doi:10.1039/D0NJ00985G.

151. Design of Carbon Dots for Metal-Free Photoredox Catalysis | ACS Applied Materials & Interfaces Available online: <https://pubs.acs.org/doi/10.1021/acsami.8b14188> (accessed on 10 May 2024).

152. Highly Biocompatible, Fluorescence, and Zwitterionic Carbon Dots as a Novel Approach for Bioimaging Applications in Cancerous Cells | ACS Applied Materials & Interfaces Available online: <https://pubs.acs.org/doi/10.1021/acsami.8b13217> (accessed on 10 May 2024).

153. Christé, S.; Esteves da Silva, J.C.G.; Pinto da Silva, L. Evaluation of the Environmental Impact and Efficiency of N-Doping Strategies in the Synthesis of Carbon Dots. *Materials* **2020**, *13*, 504, doi:10.3390/ma13030504.

154. Carbonaro, C.M.; Corpino, R.; Salis, M.; Mocci, F.; Thakkar, S.V.; Olla, C.; Ricci, P.C. On the Emission Properties of Carbon Dots: Reviewing Data and Discussing Models. *C* **2019**, *5*, 60, doi:10.3390/c5040060.
155. Qu, D.; Sun, Z. The Formation Mechanism and Fluorophores of Carbon Dots Synthesized via a Bottom-up Route. *Mater. Chem. Front.* **2020**, *4*, 400–420, doi:10.1039/C9QM00552H.
156. Sharma, V.; Tiwari, P.; Mobin, S.M. Sustainable Carbon-Dots: Recent Advances in Green Carbon Dots for Sensing and Bioimaging. *J. Mater. Chem. B* **2017**, *5*, 8904–8924, doi:10.1039/C7TB02484C.
157. Hsu, P.-C.; Shih, Z.-Y.; Lee, C.-H.; Chang, H.-T. Synthesis and Analytical Applications of Photoluminescent Carbon Nanodots. *Green Chem.* **2012**, *14*, 917–920, doi:10.1039/C2GC16451E.
158. Kalita, H.; Mohapatra, J.; Pradhan, L.; Mitra, A.; Bahadur, D.; Aslam, M. Efficient Synthesis of Rice Based Graphene Quantum Dots and Their Fluorescent Properties. *RSC Adv.* **2016**, *6*, 23518–23524, doi:10.1039/C5RA25706A.
159. Crista, D.M.A.; El Mragui, A.; Algarra, M.; Esteves da Silva, J.C.G.; Luque, R.; Pinto da Silva, L. Turning Spent Coffee Grounds into Sustainable Precursors for the Fabrication of Carbon Dots. *Nanomaterials (Basel)* **2020**, *10*, 1209, doi:10.3390/nano10061209.
160. Zhou, J.; Zhou, H.; Tang, J.; Deng, S.; Yan, F.; Li, W.; Qu, M. Carbon Dots Doped with Heteroatoms for Fluorescent Bioimaging: A Review. *Microchim Acta* **2017**, *184*, 343–368, doi:10.1007/s00604-016-2043-9.
161. Liao, J.; Cheng, Z.; Zhou, L. Nitrogen-Doping Enhanced Fluorescent Carbon Dots: Green Synthesis and Their Applications for Bioimaging and Label-Free Detection of Au³⁺ Ions. *ACS Sustainable Chem. Eng.* **2016**, *4*, 3053–3061, doi:10.1021/acssuschemeng.6b00018.
162. Martindale, B.C.M.; Hutton, G.A.M.; Caputo, C.A.; Reisner, E. Solar Hydrogen Production Using Carbon Quantum Dots and a Molecular Nickel Catalyst. *J. Am. Chem. Soc.* **2015**, *137*, 6018–6025, doi:10.1021/jacs.5b01650.
163. Xu, M.; He, G.; Li, Z.; He, F.; Gao, F.; Su, Y.; Zhang, L.; Yang, Z.; Zhang, Y. A Green Heterogeneous Synthesis of N-Doped Carbon Dots and Their Photoluminescence Applications in Solid and Aqueous States. *Nanoscale* **2014**, *6*, 10307–10315, doi:10.1039/C4NR02792B.

164. Liu, J.; Li, R.; Yang, B. Carbon Dots: A New Type of Carbon-Based Nanomaterial with Wide Applications. *ACS Cent. Sci.* **2020**, *6*, 2179–2195, doi:10.1021/acscentsci.0c01306.
165. Carbon Dots–Implanted Graphitic Carbon Nitride Nanosheets for Photocatalysis: Simultaneously Manipulating Carrier Transport in Inter- and Intralayers - Han - 2020 - Solar RRL - Wiley Online Library Available online: <https://onlinelibrary.wiley.com/doi/10.1002/solr.201900517> (accessed on 29 May 2024).
166. Cailotto, S.; Negrato, M.; Daniele, S.; Luque, R.; Selva, M.; Amadio, E.; Perosa, A. Carbon Dots as Photocatalysts for Organic Synthesis: Metal-Free Methylene–Oxygen-Bond Photocleavage. *Green Chem.* **2020**, *22*, 1145–1149, doi:10.1039/C9GC03811F.
167. Xu, L.; Bai, X.; Guo, L.; Yang, S.; Jin, P.; Yang, L. Facial Fabrication of Carbon Quantum Dots (CDs)-Modified N-TiO₂-x Nanocomposite for the Efficient Photoreduction of Cr(VI) under Visible Light. *Chemical Engineering Journal* **2019**, *357*, 473–486, doi:10.1016/j.cej.2018.09.172.
168. Wang, S.; Li, L.; Zhu, Z.; Zhao, M.; Zhang, L.; Zhang, N.; Wu, Q.; Wang, X.; Li, G. Remarkable Improvement in Photocatalytic Performance for Tannery Wastewater Processing via SnS₂ Modified with N-Doped Carbon Quantum Dots: Synthesis, Characterization, and 4-Nitrophenol-Aided Cr(VI) Photoreduction. *Small* **2019**, *15*, 1804515, doi:10.1002/sml.201804515.
169. Huang, S.; Jiang, S.; Pang, H.; Wen, T.; Asiri, A.M.; Alamry, K.A.; Alsaedi, A.; Wang, X.; Wang, S. Dual Functional Nanocomposites of Magnetic MnFe₂O₄ and Fluorescent Carbon Dots for Efficient U(VI) Removal. *Chemical Engineering Journal* **2019**, *368*, 941–950, doi:10.1016/j.cej.2019.03.015.
170. Luo, H.; Liu, Y.; Dimitrov, S.D.; Steier, L.; Guo, S.; Li, X.; Feng, J.; Xie, F.; Fang, Y.; Sapelkin, A.; et al. Pt Single-Atoms Supported on Nitrogen-Doped Carbon Dots for Highly Efficient Photocatalytic Hydrogen Generation. *J. Mater. Chem. A* **2020**, *8*, 14690–14696, doi:10.1039/D0TA04431H.
171. Xu, X.; Tang, W.; Zhou, Y.; Bao, Z.; Su, Y.; Hu, J.; Zeng, H. Steering Photoelectrons Excited in Carbon Dots into Platinum Cluster Catalyst for Solar-Driven Hydrogen Production. *Advanced Science* **2017**, *4*, 1700273, doi:10.1002/advs.201700273.

172. Yan, Y.; Chen, J.; Li, N.; Tian, J.; Li, K.; Jiang, J.; Liu, J.; Tian, Q.; Chen, P. Systematic Bandgap Engineering of Graphene Quantum Dots and Applications for Photocatalytic Water Splitting and CO₂ Reduction. *ACS Nano* **2018**, *12*, 3523–3532, doi:10.1021/acsnano.8b00498.
173. Li, M.; Wang, M.; Zhu, L.; Li, Y.; Yan, Z.; Shen, Z.; Cao, X. Facile Microwave Assisted Synthesis of N-Rich Carbon Quantum Dots/Dual-Phase TiO₂ Heterostructured Nanocomposites with High Activity in CO₂ Photoreduction. *Applied Catalysis B: Environmental* **2018**, *231*, 269–276, doi:10.1016/j.apcatb.2018.03.027.
174. Sarma, D.; Majumdar, B.; Sarma, T.K. Visible-Light Induced Enhancement in the Multi-Catalytic Activity of Sulfated Carbon Dots for Aerobic Carbon–Carbon Bond Formation. *Green Chem.* **2019**, *21*, 6717–6726, doi:10.1039/C9GC02658D.
175. Bhattacharyya, S.; Ehrat, F.; Urban, P.; Teves, R.; Wyrwich, R.; Döblinger, M.; Feldmann, J.; Urban, A.S.; Stolarczyk, J.K. Effect of Nitrogen Atom Positioning on the Trade-off between Emissive and Photocatalytic Properties of Carbon Dots. *Nat Commun* **2017**, *8*, 1401, doi:10.1038/s41467-017-01463-x.
176. Kondratenko, E.V.; Mul, G.; Baltrusaitis, J.; Larrazábal, G.O.; Pérez-Ramírez, J. Status and Perspectives of CO₂ Conversion into Fuels and Chemicals by Catalytic, Photocatalytic and Electrocatalytic Processes. *Energy Environ. Sci.* **2013**, *6*, 3112–3135, doi:10.1039/C3EE41272E.
177. Cokoja, M.; Wilhelm, M.E.; Anthofer, M.H.; Herrmann, W.A.; Kühn, F.E. Synthesis of Cyclic Carbonates from Epoxides and Carbon Dioxide by Using Organocatalysts. *ChemSusChem* **2015**, *8*, 2436–2454, doi:10.1002/cssc.201500161.
178. Franklin M. Orr, J. CO₂ Capture and Storage: Are We Ready? *Energy Environ. Sci.* **2009**, *2*, 449–458, doi:10.1039/B822107N.
179. Cokoja, M.; Bruckmeier, C.; Rieger, B.; Herrmann, W.A.; Kühn, F.E. Transformation of Carbon Dioxide with Homogeneous Transition-Metal Catalysts: A Molecular Solution to a Global Challenge? *Angew Chem Int Ed Engl* **2011**, *50*, 8510–8537, doi:10.1002/anie.201102010.
180. Cokoja, M.; Wilhelm, M.E.; Anthofer, M.H.; Herrmann, W.A.; Kühn, F.E. Synthesis of Cyclic Carbonates from Epoxides and Carbon Dioxide by Using Organocatalysts. *ChemSusChem* **2015**, *8*, 2436–2454, doi:10.1002/cssc.201500161.
181. Yu, B.; He, L.-N. Upgrading Carbon Dioxide by Incorporation into Heterocycles. *ChemSusChem* **2015**, *8*, 52–62, doi:10.1002/cssc.201402837.

182. Aresta, M.; Dibenedetto, A.; Angelini, A. Catalysis for the Valorization of Exhaust Carbon: From CO₂ to Chemicals, Materials, and Fuels. Technological Use of CO₂. *Chem. Rev.* **2014**, *114*, 1709–1742, doi:10.1021/cr4002758.
183. Gómez, J.E.; Kleij, A.W. Recent Progress in Stereoselective Synthesis of Cyclic Organic Carbonates and Beyond. *Current Opinion in Green and Sustainable Chemistry* **2017**, *3*, 55–60, doi:10.1016/j.cogsc.2016.11.005.
184. He, Q.; O'Brien, J.W.; Kitselman, K.A.; Tompkins, L.E.; Curtis, G.C.T.; Kerton, F.M. Synthesis of Cyclic Carbonates from CO₂ and Epoxides Using Ionic Liquids and Related Catalysts Including Choline Chloride–Metal Halide Mixtures. *Catal. Sci. Technol.* **2014**, *4*, 1513–1528, doi:10.1039/C3CY00998J.
185. Wu, Z.; Xie, H.; Yu, X.; Liu, E. Lignin-Based Green Catalyst for the Chemical Fixation of Carbon Dioxide with Epoxides To Form Cyclic Carbonates under Solvent-Free Conditions. *ChemCatChem* **2013**, *5*, doi:10.1002/cctc.201200894.
186. Simões, E.; Leitão, J.; Silva, J. Sulphur and Nitrogen Co-Doped Carbon Dots for Nitric Oxide Fluorescence Quantification. *Analytica Chimica Acta* **2017**, *960*, doi:10.1016/j.aca.2017.01.007.
187. Peroxynitrite and Nitric Oxide Fluorescence Sensing by Ethylenediamine Doped Carbon Dots - ScienceDirect Available online: <https://www.sciencedirect.com/science/article/pii/S0925400515008473> (accessed on 21 June 2024).
188. Algarra, M.; Campos, B.B.; Radotić, K.; Mutavdžić, D.; Bandosz, T.; Jiménez-Jiménez, J.; Rodríguez-Castellón, E.; Silva, J.C.G.E. da Luminescent Carbon Nanoparticles: Effects of Chemical Functionalization, and Evaluation of Ag⁺ Sensing Properties. *J. Mater. Chem. A* **2014**, *2*, 8342–8351, doi:10.1039/C4TA00264D.
189. Campos, B.B.; Contreras-Cáceres, R.; Bandosz, T.J.; Jiménez-Jiménez, J.; Rodríguez-Castellón, E.; Esteves da Silva, J.C.G.; Algarra, M. Carbon Dots as Fluorescent Sensor for Detection of Explosive Nitrocompounds. *Carbon* **2016**, *106*, 171–178, doi:10.1016/j.carbon.2016.05.030.
190. Desens, W.; Werner, T. Convergent Activation Concept for CO₂ Fixation in Carbonates. *Advanced Synthesis & Catalysis* **2016**, *358*, 622–630, doi:10.1002/adsc.201500941.

191. Shen, Y.; Duan, W.-L.; Shi, M. Phenol and Organic Bases Co-Catalyzed Chemical Fixation of Carbon Dioxide with Terminal Epoxides to Form Cyclic Carbonates. *Advanced Synthesis & Catalysis* **2003**, 345, 337–340, doi:10.1002/adsc.200390035.

192. Sankar, M.; Tarte, N.H.; Manikandan, P. Effective Catalytic System of Zinc-Substituted Polyoxometalate for Cycloaddition of CO₂ to Epoxides. *Applied Catalysis A: General* **2004**, 276, 217–222, doi:10.1016/j.apcata.2004.08.008.

Attachments

The work presented here refers to work developed in the scope of the project, in which I am not the first author.

1. Normal breast epithelial MCF-10A cells to evaluate the safety of carbon dots

Article 1

Normal breast epithelial MCF-10A cells to evaluate the safety of carbon dots

Nuno Vale, Sara Silva, Diana Duarte, Diana M.A.Crista, Luís Pinto da Silva and Joaquim C.G. Esteves da Silva, RSC Medicinal Chemistry, **2021**, 12, 245-253.

1.1. Contextualization

The human normal breast cell line MCF-10A is extensively used as a model in toxicity studies due to its structural similarity to normal human mammary epithelium. The application of CDs in biomedicine has been growing over the years, attributed to their photoluminescence properties, biocompatibility, biosafety, and potential uses in bioimaging and drug delivery.

In this study, we prepared three different types of CDs from the same carbon and nitrogen precursors (citric acid and urea) via three distinct bottom-up synthetic routes and tested their safety against the MCF-10A cell line. Characterization results indicated that all CDs had similar size ranges and compositions. MCF-10A cells were treated with varying concentrations of CDs for 24, 48, and 72 hours to assess cell viability over time. After 24 hours, there were no significant decreases in cell viability. However, after 48 hours, a significant decrease in viability was observed in cells treated with calcination-based CDs, while no significant changes were noted for microwave and hydrothermal-based CDs. After 72 hours, hydrothermal-based CDs showed the most promising viability profile. Additionally, compared to paclitaxel, these CDs demonstrated a safety profile similar to that of an antineoplastic agent in non-tumor cells.

Our findings suggest that these novel CDs have potential as imaging candidates, biosensing tools, or drug carriers, warranting further investigation in animal models for future medical applications.

1.2. Contributions to this work

In this work, I was responsible for the synthesis and characterization of the nanoparticles under the supervision of Professor Dr. Joaquim Esteves da Silva and Professor Dr. Luís Pinto da Silva. The study with MCF-10A cell line were conducted by the team of Dr. Nuno Vale at Faculty of Medicine of University of Porto.

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Normal breast epithelial MCF-10A cells to evaluate the safety of carbon dots†

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The human normal breast cell line MCF-10A is being widely used as a model in toxicity studies due to its structural similarity to the normal human mammary epithelium. Over the years, application of carbon dots (C-dots) in biomedicine has been increasing due to their photoluminescence properties, biocompatibility, biosafety and possible applications in bioimaging and as drug carriers. In this work we prepared three different C-dots from the same set of carbon and nitrogen precursors (citric acid and urea, respectively) via three distinct bottom-up synthetic routes and their safety was tested against the normal breast cell line MCF-10A. The characterization results demonstrated a similar size range and composition for all the C-dots. The MCF-10A cells were treated with different concentrations of C-dots for 24, 48 and 72 h to evaluate the cell viability over time. For the 24 h incubation, there were no significant decreases in the viability of the MCF-10A cells. For the 48 h treatment, there was a significant decrease in the viability of the cells treated with calcination-based C-dots, but without significant cellular viability changes for microwave and hydrothermal-based C-dots. For 72 h, cells treated with hydrothermal-based C-dots have the most promising viability profile. Also, compared with paclitaxel, these C-dots have a safety profile very close to that of an antineoplastic in non-tumor cells. Our results suggest that these new C-dots have potential as imaging candidates or biosensing tools as well as drug carriers, and further investigation in animal models is needed for future application in medicine.

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Introduction

Breast cancer is the most common type of cancer in women and the second leading cause of cancer death worldwide.^{1,2} The study of the mechanisms of development and progression of breast cancer is of particular importance and

has led to the development of different *in vivo* and *in vitro* models. Although *in vivo* studies give results closer to reality, they are more complicated models and it is difficult to recapitulate between normal human breast and breast cancer development. Thus, *in vitro* studies have a complementary role in these studies and are widely used.³ Despite the recent technological advances, the fields of diagnostics and treatment in breast cancer still have a lot of potential to be explored. Novel nanomaterials can be essential tools for the diagnosis of diseases or transport/delivery of drugs in tumor cells.^{4,5}

Fluorescent nanomaterials, such as carbon dots (C-dots),⁶ semiconductor carbon dots,^{7,8} polymer dots,⁹ etc., have gained increasing interest due to their properties, namely environment-friendly and stability,^{7,10,11} and to their wide range of applications, such as fluorescence imaging, transport of drugs and genes by conjugation, controlled drug delivery, medical diagnosis, and biosensing, among others.^{12–17} Recently, these C-dots have already been applied as potential antiviral and antibacterial therapeutics.^{18–20} C-Dots were first reported in 2004 by Xu *et al.*²¹ and are small quasi-spherical photoluminescent nanomaterials (usually less than 10 nm)²² that have been used in imaging, biological labeling and analyte sensing (biosensing). The properties of C-dots include high photoluminescence,²³ low cost

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† Electronic supplementary information (ESI) available: Fig. S1 – XPS C 1s spectra for calcination-, microwave- and hydrothermal-based C-dots. Fig. S2 – XPS O 1s spectra for calcination-, microwave- and hydrothermal-based C-dots. Fig. S3 – XPS N 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) C-dots. Fig. S4 – absorption spectra for calcination-, hydrothermal- and microwave-based C-dots. Fig. S5 – emission spectra for hydrothermal-, microwave- and calcination-based C-dots, at different pH values (5, 7 and 10). See DOI: 10.1039/d0md00317d

(precursors might even be coffee grounds,²⁴ tea,²⁵ grass,²⁶ etc.), stability, high absorption, light-stable²³ and environment-friendly features,^{27,28} and biocompatibility,²⁹ which make them ideal for imaging and biological labeling.

C-Dots can be prepared from different materials and through different synthesis strategies, which are classified into top-down and bottom-up approaches.^{6,30} The top-down synthesis is based on large starting materials such as graphite powder and multi-walled carbon nanotubes (MWCNTs), with their subsequent breakdown into smaller carbon materials.^{31–33} Nevertheless, the bottom-up synthesis is the most widely used type of synthesis. Contrary to top-down procedures, bottom-up routes produce C-dots from smaller carbon sources, such as carbohydrates^{34,35} and citric acid,^{36–39} *via* hydrothermal,^{36,40} calcination²⁸ and microwave-assisted syntheses.^{37,41}

The addition of other small molecules containing heteroatoms (such as N, S and B) leads to heteroatom doping of the C-dots.⁴² Among them, N-doping is the most often employed strategy, and nitrogen and carbon atoms have similar size. Moreover, N-doping has been linked to an increase of the fluorescence quantum yield of these nanomaterials.^{42,43} N-Doping can be achieved by adding a nitrogen-rich small molecule to the carbon precursor, with typical nitrogen sources being molecules such as urea and ethylenediamine.³⁷

MCF-10A cells are the most common cell line used as a model for normal human breast cells.³ They are very structurally similar to normal human mammary epithelial cells and are adherent at normal calcium levels.⁴⁴ In collagen, these cells grow in 3D structures and express sialomucins, keratins, hemidesmosomes and desmosomes.^{45,46} In addition, they are controlled by hormones and growth factors, although they do not show estrogen receptors or signs of terminal differentiation or senescence.⁴⁷ In mice, these cells were also found to be non-tumorigenic.⁴⁶ Studies suggest that these cells respond to insulin, glucocorticoids, cholera endotoxin and epidermal growth factor (EPG).⁴⁷ Given that their characteristics are very similar to the normal human breast epithelium, these cells have been widely used in cytotoxicity studies as a control to evaluate the safety of different compounds, in comparison with breast tumor cell lines, such as MCF-7 or MDA-MB-231, among others.^{48–50} These studies included the evaluation of the cytotoxicity of different compounds, from natural molecules such as eupatorin⁵¹ to the study of antineoplastics, such as doxorubicin.⁵² In the last five years, promising carbon-based materials for breast cancer chemotherapy application have been reported.^{53–57} In the field of nanotechnology, MCF-10A cells also continue to be used to assess the toxicity of nanoparticles containing drugs.⁵⁸ These cells have already been used to evaluate the toxicity of different carbon dots, since they are a good model to evaluate the biosafety and biocompatibility of these nanomaterials.

In a study with C-dots obtained from green tea, these nanoparticles were tested on 5 cell lines such as human breast cancer cell lines (MCF-7 and MDA-MB-231), normal

mammary epithelial cells (MCF-10A), human cervical cancer cells (HeLa) and normal porcine kidney cells (LLC-PK1).²⁵ The synthesized C-dots effectively decreased the viability of the tumor cell lines MCF-7, MDA-MB-231 and HeLa by 20%, 18% and 68%, respectively, for the highest concentration tested, while the normal cells (MCF-10A and LLC-PK1) only present a 5% inhibition, which demonstrates the high efficiency of these C-dots in tumor cells and their safety in normal cells.²⁵ In addition, compared with antineoplastic drugs that are already on the market, such as doxorubicin and paclitaxel, it was found that these C-dots were less toxic.²⁵ Paclitaxel is currently used against a wide range of solid tumors, including lung, ovarian, urothelial and breast tumors⁵⁹ and its clinical use assumes that this antineoplastic is safe for non-tumoral cells.

Another study tested fluorescent C-dots obtained from ginger on different tumor cell lines, namely human lung cancer cell line A549, human breast cancer cell line MDA-MB-231, human cervical cancer cell line HeLa and human hepatocellular carcinoma cell line HepG2, with high suppression of the growth of these cells, mainly HepG2 cells.⁶⁰ These C-dots have also been tested on normal lines such as normal mammary epithelial cells (MCF-10A) and normal liver cells (FL83B) to assess their biosafety and have demonstrated low toxicity to these cells.⁶⁰ Ray *et al.* synthesized C-dots with diameters between 2 and 6 nm and it was also demonstrated that the toxicity of these nanomaterials was negligible at the concentrations normally used for bioimaging.⁶¹

A relatively recent *in vivo* study (conducted in rats) performed by Zhan *et al.* showed that aspirin-based C-dots had no significant cytotoxicity in the liver, spleen, lungs, kidneys or heart, while providing anti-inflammatory protection.⁶² Leblanc's group produced C-dots from carbon nanopowder, which were revealed to be non-toxic while possessing high affinity and specificity for bone binding.⁶³ Du and co-workers produced gadolinium-doped C-dots with stable fluorescence that accumulated in the kidneys after vein injection and could be cleared away by urination, demonstrating relevant biocompatibility.⁶⁴ Finally, a relevant amount of research supports the biocompatibility of C-dots in both mouse and zebrafish testing.⁶⁵

Despite these results, it remains necessary to explore the cytotoxicity of these C-dots for more comprehensive applications, such as *in vivo* imaging and pharmaceuticals. This work aims to evaluate the safety and compatibility of these new nanoparticles in the MCF-10A cell line. To this end, three different C-dots were prepared from the same set of carbon and nitrogen precursors (citric acid and urea, respectively) *via* three distinct bottom-up synthesis routes: hydrothermal, calcination and microwave-assisted syntheses, following a previous report from our group.⁶⁶ Their cytotoxicity toward the human normal breast cell line MCF-10A was subsequently tested. With this approach we will be able to analyze the biosafety and biocompatibility of these nanomaterials, when considering widely used starting

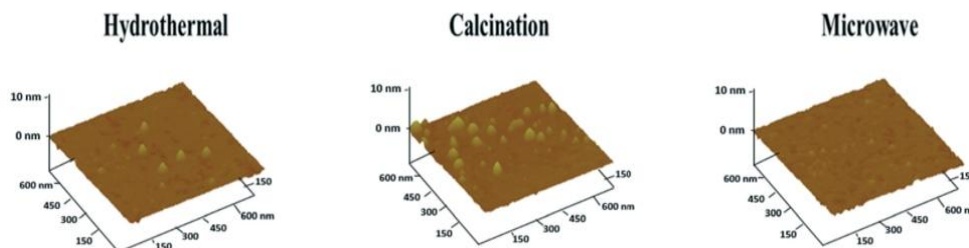


Fig. 1 3D AFM images relative to the size of hydrothermal-, calcination- and microwave-based C-dots.

materials and procedures, while providing insight into the different effects exerted by distinct bottom-up strategies.

Results and discussion

Characterization of C-dots

The different syntheses led to similar size distributions, which were obtained by AFM measurements (Fig. 1). Namely, we obtained the following average sizes ($\pm$ standard deviation) for the three types of C-dots: 6.9 ± 2.0 nm (hydrothermal), 6.1 ± 1.7 nm (calcination) and 7.3 ± 1.7 nm (microwave-assisted synthesis). These are sizes typically associated with C-dots, and indicate that the obtained particles are indeed nano-sized. It should be noted that while microwave-based C-dots present higher average sizes for individual nanoparticles, calcination and hydrothermal-based C-dots appear to have higher tendency toward agglomeration under the employed AFM measurements, which explains some higher-size agglomerates for these types of CDs (Fig. 1).

An XPS analysis was made to analyze the surface compositions of the three C-dot samples (Fig. S1–S3†) and the XPS atomic composition (at wt%) for each sample (Table 1). As expected, all C-dots are composed mainly of C (60.0–62.0%), followed by O (24.7–28.8%) and N (9.1–13.5%). A detailed scan for internal levels of C 1s, O 1s and N 1s was subsequently made, towards deconvolution and chemical state and quantitative analysis (Fig. S1–S3†).

The C 1s spectra (Fig. S1†) could be split into three peaks for calcination- and microwave-based C-dots: at binding energies of ~ 285 eV (attributed to C–C/C–H groups), ~ 286 eV (attributed to C–N/C–O groups) and ~ 289 eV (attributed to O–C=O groups). As for hydrothermal-based C-dots, the C 1s spectrum was split into four peaks instead, with the new one being found at a binding energy of ~ 287 eV (attributed to C=O groups).

By their turn, the O 1s spectra (Fig. S2†) were split into two peaks for all syntheses: at binding energies of ~ 531 eV (attributed to C=O) and ~ 532 eV (attributed to C–O groups).

Finally, the N 1s spectra were also split into two peaks: at binding energies of ~ 400 eV (attributed to amine–amide groups) and ~ 401 eV (attributed to protonated amides). Thus, the surface compositions of these C-dots appear to be similar.

The normalized excitation and fluorescence spectra of the three C-dots can be found in Fig. 2. The fluorescence quantum yields of these C-dots were calculated previously by our group⁶⁶ and are: 3.7% for hydrothermal-based C-dots, 25.1% for microwave-based C-dots, and 29.3% for calcination-based C-dots. All nanoparticles present similar blue emission: maxima at 435 nm for hydrothermal- and calcination-based C-dots, and at 430 nm for microwave-based C-dots. The excitation maxima of these C-dots can be found in the ultraviolet A (UV-A) range. Nevertheless, the variation in the excitation wavelength maximum between the C-dots is greater than for the emission. Namely, hydrothermal-based C-dots can be excited at 325 nm, while microwave-based C-dots can be excited at 340 nm. Calcination-based C-dots present the most red-shifted excitation at 345 nm. All three C-dots present identical absorption spectra (Fig. S4†), which consist of a main band at ~ 350 nm and a shoulder at ~ 245 nm. These can be attributed to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions.⁵² Regarding the luminescence of C-dots, it should be noted that their emission cannot be explained by just a single-dominating mechanism, but rather by two or more processes (such as quantum size effects, bandgap transitions or defect-derived emission).⁶⁷

The effect of pH on emission of the three C-dots was also assessed (Fig. S5†). The pH affects all the C-dots in a similar manner. More specifically, both the emission intensity and wavelength are identical at neutral (pH 7) and basic (pH 10) pH. At acidic pH (pH 5) a very slight blue-shift with a relevant decrease in intensity occurs. These changes can be attributed to protonation of some functional groups of the C-dots, which leads to changes in fluorescence. In fact, previous studies have also observed that the fluorescence of C-dots produced from citric acid and urea can be pH-sensitive.^{68,69}

Table 1 Atomic surface compositions (%) for hydrothermal-, calcination- and microwave-based C-dots, determined by XPS analysis

Atomic surface composition (%)	Hydrothermal-based C-dots	Microwave-based C-dots	Calcination-based C-dots
C (%)	62.0	60.0	61.9
N (%)	9.1	13.1	13.5
O (%)	28.8	26.9	24.7

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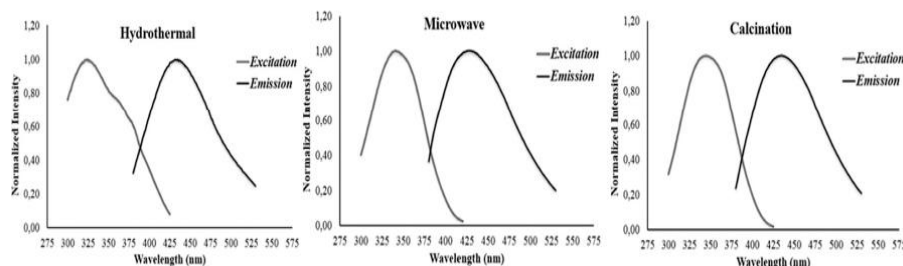


Fig. 2 Normalized excitation and emission spectra (in water) for hydrothermal-, calcination- and microwave-based C-dots.

These authors attributed this pH-sensitivity to the presence of surface moieties resembling molecular fluorophores (as citrazinic acid), which can be present in various molecular forms at different pH values.^{68,69} Nevertheless, the reduction in intensity is significantly lower (~10%) for calcination-based C-dots than for both hydrothermal- and microwave-based C-dots (~30%).

While there is a significant amount of work trying to evaluate the effect of using different carbon precursors and/or heteroatom dopants, there is limited information regarding which way different synthetic routes can affect the properties of C-dots (such as their biocompatibility). Thus, the next step of this study was to assess what way different bottom-up strategies can be used to optimize the biocompatibility of the new C-dots, which are produced from the same carbon precursor (citric acid) and N-dopant (urea).

Cytotoxic effect of C-dots

After the synthesis of C-dots, we intended to evaluate the cytotoxicity of these nanomaterials to normal breast cells.

As the purpose of these nanoparticles is to assist in the diagnosis of breast cancer and transport of drugs to tumor cells, it is important to ensure, at an early stage, that the safety of these particles is guaranteed in normal cells. Although the formulations are different, our goal was to find the most appropriate (and safe) C-dots for this type of application. For this, the cytotoxicity of these three formulations of carbon dots was tested against the human normal breast cell line MCF-10A. The cells were incubated with the C-dots (0.01–1 g L⁻¹) for 24, 48 and 72 hours. When selecting these incubation times, we ensure that the safety of these nanomaterials is guaranteed over time, with no cytotoxicity associated. To assess the cell viability, the MTT assay was performed. In this assay, the control well corresponds to 100% viable cells and the percentage of cell viability in each well is represented as relative to the control. In order to study the compatibility of these nanoparticles with this assay, incubation of the C-dots was performed with the MTT reagent. The results for the 24 h incubation confirm the low cytotoxicity of these nanoparticles (Fig. 3). When treated with hydrothermal-

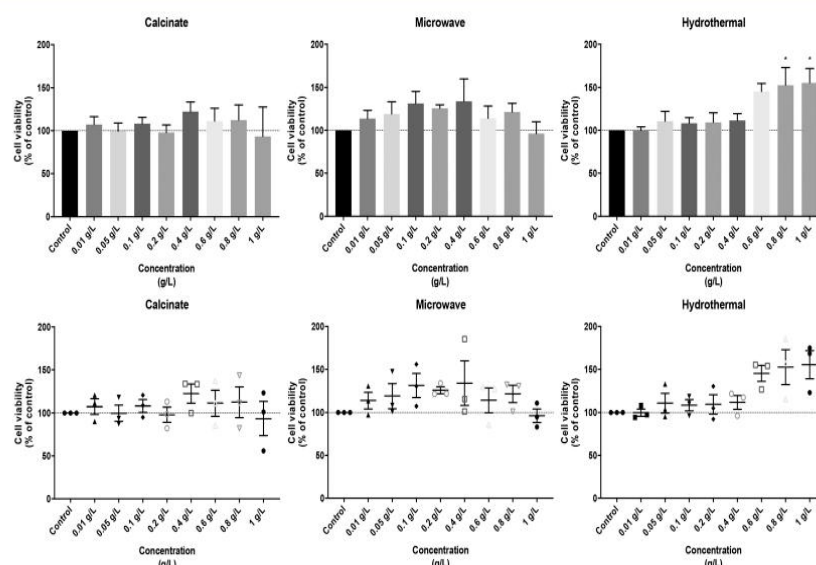


Fig. 3 Relative C-dot cytotoxicity against MCF-10A cells after 24 h incubation through the MTT assay. The percentage of cell viability is represented as relative to the negative control. The values represent mean $\pm$ SEM ($n = 3$) ($*p < 0.05$).

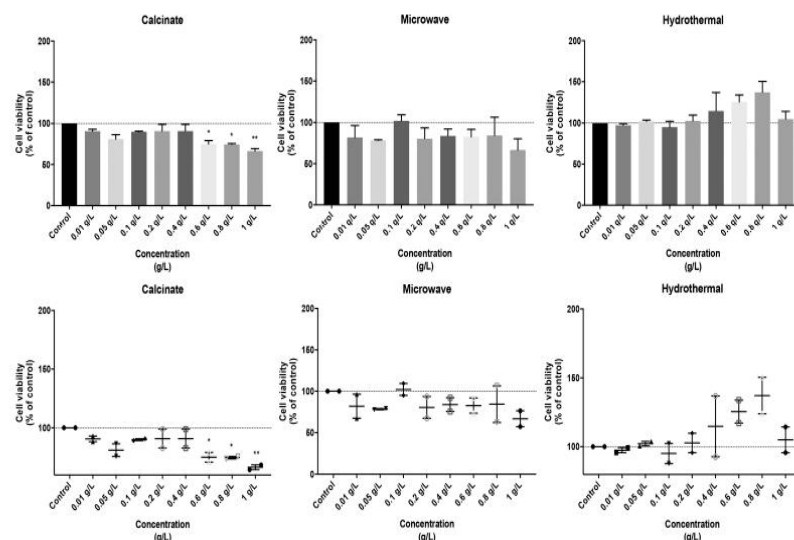


Fig. 4 Relative C-dot cytotoxicity against MCF-10A cells after 48 h incubation through the MTT assay. The percentage of cell viability is represented as relative to the negative control. The values represent mean $\pm$ SEM ($n = 2$) (* $p < 0.05$) (** $p < 0.01$).

based C-dots, the cell viability significantly increases, which may be related to an increase in the number of cells in the well or higher metabolic activity of the cells. Due to natural variations of cellular metabolism or because slightly more cells were seeded in that well due to small pipetting errors, it is totally normal to have samples that exceed the 100% viability. In this case, we found out that these C-dots may also have an influence on the cell growth, confirmed by the cell count with trypan blue. The upper graphs allow faster reading of the results while the lower graphs allow evaluation of the precision and accuracy of the values obtained in the cellular assays.

As expected, after 48 h treatment, there was a general decrease in cell viability compared to the results obtained at 24 h, mainly for calcination and microwave-based carbon dots at the highest concentrations. For hydrothermal-based C-dots, the same trend was observed as that in 24 h treatment, but with an attenuated increase in cell viability (Fig. 4).

For the 72 h incubation, the decrease of cell viability was accentuated in the microwave-based formulation (Fig. 5) when compared with the same formulation for the 48 h (Fig. 4) and 24 h incubation (Fig. 3). In addition, the toxicity based on the MTT assay showed that there is no significant

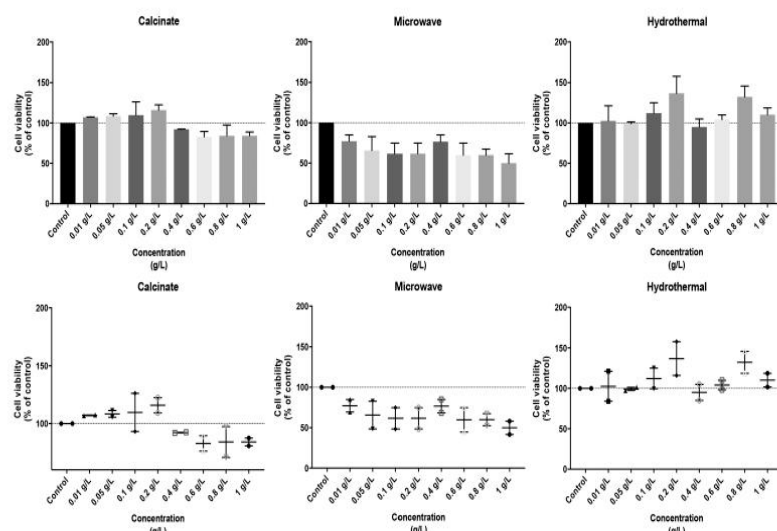


Fig. 5 Relative C-dot cytotoxicity against MCF-10A cells after 72 h incubation through the MTT assay. The percentage of cell viability is represented as relative to the negative control. The values represent mean $\pm$ SEM ($n = 2$).

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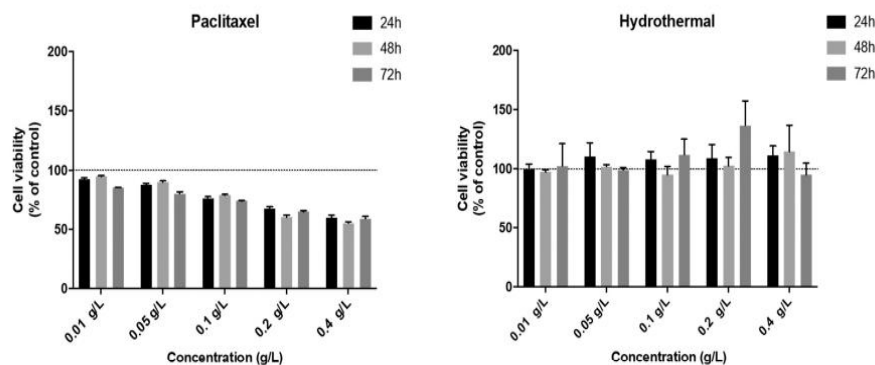


Fig. 6 Relative viabilities of MCF-10A cells after 24, 48 and 72 h treatments with paclitaxel vs. hydrothermal-based C-dots. The percentage of cell viability is represented as relative to the negative control. The values represent mean $\pm$ SEM. The data for paclitaxel are in agreement with the literature.⁶³

sign of toxicity observed for all concentrations of hydrothermal-based C-dots over time (Fig. 3–5).

As hydrothermal-based carbon dots demonstrated less cytotoxicity, we compared the toxicity of these C-dots with an antineoplastic commonly used in clinical practice, paclitaxel. Paclitaxel has low toxicity, is considered safe and is approved by the FDA for tumor therapy. Since this drug has a safety profile compatible for its clinical use, we compared the toxicity of our C-dots with paclitaxel. The hydrothermal-based C-dots formulated in this study cause less decreases in cell viability than paclitaxel for the same range of concentrations, so their use in normal breast cells can be considered safe (Fig. 6).

In this work, the characterization results of the new C-dots showed similar particle size and surface composition. The structural properties of the synthesized C-dots proved to be favorable to the study of the bioapplication of these nanomaterials as non-toxic fluorescent agents for bioimaging. As C-dots usually have promising biocompatibility profiles, MTT assays were performed to assess the toxicity of these nanoparticles against MCF-10A cells (normal breast cells). The MTT assay is a colorimetric assay that is based on the ability of cell mitochondria to reduce the MTT dye to the purple-colored formazan. This reduction is greater as cells are more viable, so there is a correlation between the MTT signal and the cell viability. Thus, the greater the toxicity of a given agent, the less the reduction of MTT into formazan (less viable cells, less functional mitochondria). The MTT assay demonstrated that the prepared calcination, microwave and hydrothermal-based C-dots showed high cell viabilities for MCF-10A cells, even in high concentrations of 1 g L^{-1} for 24 h (Fig. 3). The most significant decrease in cell viability was observed for calcination-based C-dots in concentrations above 0.6 g L^{-1} for 48 h incubation (Fig. 4). The probable mechanism for this process can be associated with ROS produced in the mitochondria. The ROS generation process plays a decisive role in biological processes such as cell division, differentiation, apoptosis, cellular senescence and radical-mediated oxidative damage.

Despite the physicochemical similarities of all the C-dots, the hydrothermal-based C-dots were considered the best formulation, as they showed no effect on cell viability for all tested concentrations and incubation. For cross checking the toxicity, the cells were treated with paclitaxel, an antineoplastic widely used in breast cancer therapy that has a considered safe toxicological profile, so the toxicity of these C-dots was compared with the toxicity of paclitaxel to MCF-10A cells. In Fig. 6, it is possible to conclude that the hydrothermal-based C-dots are significantly less toxic than paclitaxel for the same range of concentrations. Two possibly complementary explanations can be given to justify the higher biocompatibility of the hydrothermal-based C-dots. Wang and co-workers have found for graphene quantum dots a positive correlation between a higher surface oxygen content and an improved antioxidant activity of the nanoparticles.⁷⁰ In fact, the XPS analysis (Table 1) performed by our group has shown that hydrothermal-based C-dots have a higher surface oxygen content (28.8%) than both microwave- and calcination-based C-dots (24.7–26.9%). Thus, it is possible that by exhibiting a higher antioxidant activity, hydrothermal-based C-dots can provide some protection toward oxidative stress to the cells, and so, maintain their cellular viability. Liu *et al.*⁷¹ have also recently studied the influence of surface functional groups on the toxicity of carbon-based nanomaterials. Interestingly, they have found that the presence of epoxide (C–O–C) functional groups enhances the oxidation potential of these nanomaterials, which leads to higher toxicity.⁷¹ Consistent with this scenario, hydrothermal-based C-dots present a lower contribution of C–O functional groups (9.2%) to the C 1s spectra (Fig. S1†) than both microwave- (11.0%) and calcination-based (12.1%) C-dots. Taken together, both hypotheses might help to explain the higher biocompatibility of C-dots.

Conclusions

The increase in biomedical application of C-dots is due to their incredible photoluminescence properties, photostability, biocompatibility and biosafety. The carbon dots synthesized

in this study are obtained through different synthesis strategies that reflect in the *in vitro* results. Among the C-dots tested in these study, hydrothermal-based carbon dots have the lowest toxicity against the normal breast cells, MCF-10A, demonstrating high biocompatibility and biosafety, with potential for use in medicine as bioimaging and biosensing agents and drug or gene delivery.⁷²

Experimental section

Chemicals

DMEM/nutrient mixture F-12 Ham (DMEM-F12), fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from Millipore Sigma (Merck KGaA, Germany). Other cell culture reagents were purchased from Gibco (Thermo Fisher Scientific, Inc, MA, USA). Paclitaxel (cat. no. 1097, from Tocris) was obtained from Biogen Científica (Madrid, Spain). Thiazolyl blue tetrazolium bromide (MTT, cat. no. M5655) was obtained from Sigma-Aldrich (Merck KGaA, Germany).

Synthesis of C-dots

The C-dots were obtained by three different methods: hydrothermal, calcination and microwave-assisted syntheses, following a previous report from our group.⁶⁶ A mixture of citric acid (0.75 g) and urea (0.25 g) was used as a starting material in all synthesis procedures. For the hydrothermal synthesis, the citric acid-urea mixture was dissolved in 5 mL of deionized water. The solution was placed in a Teflon-lined reactor and heated for 2 hours in an oven at 200 °C. For the calcination-based procedure, the citric acid-urea powder mixture was placed in a beaker and heated for 2 hours in an oven at 200 °C. Finally, for the microwave-assisted synthesis, the citric acid-urea mixture was dissolved in 5 mL of deionized water and subsequently placed in a glass petri dish. The reaction mixture was subjected to microwave irradiation (700 W in a domestic microwave) for 5 minutes.

The obtained C-dots were subsequently suspended in water and initially purified by centrifugation (10 minutes at 12000 rpm) to eliminate suspended impurities. The samples were further purified by dialysis for 24 hours, by using a Float-A-Lyzer®G2 Dialysis Device Spectrum® with a molecular weight cut-off of 500 Da.

The synthesis yields (in %) for the three C-dots were: 1.8% for hydrothermal-based C-dots, 28.5% for microwave-based C-dots and 26.9% for calcination-based C-dots.⁶⁶

Characterization of C-dots

Atomic force microscopy (AFM) was carried out using a Veeco Metrology Multimode/Nanoscope IVA in tapping mode, using a Bruker silicon probe (model TESP-SS, resonant frequency 320 kHz, nominal force constant 42 N m⁻¹, estimated tip radius 2 nm). X-ray photoelectron spectroscopy (XPS) was performed with a Fi Kratos Axis Ultra HAS-VISION, using monochromatic Al-K α radiation (15 kV, 90 W). The spectra

were analyzed and quantified using CasaXPS software employing sensitivity factors supplied by the manufacturer. Analysis included the subtraction of a linear background and charge referencing to the adventitious carbon signal at 285 eV. Fluorescence was measured in standard 10 mm fluorescence quartz cells by using a Horiba Jovin Yvon Fluoromax-4 spectrofluorometer. The spectra were obtained with a 1 nm interval and 5 nm slit widths. The average sizes were calculated by quantifying the size of 40 individual nanoparticles for each CD sample.

Cell incubation

The human normal breast MCF-10A cell line was obtained from the American Type Culture Collection (ATCC; Virginia, USA) and maintained according to ATCC's recommendations at 37 °C and 5% CO₂ in DMEM-F12 medium supplemented with 10% fetal bovine serum, 100 μ g mL⁻¹ epidermal growth factor (EGF), 1 mg mL⁻¹ hydrocortisone, 10 mg mL⁻¹ insulin, 100 U mL⁻¹ penicillin G and 100 μ g mL⁻¹ streptomycin. The cells were maintained in the logarithmic growth phase at all times. The medium was renewed every 2 days and the cells were trypsinized with 0.25% trypsin-EDTA and subcultured in the same medium. MCF-10A cells (5000 cells per well) were seeded in 96-well plates and allowed to adhere overnight prior to drug exposure. After that, the cell culture medium was replaced with C-dot-containing media. The cells were exposed to C-dots for 24, 48 and 72 h, followed by the MTT assay to evaluate the cell viability of these cells in the C-dot treatments.

Cytotoxicity assays

To determine the effect of C-dots on the viability of MCF-10A cells, the MTT assay was used. For the MTT protocol, after C-dot treatment, the cell medium was removed, and 100 μ L per well of MTT solution (0.5 mg mL⁻¹ in PBS) was added. The cells were incubated for 3 h, protected from light. After this period, the MTT solution was removed, and DMSO (100 μ L per well) was added to solubilize the formazan crystals. Absorbance was measured at 570 nm using an automated microplate reader (Synergy HT, Biotek Instruments Inc., Winooski, VT, USA). The results are expressed as percentage of the respective control (vehicle). All conditions were performed in triplicate.

Statistical analysis

The results are presented as mean $\pm$ SEM for *n* experiments performed. Statistical comparisons between groups, at the same time point, were performed with one-way ANOVA, after Shapiro-Wilk test normality evaluation. Statistical significance was accepted at *p* values < 0.05. The Student-Newman-Keuls *post-hoc* test was used once a significant *p* value was achieved.

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Conflicts of interest

The authors declare no conflict of interest.

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References

- O. A. Bamodu, W.-C. Huang, D. T. W. Tzeng, A. Wu, L. S. Wang, C.-T. Yeh and T.-Y. Chao, *Cancer Lett.*, 2015, **364**, 125–134.
- I. Ahmad and Shagufta, *Eur. J. Med. Chem.*, 2015, **102**, 375–386.
- Y. Qu, B. Han, Y. Yu, W. Yao, S. Bose, B. Y. Karlan, A. E. Giuliano and X. Cui, *PLoS One*, 2015, **10**, e0131285.
- Y. Xin, M. Yin, L. Zhao, F. Meng and L. Luo, *Cancer Biol. Med.*, 2017, **14**, 228–241.
- Y. Dang and J. Guan, *Smart Mater. Med.*, 2020, **1**, 10–19.
- S. N. Baker and G. A. Baker, *Angew. Chem., Int. Ed.*, 2010, **49**, 6726–6744.
- N. Hildebrandt, C. M. Spillmann, W. R. Algar, T. Pons, M. H. Stewart, E. Oh, K. Susumu, S. A. Díaz, J. B. Delehanty and I. L. Medintz, *Chem. Rev.*, 2017, **117**, 536–711.
- X. Dai, Y. Deng, X. Peng and Y. Jin, *Adv. Mater.*, 2017, **29**, 1607022.
- K. Sun, Y. Tang, Q. Li, S. Yin, W. Qin, J. Yu, D. T. Chiu, Y. Liu, Z. Yuan, X. Zhang and C. Wu, *ACS Nano*, 2016, **10**, 6769–6781.
- S. Y. Y. Lim, W. Shen and Z. Gao, *Chem. Soc. Rev.*, 2015, **44**, 362–381.
- J. M. M. Yoo, J. H. H. Kang and B. H. H. Hong, *Chem. Soc. Rev.*, 2015, **44**, 4835–4852.
- Z. Peng, X. Han, S. Li, A. O. Al-Youbi, A. S. Bashammakh, M. S. El-Shahawi and R. M. Leblanc, *Coord. Chem. Rev.*, 2017, **343**, 256–277.
- P. Namdari, B. Negahdari and A. Eatemadi, *Biomed. Pharmacother.*, 2017, **87**, 209–222.
- M. Tuerhong, Y. Xu and X.-B. Yin, *Fenxi Huaxue*, 2017, **45**, 139–150.
- X. Sun and Y. Lei, *TrAC, Trends Anal. Chem.*, 2017, **89**, 163–180.
- D. Bhattacharyya, P. K. Sarswat and M. L. Free, *Vacuum*, 2017, **146**, 606–613.
- M. Algarra, M. Pérez-Martin, M. Cifuentes-Rueda, J. Jiménez-Jiménez, J. C. G. Esteves da Silva, T. J. Badosz, E. Rodríguez-Castellón, J. T. López Navarrete and J. Casado, *Nanoscale*, 2014, **6**, 9071–9077.
- P. Li, F. Han, W. Cao, G. Zhang, J. Li, J. Zhou, X. Gong, G. Turnbull, W. Shu, L. Xia, B. Fang, X. Xing and B. Li, *Appl. Mater. Today*, 2020, **19**, 100601.
- M. Khanal, T. Vausselein, A. Barras, O. Bande, K. Turcheniuk, M. Benazza, V. Zaitsev, C. M. Teodorescu, R. Boukherroub, A. Siriwardena, J. Dubuisson and S. Szunerits, *ACS Appl. Mater. Interfaces*, 2013, **5**, 12488–12498.
- P. Innocenzi and L. Stagi, *Chem. Sci.*, 2020, **11**, 6606–6622.
- X. Xu, R. Ray, Y. Gu, H. J. Ploehn, L. Gearheart, K. Raker and W. A. Scrivens, *J. Am. Chem. Soc.*, 2004, **126**, 12736–12737.
- K. A. S. Fernando, S. Sahu, Y. Liu, W. K. Lewis, E. A. Gulians, A. Jafariyan, P. Wang, C. E. Bunker and Y.-P. Sun, *ACS Appl. Mater. Interfaces*, 2015, **7**, 8363–8376.
- C. Xia, S. Zhu, T. Feng, M. Yang and B. Yang, *Adv. Sci.*, 2019, **6**, 1901316.
- D. M. A. Crista, A. El Mragui, M. Algarra, J. C. G. Esteves da Silva, R. Luque and L. Pinto da Silva, *Nanomaterials*, 2020, **10**, 1209.
- P.-C. Hsu, P.-C. Chen, C.-M. Ou, H.-Y. Chang and H.-T. Chang, *J. Mater. Chem. B*, 2013, **1**, 1774.
- S. Liu, J. Tian, L. Wang, Y. Zhang, X. Qin, Y. Luo, A. M. Asiri, A. O. Al-Youbi and X. Sun, *Adv. Mater.*, 2012, **24**, 2037–2041.
- X. Zhang, M. Jiang, N. Niu, Z. Chen, S. Li, S. Liu and J. Li, *ChemSusChem*, 2018, **11**, 11–24.
- P. C. Hsu, Z. Y. Shih, C. H. Lee and H. T. Chang, *Green Chem.*, 2012, **14**, 917–920.
- J. Liu, S. Lu, Q. Tang, K. Zhang, W. Yu, H. Sun and B. Yang, *Nanoscale*, 2017, **9**, 7135–7142.
- H. Li, Z. Kang, Y. Liu and S.-T. Lee, *J. Mater. Chem.*, 2012, **22**, 24230.
- Y.-P. Sun, B. Zhou, Y. Lin, W. Wang, K. A. S. Fernando, P. Pathak, M. J. Mezziani, B. A. Harruff, X. Wang, H. Wang, P. G. Luo, H. Yang, M. E. Kose, B. Chen, L. M. Veca and S.-Y. Xie, *J. Am. Chem. Soc.*, 2006, **128**, 7756–7757.
- H. Liu, T. Ye and C. Mao, *Angew. Chem., Int. Ed.*, 2007, **46**, 6473–6475.
- H. Gonçalves, P. A. S. Jorge, J. R. A. Fernandes and J. C. G. Esteves da Silva, *Sens. Actuators, B*, 2010, **145**, 702–707.
- H. Zhu, X. Wang, Y. Li, Z. Wang, F. Yang and X. Yang, *Chem. Commun.*, 2009, 5118.
- H. Li, X. He, Y. Liu, H. Huang, S. Lian, S.-T. Lee and Z. Kang, *Carbon*, 2011, **49**, 605–609.
- R. Sendão, M. D. V. M. de Yuso, M. Algarra, J. C. G. E. da Silva and L. P. da Silva, *J. Cleaner Prod.*, 2020, **254**, 120080.
- S. Christé, J. C. G. E. da Silva and L. P. da Silva, *Materials*, 2020, **13**, 504.

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- 38 R. M. S. Sendão, D. M. A. Crista, A. C. P. Afonso, M. D. V. M. de Yuso, M. Algarra, J. C. G. E. da Silva and L. P. Da Silva, *Phys. Chem. Chem. Phys.*, 2019, **21**, 20919–20926.
- 39 Z. Tian, X. Zhang, D. Li, D. Zhou, P. Jing, D. Shen, S. Qu, R. Zboril and A. L. Rogach, *Adv. Opt. Mater.*, 2017, **5**, 1700416.
- 40 E. F. C. Simões, L. P. da Silva, J. C. G. E. da Silva and J. M. M. Leitão, *Talanta*, 2020, **208**, 120447.
- 41 E. F. C. Simões, J. M. M. Leitão and J. C. G. E. da Silva, *Microchim. Acta*, 2016, **183**, 1769–1777.
- 42 J. Zhou, H. Zhou, J. Tang, S. Deng, F. Yan, W. Li and M. Qu, *Microchim. Acta*, 2017, **184**, 343–368.
- 43 Y. Du and S. Guo, *Nanoscale*, 2016, **8**, 2532–2543.
- 44 T. M. Paine, H. D. Soule, R. J. Pauley and P. J. Dawson, *Int. J. Cancer*, 1992, **50**, 463–473.
- 45 J. Russo, G. Calaf and I. H. Russo, *Crit. Rev. Oncog.*, 1993, **4**, 403–417.
- 46 H. D. Soule, T. M. Maloney, S. R. Wolman, W. D. Peterson, R. Brenz, C. M. McGrath, J. Russo, R. J. Pauley, R. F. Jones and S. C. Brooks, *Cancer Res.*, 1990, **50**, 6075–6086.
- 47 L. Tait, H. D. Soule and J. Russo, *Cancer Res.*, 1990, **50**, 6087–6094.
- 48 G. Cheng, J. Zielonka, D. M. McAllister, A. C. Mackinnon, J. Joseph, M. B. Dwinell and B. Kalyanaraman, *BMC Cancer*, 2013, **13**, 285.
- 49 D. Pitchai, A. Roy and C. Ignatius, *J. Adv. Pharm. Technol. Res.*, 2014, **5**, 179.
- 50 E. A. Thompson, E. Graham, C. M. MacNeill, M. Young, G. Donati, E. M. Wailes, B. T. Jones and N. H. Levi-Polyachenko, *Int. J. Hyperthermia*, 2014, **30**, 312–323.
- 51 N. A. Razak, N. Abu, W. Y. Ho, N. R. Zamberi, S. W. Tan, N. B. Alitheen, K. Long and S. K. Yeap, *Sci. Rep.*, 2019, **9**, 1514.
- 52 E. Gajewski, S. Gaur, S. A. Akman, L. Matsumoto, J. N. A. van Balgooy and J. H. Doroshov, *Biochem. Pharmacol.*, 2007, **73**, 1947–1956.
- 53 J. Li, M. Li, L. Tian, Y. Qiu, Q. Yu, X. Wang, R. Guo and Q. He, *Int. J. Pharm.*, 2020, **578**, 119122.
- 54 T. Kong, L. Hao, Y. Wei, X. Cai and B. Zhu, *Cell Proliferation*, 2018, **51**, e12488.
- 55 N. R. Ko, M. Nafijjuman, J. S. Lee, H.-N. Lim, Y.-k. Lee and I. K. Kwon, *RSC Adv.*, 2017, **7**, 11420–11427.
- 56 P. Pedram, M. Mahani, M. Torkzadeh-Mahani, Z. Hasani and H. Ju, *Microchim. Acta*, 2016, **183**, 67–71.
- 57 Z. Wang, H. Liao, H. Wu, B. Wang, H. Zhao and M. Tan, *Anal. Methods*, 2015, **7**, 8911–8917.
- 58 L. Khorsandi, M. Orazizadeh, F. Niazvand, M. R. Abbaspour, E. Mansouri and A. Khodadadi, *Bratisl. Lek. Listy*, 2017, **118**, 123–128.
- 59 B. Ramanathan, K.-Y. Jan, C.-H. Chen, T.-C. Hour, H. J. Yu and Y.-S. Pu, *Cancer Res.*, 2005, **65**, 8455–8460.
- 60 C. L. Li, C. M. Ou, C. C. Huang, W. C. Wu, Y. P. Chen, T. E. Lin, L. C. Ho, C. W. Wang, C. C. Shih, H. C. Zhou, Y. C. Lee, W. F. Tzeng, T. J. Chiou, S. T. Chu, J. Cang and H. T. Chang, *J. Mater. Chem. B*, 2014, **2**, 4564–4571.
- 61 S. C. Ray, A. Saha, N. R. Jana and R. Sarkar, *J. Phys. Chem. C*, 2009, **113**, 18546–18551.
- 62 Y. Zhan, T. Geng, Y. Liu, C. Hu, X. Zhang, B. Lei, J. Zhuang, X. Wu, D. Huang, G. Xiao and B. Zou, *ACS Appl. Mater. Interfaces*, 2018, **10**, 27920–27927.
- 63 Z. Peng, E. H. H. Miyajiri, Y. Zhou, J. Pardo, S. D. D. Hettiarachchi, S. Li, P. L. L. Blackwelder, I. Skromne and R. M. M. Leblanc, *Nanoscale*, 2017, **9**, 17533–17543.
- 64 F. Du, L. Zhang, L. Zhang, M. Zhang, A. Gong, Y. Tan, J. Miao, Y. Gong, M. Sun, H. Ju, C. Wu and S. Zou, *Biomaterials*, 2017, **121**, 109–120.
- 65 H. Li, X. Yan, D. Kong, R. Jin, C. Sun, D. Du, Y. Lin and G. Lu, *Nanoscale Horiz.*, 2020, **5**, 218–234.
- 66 D. M. A. Crista, J. C. G. Esteves da Silva and L. Pinto da Silva, *Nanomaterials*, 2020, **10**, 1316.
- 67 P. K. K. Sarswat and M. L. L. Free, *Phys. Chem. Chem. Phys.*, 2015, **17**, 27642–27652.
- 68 S. Sarkar, J. Chowdhury, S. Dutta and T. Pal, *Spectrochim. Acta, Part A*, 2016, **169**, 108–115.
- 69 S. Mura, L. Stagi, R. Ludmerczki, L. Malfatti and P. Innocenzi, *Materials*, 2020, **13**, 3654.
- 70 Y. Wang, W. Kong, L. Wang, J. Z. Zhang, Y. Li, X. Liu and Y. Li, *Phys. Chem. Chem. Phys.*, 2019, **21**, 1336–1343.
- 71 Y. Liu, H. Jiang, C. Liu, Y. Ge, L. Wang, B. Zhang, H. He and S. Liu, *Atmos. Chem. Phys.*, 2019, **19**, 8175–8187.
- 72 K. O. Boakye-Yiadom, S. Kesse, Y. Opoku-Damoah, M. S. Filli, M. Aquib, M. M. B. Joelle, M. A. Farooq, R. Mavlyanova, F. Raza, R. Bavi and B. Wang, *Int. J. Pharm.*, 2019, **564**, 308–317.

1.3. Supplementary Information

Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.
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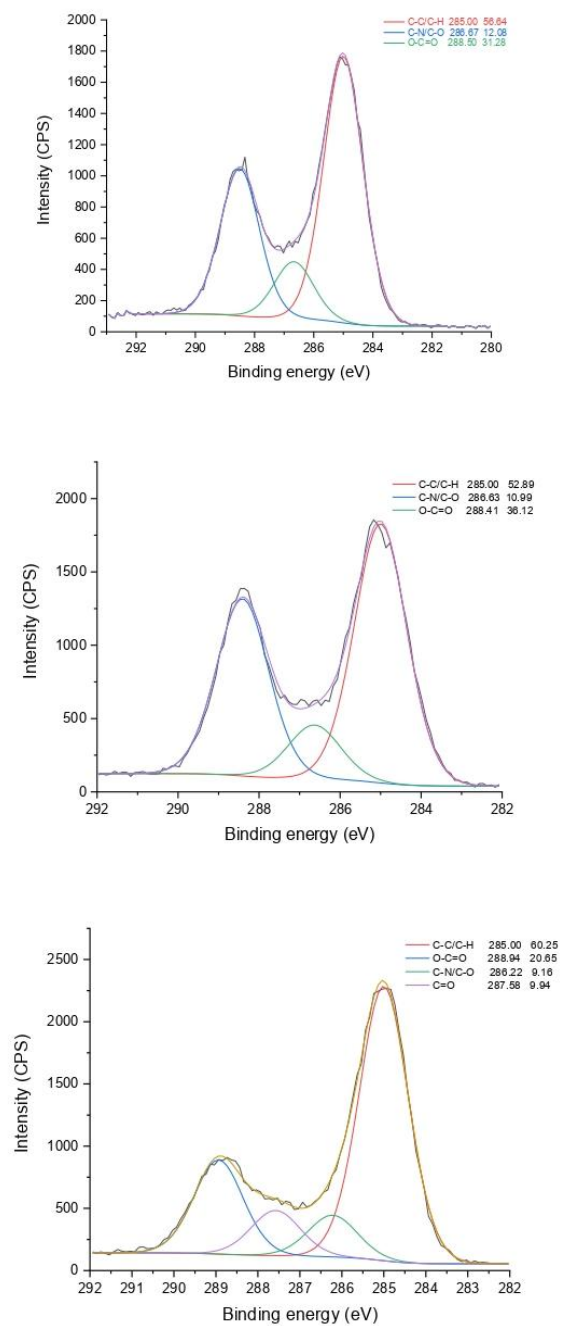


Figure S1 – XPS C 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) C-dots.

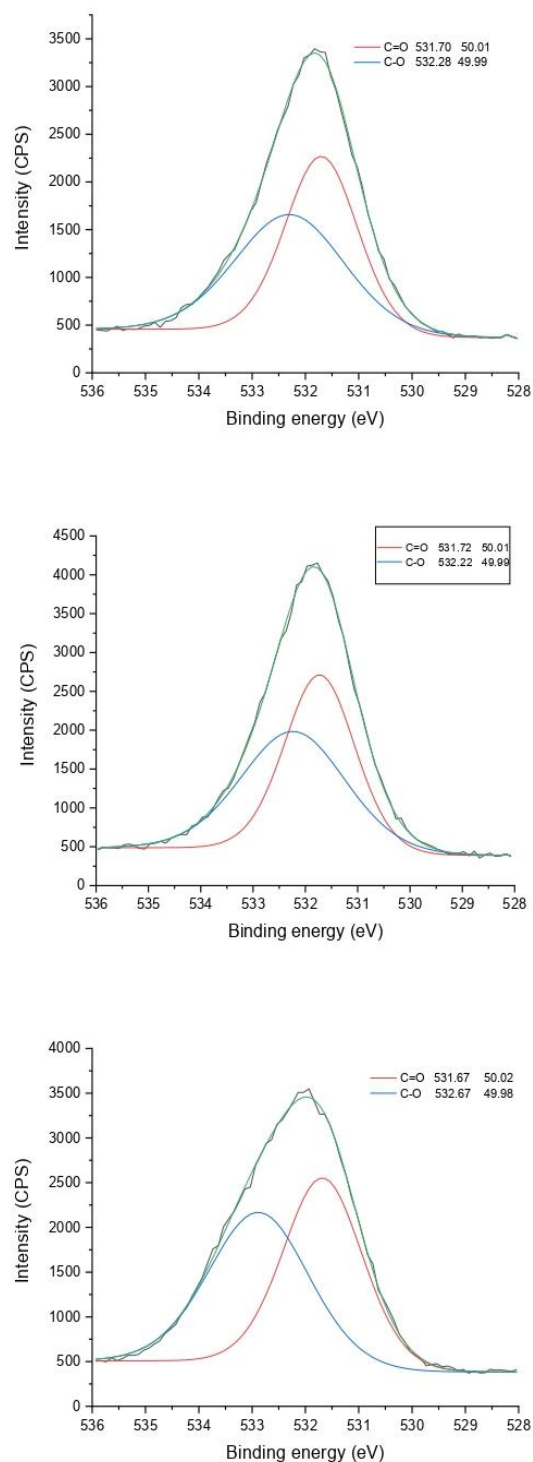


Figure S2 – XPS O 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) C-dots.

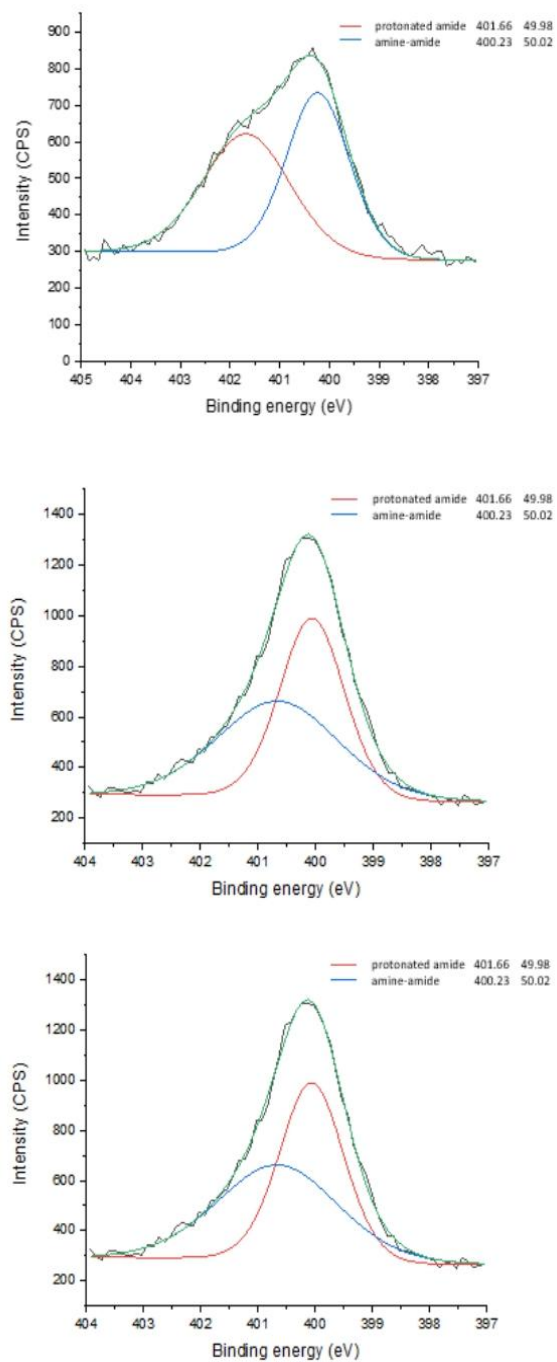


Figure S3 – XPS N 1s spectra for calcinated- (top), microwave- (middle) and hydrothermal-based (bottom) C-dots.

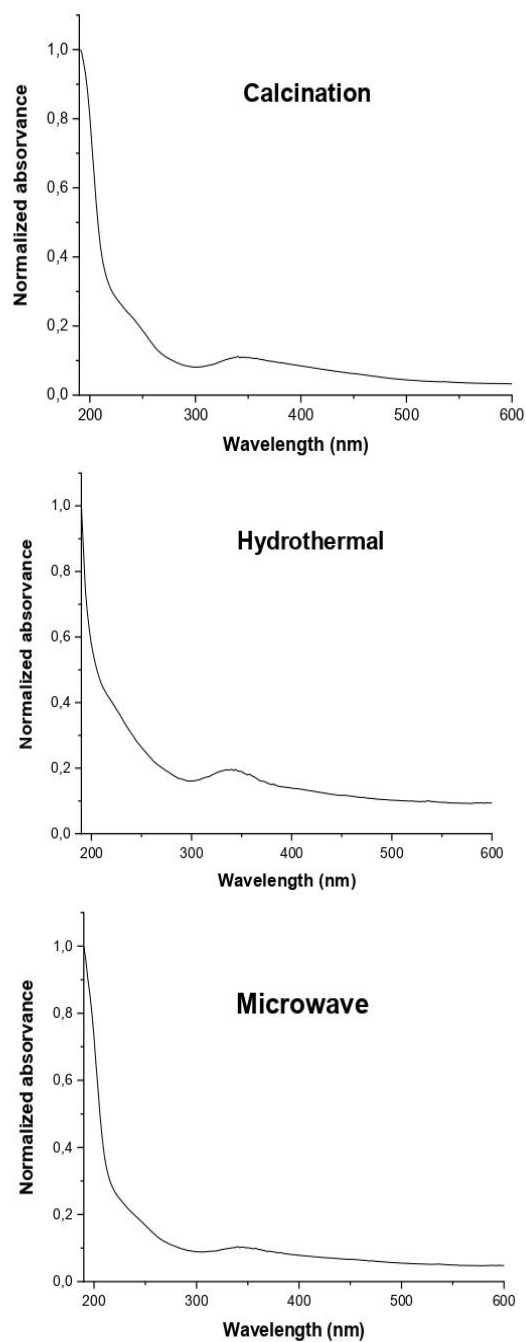


Figure S4 – Normalized absorbance spectra for calcinated- (top), hydrothermal- (middle) and microwave-based (bottom) C-dots, in aqueous solution.

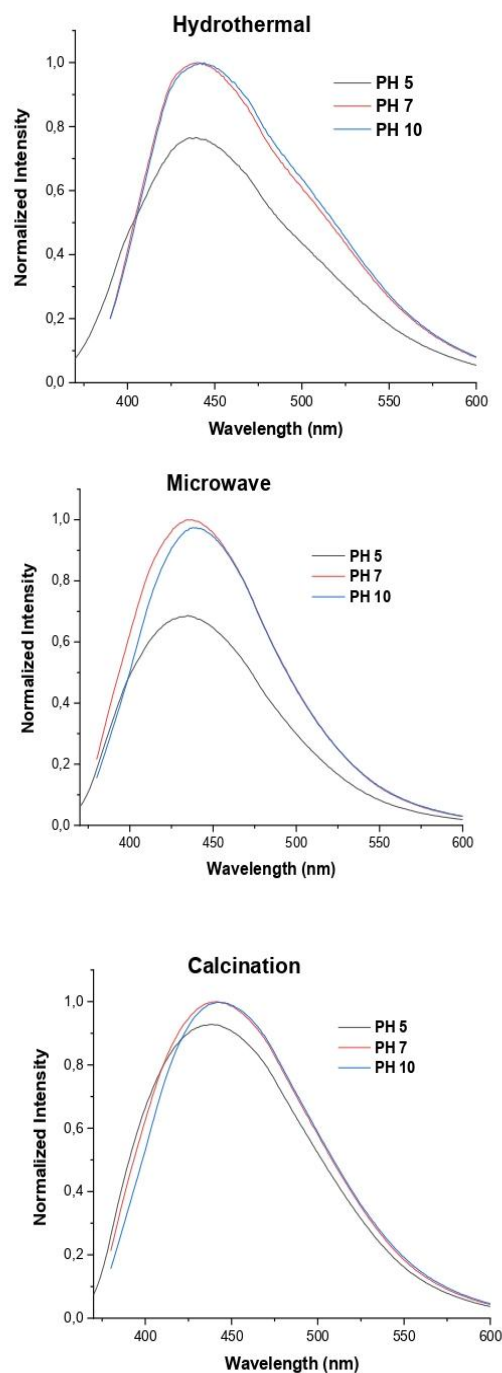


Figure S5 – Normalized emission spectra for hydrothermal- (top), microwave- (middle) and calcination-based (bottom) C-dots, in aqueous solution at different pHs (5, 7 and 10).

1.3. Conclusions

The increasing biomedical application of CDs is driven by their remarkable photoluminescence properties, photostability, biocompatibility, and biosafety. In this study, CDs were synthesized using different strategies, resulting in varied in vitro outcomes. Among the CDs tested, those produced via hydrothermal synthesis exhibited the lowest toxicity against normal breast MCF-10A cells, demonstrating high biocompatibility and biosafety. These hydrothermal-based CDs show potential for use in medicine as bioimaging and biosensing agents, as well as for drug or gene delivery.