M 2021

U.PORTO
FEUP FACULDADE DE ENGENHARIA
UNIVERSIDADE DO PORTO

SUSTAINABLE MICROCAPSULES PRODUCTION FOR TEXTILES APPLICATIONS

MARIA SUSANA REGO DINIS
MASTER'S DISSERTATION SUBMITTED TO
THE FACULTY OF ENGINEERING OF THE UNIVERSITY OF PORTO IN
CHEMICAL ENGINEERING

Master in Chemical Engineering

Sustainable Microcapsules Production for Textiles Applications

Master Dissertation

of

Maria Susana Rego Dinis

Developed within the course of dissertation

held in

HeiQ Iberia Unipessoal Lda. and Laboratory of Separation and Reaction Engineering





Supervisor at FEUP: **Dra. Isabel Martins**

Joint supervisor at FEUP: **Prof. Alírio Rodrigues**

Coordinator at HeiQ Iberia: **Dra. Raquel Vaz Vieira**





July 2021

CLASSIFIED DOCUMENT. USE ONLY FOR EVALUATION PURPOSES

Acknowledgment

I would like to sincerely thank my supervisor, Dra. Isabel Martins, for her continuous support, dedication and availability through all these past months. Her vast knowledge has positively influenced me. I also would like to thank my joint supervisor, Prof. Alírio Rodrigues, for his insightful comments and advice and to Dra. Yaidelin Manrique for her willingness to help me when I encountered difficulties and her assistance on obtaining SEM images.

I also want to thank to the *HeiQ Iberia* team, who supported and helped me. I really appreciate the opportunity to work with such amazing people. I want to thank Pedro for his availability to perform the antimicrobial tests. To Dra. Raquel Vaz Vieira, my sincere thanks for her empathy and support throughout the dissertation.

I thank my friends, who always made me smile, I know I can count on them through good and bad times. To my childhood friend Vivi, to my caring friend Joana, to my thoughtful friend Andreia, to my everlasting partner Joaninha, to my kindhearted buddy Edu, to my sincere friend Naná and to my cheerful friend Maria: Thank you all for saying the right words at the right time, for the patience, for the motivation and for all the good times we shared. I am sure our friendship will always be like this.

Last but not least, I would like to express my deepest gratitude towards my mother and my brother, for their unconditional love. All their support through all my life has been the driven key to who I am today. I hope from now on I will also be able to bring you many joys. And also, to my boyfriend, for his patience, love and undeniable support.

Abstract

Nowadays textile industries are increasingly more interested on innovative biobased products. In this context, the present project has the main goal to produce biobased and aroma-loaded microcapsules by complex coacervation at large scale production and impregnate them to textile fabrics. The biopolymers used as shell materials were chitosan and gum Arabic and the core materials were peppermint oil and thyme oil. Different parameters were evaluated throughout the present project: effect of biopolymers concentration, influence of the increase of lactic acid concentration, effect of speed and time on the homogenization step, influence of the emulsifier and, at last, influence of core material proportions. A biopolymers ratio, $R_{GA/CH,}$ of 2 was preferred since it decreased the aggregation observed when a ratio of 1 was used. The increase of lactic acid concentration to 1.5 % (w/v) and decrease of tannic acid 10 % (w/v) to 10 mL was beneficial since the residues at the bottom of the flask disappeared. A better emulsion was attained with a homogenization step of 9500 rpm with a time of 4 min, because no more surface stains were observed on the solution. PGPR was chosen as emulsifier since it achieved better results than Brij C2 on terms of dispersion. At last, the proportion of essential oils on the core material did not influence the microcapsules production. Afterwards, the produced microcapsules were characterized in terms of morphology, particle size, solid content and encapsulation efficiency. The produced microcapsules exhibited spherical shape and polynuclear morphology in all formulations. The mean particle size was between 10.7 to 42.6 µm, which is in accordance with the values reported on literature for complex coacervation. The solid content ranged from 3.90 % to 5.02 %, what is expected since the main constituent on the microcapsules solution is water. A high global encapsulation efficiency was achieved for two formulations, with values of (99.9±1.5) % and (99.9±1.9) %. Thereafter, the produced microcapsules were impregnated on two textile substrates with the pad-drying method and this impregnation was evaluated through scanning electron microscopy. The obtained images revealed that the smaller microcapsules, ranging from 2.5 to 20 µm, were resistant enough to the application process. The durability to wash was merely observed on a specific fabric. Another formulation containing citric acid on the immersive bath was also studied and it was verified the improvement of microcapsules attachment. At last, the antimicrobial activity of the two treated fabrics was evaluated and it was verified that one of them presented a bacterial reduction of 99.70 %, being considered as bacteriostatic. With this work, it was possible to demonstrate a methodology for the production of sustainable microcapsules for their application onto textile substrates.

Keywords: Textile industry, Complex Coacervation, Biopolymers, Textile Fabrics.

Resumo

Atualmente, as indústrias têxteis estão cada vez mais interessadas em produtos inovadores de base biológica. Neste contexto, o presente projeto tem como principal objetivo a produção de microcápsulas biodegradáveis e carregadas de aroma através da coacervação complexa em grande escala e a sua impregnação em tecidos têxteis. Os biopolímeros utilizados como materiais de revestimento foram quitosano e goma arábica e usou-se óleo de hortelã-pimenta e óleo de tomilho como princípios ativos. Foram avaliados diferentes parâmetros ao longo do presente projeto: efeito da concentração dos biopolímeros, influência do aumento da concentração de ácido láctico, efeito da velocidade de agitação e do tempo na etapa de homogeneização, influência do surfactante e, finalmente, influência das proporções do material do núcleo. Foi preferida uma razão de biopolímeros de 2, uma vez que diminuiu a agregação observada quando foi utilizada uma razão de 1. O aumento da concentração de ácido láctico para 1,5 % (p/v) e a diminuição do ácido tânico 10 % (p/v) para 10 mL foi benéfico uma vez que os resíduos no fundo do frasco desapareceram. Obteve-se uma emulsão mais estável usando uma velocidade de agitação de 9500 rpm com um tempo de 4 min, uma vez que não foram observadas manchas superficiais na solução. O PGPR foi escolhido como tensioativo uma vez que obteve melhores resultados do que com o Brij C2 em termos de dispersão. Finalmente, verificou-se que a proporção de óleos essenciais sobre o material do núcleo não influenciou a produção de microcápsulas. Posteriormente, as microcápsulas produzidas foram caraterizadas em termos de morfologia, tamanho das partículas, conteúdo sólido e eficiência da encapsulação. As microcápsulas produzidas exibiram forma esférica e morfologia polinuclear em todas as formulações. A dimensão média das partículas situou-se entre 10,7 e 42,6 μm, o que está de acordo com os valores reportados na literatura para a coacervação complexa. O conteúdo sólido variou entre 3,90 % e 5,02 %, o que é esperado uma vez que o principal constituinte da solução de microcápsulas é a água. Foi alcançada uma elevada eficiência global de encapsulação para duas formulações, com valores de (99,9±1,5) % e (99,9±1,9) %. Posteriormente, as microcápsulas produzidas foram impregnadas em dois substratos têxteis usando o método de *pad-drying*, sendo a impregnação avaliada através de microscopia eletrónica de varrimento. As imagens obtidas revelaram que as microcápsulas mais pequenas, entre 2,5 e 20 μm, eram suficientemente resistentes ao processo de aplicação. A durabilidade à lavagem foi apenas observada num tecido específico. Outra formulação contendo ácido cítrico no banho imersivo foi também estudada e verificou-se a melhoria da fixação das microcápsulas. Finalmente, foi avaliada a atividade antimicrobiana dos dois tecidos tratados e verificou-se que um deles apresentava uma redução bacteriana de 99,70 %, sendo considerado bacteriostático. Com este trabalho, foi possível demonstrar uma metodologia para a produção de microcápsulas sustentáveis para a sua aplicação em substratos têxteis.

Palavras-chave: Indústria têxtil, Coacervação Complexa, Biopolímeros, Tecidos têxteis.

Declaration

I hereby declare, under word of honor, that this work is original and that all non-original contributions were properly referenced with source identification.



Index

1 Introduction 1

1.1 Framing and presentation of the work 1

1.2 Presentation of the company 2

1.3 Contribution of the author to the work 2

1.4 Organization of the dissertation 3

2 Context and state of the art 4

2.1 Textile industry 4

2.2 Microencapsulation 5

2.3 Microencapsulation processes 6

2.4 Complex coacervation 7

2.5 Biodegradable polymers as coating material 11

2.5.1 Chitosan 11

2.5.2 Gum Arabic 12

2.6 Core materials: Essential oils 13

2.6.1 Peppermint oil 13

2.6.2 Thyme oil 14

2.7 Microcapsules application: Textile industry 14

3 Materials and methods 16

3.1 Materials 16

3.2 Production process of microcapsules 17

3.2.1 Laboratory scale microcapsules formation 17

3.2.2 Large-scale microcapsules formation 17

3.3 Characterization methods 19

3.3.1 Optical microscopy (OM) 19

3.3.2 Solid content 19

3.3.3 Particle size evaluation 19

3.3.4 Scanning electron microscopy (SEM) 20

3.3.5 Gas chromatography (GC/MS) 20
3.4 Application of microcapsules onto fabrics 21
3.5 Characterization of fabrics impregnated with microcapsules 23
3.5.1 Scanning electron microscopy (SEM) 23
3.5.2 Antimicrobial activity 23
4 Results and discussion 24
4.1 Microcapsules production 24
4.1.1 Effect of biopolymers concentration 26
4.1.2 Effect of increasing lactic acid concentration 27
4.1.3 Effect of speed and time on the homogenization step 28
4.1.4 Effect of the emulsifier 29
4.1.5 Effect of the proportions of the core material 30
4.2 Microcapsules characterization 32
4.2.1 Particle size evaluation 32
4.2.2 Encapsulation efficiency 34
4.2.3 Solid content 36
4.3 Fabric with impregnated microcapsules characterization 37
4.3.1 Simple coating method: pad-drying 37
4.3.2 Coating method with citric acid: pad-drying 39
4.3.3 Antimicrobial activity 40
4.3.4 Reduction rate of odor 40
5 Conclusion 41
6 Assessment of the work done 43
6.1 Objectives achieved 43
6.2 Limitations and future work 43
6.3 Final assessment 43
7 References 44
Appendix A – Optical and scanning electron microscopy images 47
A.1 – Optical microscopy images 47

A.2 – Scanning electron microscopy images 49

Appendix B – Effect of the speed and time at the homogenization step images 52

Appendix C - Particle size evaluation..... 53

Appendix D - Calibration curves 58

Appendix E - Percentage of each compound for all assays 59

Appendix F - Reduction rate of odor..... 60

List of Figures

Figure 1. Number of published articles over the last thirty years regarding microencapsulation on textile substrates, taken from the Scopus database, keywords " microencapsulation OR microcapsule OR microencapsulat AND textile OR garment OR fabric OR cloth".Scopus[7]. *4*

Figure 2. Morphology of microcapsules. Created with BioRender.com. Adapted from Ref.[16]. *6*

Figure 3. Diagram illustrating statistical distribution of the use of microencapsulation in different industries. Adapted from Ref.[12]. *8*

Figure 4. Schematic diagram of complex coacervation. Created with BioRender.com. Adapted from Ref.[6]. *8*

Figure 5. Schematic representation of Sharkawy et al. procedure [4]. *9*

Figure 6. Electrostatic attraction between NH3+ of chitosan and COO- of an anionic polymer. Created with BioRender.com. Adapted from Ref. [3]. *10*

Figure 7. Chitin and chitosan chemical structures. Created with BioRender.com. Adapted from Ref. [26]. *12*

Figure 8. Gum Arabic exudate. Adapted from Ref. [27]. *13*

Figure 9. Schematic representation of impregnating microcapsules to textile substrates. Adapted from Ref.[35]. *15*

Figure 10. Thermostatic bath, Hubber CC308 with compatible control CC3 (left) Jacketed batch reactor, IKA LR-2.ST (right): (1) reactor vessel; (2) ultraturrax; (3) overhead stirring drive. *18*

Figure 11. Experimental set-up used at HeiQ: (1) reactor vessel; (2) overhead stirring drive; (3) thermostatic bath. *18*

Figure 12. Optical microscope, ZEISS Axiotech 100 HD. *19*

Figure 13. Laser diffraction particle size analyzer, Beckman Coulter LS 230[36]. *20*

Figure 14. Scanning electron microscope, Phenom ProX Desktop [37]. *20*

Figure 15. Shimadzu GCMS-TQ8040 (left) Himac CT15 Series Tabletop High Speed Micro Centrifuge (right). *21*

Figure 16. Foulard, Rapid model P-A1 (left) Mechanical convection oven, Xutemp XT5118-IO140 (middle) Wascator, Eletrolux FOM71 CLS (right). *22*

Figure 17. Optical Microscopy Images of assay 1 (left) assay 2 (right) at magnification 100 x. *26*

Figure 18. Optical Microscopy Images of assay 3 (left) assay 4 (right) at magnification 100 x. *27*

Figure 19. Optical Microscopy Images of assay 5: magnification 100 x (left) and magnification 1000 x (right). *28*

Figure 20. Optical Microscopy Images of assay 6: magnification 100 x (left) and magnification 1000 x (right)..... 29

Figure 21. Optical Microscopy Images of (A) assay 7 at magnifications 100 x (left) and 1000 x (right) (B) assay 9 at magnifications 100 x (left) and 100 0x (right). 30

Figure 22. Optical Microscopy Images of formulation 8 at magnifications 100 x (left) and 1000 x (right)..... 31

Figure 23. Optical Microscopy Images of assay 10 at magnifications 100 x (left) and 1000 x (right). .. 32

Figure 24. Scanning electron microscopy images of the fabrics at magnification 1000 x: Untreated American Fleece Off White (left) Untreated Knit Beige (right). 37

Figure 25. Scanning electron microscopy images of the fabrics treated with formulation 8 at magnification 1000 x: American Fleece Off White Unwashed (left) American Fleece Off White Washed 20 x (right)..... 38

Figure 26. Scanning electron microscopy images of the fabrics treated with formulation 8 at magnification 3000 x: Knit Beige Unwashed (left) Knit Beige Washed 20 x (right). 38

Figure 27. Scanning electron microscopy images of American Fleece Off White fabric treated with formulation 8 and citric acid at magnification 3000 x. 39

Figure 28. Images relating to the visual appearance of: formulation 4 (left) formulation 5 (right). .. 52

List of Tables

Table 1. Patent examples regarding microencapsulation on textiles. 5

Table 2. Microencapsulation techniques: mechanical, chemical and physicochemical[3]. 6

Table 3. Reagents used and the respective function in the microcapsule production. 16

Table 4. Fabrics used and their respective compositions. 16

Table 5. Fabrics used and their respective WPU (%). 22

Table 6. Formulations for the different assays for the production of the microcapsules. 25

Table 7. Mean diameters and the standard deviations of the produced microcapsules. 32

Table 8. Quantification of the compounds of peppermint oil and their retentions time. 34

Table 9. Encapsulation efficiencies of formulation 5 and respective standard deviations. 35

Table 10. Encapsulation efficiencies of formulation 6 and respective standard deviations. 35

Table 11. Solid content of the produced microcapsules. 36

Table 12. Bacterial reduction (%) of the tested fabrics with formulation 8. 40

Table 13. Optical microscopy images of the microcapsules at the end of the process. 47

Table 14. Scanning electron microscopy images of the microcapsules at the end of the process. 49

Table 15. Particle size evaluation in volume and in number. 53

Table 16. Calibration curves for peppermint oil compounds. 58

Table 17. Percentage of each compound for all formulations. 59

Table 18. Odor reduction rate (%) of the textile substrate studied with formulation 8. 60

Notation and Glossary

m	Mass	g
t_r	Retention time	min
$R_{CH/GA}$	Biopolymers mass ratio (chitosan/gum Arabic)	
$R_{GA/CH}$	Biopolymers mass ratio (gum arabic/chitosan)	

Indexes

i	Compound
®	Registered trademark symbol

List of Acronyms

GA	Gum Arabic
CH	Chitosan
AA	Acetic Acid
LA	Lactic Acid
HLB	Hydrophilic-Lipophilic Balance
PGPR	Polyglycerol Polyricinoleate
TA	Tannic Acid
DDA	Degree of Deacetylation
PO	Peppermint Oil
TO	Thyme Oil
OM	Optical Microscopy
SEM	Scanning Electron Microscopy
GC	Gas Chromatography
MS	Mass Spectrometry
EE	Encapsulation Efficiency
WPU	Wet Pick Up
WOF	Weight Of Fabric
CFU	Colony-Forming Unit
PET	Polyethylene Terephthalate
IVA	Isovaleric Acid

1 Introduction

1.1 Framing and presentation of the work

Textile industry was once seen as a field to merely produce traditional technologies, which under basic specifications, had the main goal to protect consumers' bodies. With the continuous evolution of disruptive technologies, textile industry kept the pace of innovation working on smart textiles[1]. Those present functional properties, leading to higher added value textiles. Accordingly, textile field has grown all over the years achieving wider market niches by developing more product variants that meet consumers' demand. As so, microencapsulation was introduced in the market as a technology capable of producing innovative products with additional properties when compared to traditional ones, namely addition of fragrances, antimicrobials or insect repellents[1, 2].

Microencapsulation is known as a technique employed to surround a certain microscopic amount of a compound/active principle within a continuous film of polymers to obtain microcapsules for protection of the core material or to have a controlled release of it[1-3].

How the environment is affected through all the processes used to produce certain technologies has been of great concern among researchers and consumers. Therefore, enterprises are interested on developing sustainable and eco-friendly procedures and products, thus achieving differentiation from competition. Accordingly, natural, non-toxic and biodegradable materials are considered through all the microencapsulation procedure. The present work is based on a previous one [4] where microencapsulation was carried out by complex coacervation on laboratory scale. As so, aroma-loaded microcapsules will be produced using biopolymers, chitosan and gum Arabic, as the shell materials and essential oils, peppermint oil and thyme oil, as the core materials. Moreover, it is important to highlight that the present work wishes to scale-up the former, utilizing different active principles.

The present project will be followed according to the succeeding parts:

1. The first part is dedicated to the production of microcapsules in a batch reactor equipped with stirring and temperature control. The procedure is the same as the former work [4], although with adjusted modifications to scale-up in order to obtain an appropriate process.

2. The next part is committed to evaluate the properties of the produced microcapsules. In order to support the feasibility of the microcapsules, five characterization methods are applied: optical microscopy and scanning electron microscopy to examine microcapsules morphologies; laser dispersion to determine the size distribution in number and volume; solid content determination to calculate the solid content present in the microencapsulation solution; and gas chromatography to quantify indirectly the active substance encapsulated and, consequently, the encapsulation efficiency.
3. The final part is responsible for the impregnation of microcapsules on textile substrates and the evaluation of their behavior.

1.2 Presentation of the company

HeiQ is an innovative Swiss company specialized in smart and functional materials and technologies for textile industry directed to sectors of sports, home textiles, clothing and medicine. Founded as a spin-off from the Swiss Federal Institute of Technology Zurich (ETH) in 2005, *HeiQ* nowadays develops effective technologies on the market that can cool, repel, purify and stop viruses. Through continuous innovations, the company is dedicated to manufacture everyday products more useful and sustainable. Integrating scientific research, materials manufacturing and ingredient-branding, *HeiQ* was responsible for establishing over 200 technologies in partnership with major brands such as Patagonia, Speedo, Dickies, Zara, Burton and Burberry[5].

1.3 Contribution of the author to the work

The work developed throughout the present project was based on the work elaborated by Sharkawy et al.[4] that studied microencapsulation of limonene and vanillin with chitosan and gum Arabic as shell materials on laboratory scale. Gil et al. also followed up that work by increasing the scale production of microcapsules containing limonene[3]. The present project is differentiated by a further increase in the scale of production and a new study of different active principles: peppermint oil and thyme oil. Furthermore, the solvent used to dissolve chitosan was replaced by a natural one, which resulted on the production of fully biobased microcapsules.

The microencapsulation shots were performed on *LSRE – Laboratory of Separation and Reaction Engineering* in association with *HeiQ Iberia Unipessoal Lda*.

Additionally, the present project is presented as an evidence of the continuously interested topic of microencapsulation on scientific and industrial research.

1.4 Organization of the dissertation

The present project presentation is summarized in six chapters. The first chapter "Chapter 1: Introduction" underlines the importance of disruptive technologies to achieve industrial competitiveness and, as so, microencapsulation arises as an innovative technique to produce functional textiles. Also, the main objectives and the basic structure of the thesis is presented.

"Chapter 2: Context and state of the art" approaches the principal concepts of the topic, as the shell materials and active substances used, as well as microencapsulation techniques and its applications on textile fabrics.

"Chapter 3: Materials and methods" describes the microencapsulation technique utilized, the material to do so, the impregnation of the microcapsules onto the fabrics and the employed methods to characterize the produced microcapsules and treated fabrics.

"Chapter 4: Results and discussion" is dedicated to the presentation and discussion of the results obtained throughout the project.

"Chapter 5: Conclusions" presents the main conclusions, framing the obtained results with the objectives of the work.

At last, "Chapter 6: Assessment of the work done" presents an evaluation of the work in terms of the objectives achieved, mentioning the limitations encountered and proposing future work ideas.

All the contents that support this project are referenced on "Chapter 7: References".

2 Context and state of the art

2.1 Textile industry

Nowadays, enterprises are concerned about keeping the pace of innovation, since technological evolution is one of the most important drivers of the competitive success. Therefore, companies tend to develop disruptive technologies to reach market differentiation, and consequently, leading to profit. The development of eco-friendly procedures and products is increasingly more valued for consumers in general, guiding enterprises to arise with green protocols, thus achieving differentiation from traditional textile companies.

Microencapsulation arises in the 1990s as an innovative technology that, later, led to functional textiles, emerging many differentiated commercial products with irreverent properties[1, 2].The continuous study of this technique brought the development of additional benefits to the textile itself, such as UV-protection, antioxidant, antimicrobial, thermal regulation, among others[3, 6]. These newly innovative features attract consumers attention and directly add enterprises value[6].

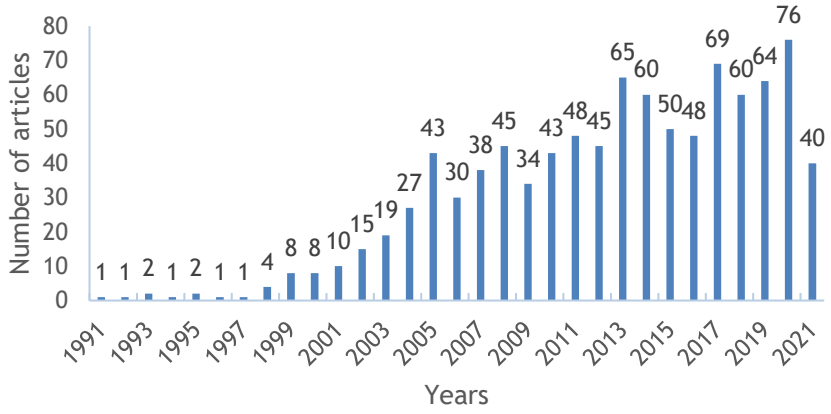


Figure 1. Number of published articles over the last thirty years regarding microencapsulation on textile substrates, taken from the Scopus database, keywords " microencapsulation OR microcapsule OR microencapsulat AND textile OR garment OR fabric OR cloth".Scopus[7].

Despite some oscillations, the number of articles on microencapsulation regarding textile substrates has been raising all over the years, as shown in Figure 1. This proves the growth of basic research on this topic. Industrial research is also invested in this

technology since the number of patents has been increasing as well. In the next table some patents related to microencapsulation on textile fabrics are presented.

Table 1. Patent examples regarding microencapsulation on textiles.

Year	Patent	Patent applicant	Assignee
2010	Functional textile material provided with microcapsules containing an active ingredient and use thereof	Bohringer [8]	Blucher GmbH (Erkrath, DE)
2011	Finished fibers and textiles	Copete Vidal [9]	Cognis IP Management GmbH (Duesseldorf, DE)
2017	Process of treatment of fibers and/or textile materials	Viladot Petit [10]	Lipotec, S.A. (Gava, ES)
2019	Microcapsules containing microorganisms	Vandendaele [11]	Devan Chemicals NV (Ronse-Renaix, BE)

2.2 Microencapsulation

Microencapsulation is a technique used to enclose a certain compound, such as a gas, liquid, or solid material, coating it with a continuous film of polymeric material, thus assembling particles in the micrometer range. This strategy provides not only protection for the active principle, as its controlled release under specific conditions over time[1, 3, 4].

Due to its intrinsic versatility, microencapsulation has been studied for several years in order to be implemented in a wide range of industries: pharmaceutical, medical, cosmetic, food, textile and so on[6, 12]. Regarding the textile field, many interesting properties can be improved by the encapsulation of an adequate active principle capable of adding value to the fabrics. Microencapsulation allowed sensitive substances, such as essential oils, microorganisms, and insecticides, to be impregnated on textiles[13]. Moreover, microencapsulation provides physical protection for the internal material from environment conditions that are not favorable, essentially pH and temperature[4].

The produced particles are denominated microparticles and, typically, their size is between 1 and 1000 μm. Depending on microparticles morphology, they can be designated microcapsules or microspheres[1, 14]. Microcapsules are comprised of a core which contains the active principle (core material) and a wall material that surrounds it. They are characterized for the complete entrapment of the active substance by a

different coating material forming a reservoir, whereas microspheres, also known as matrix-type microcapsules, are identified when the active principle is homogeneously dispersed throughout its matrix[14, 15].

Furthermore, microcapsules can be classified into mononuclear or polynuclear depending on the core material used and the deposition process of the capsule material. Just as the name implies, mononuclear microcapsules have a homogeneous core surrounded by a continuous film (shell) of capsule material. On the other hand, polynuclear microcapsules have small heterogeneous particles of the active substance dispersed throughout the core region[15, 16].

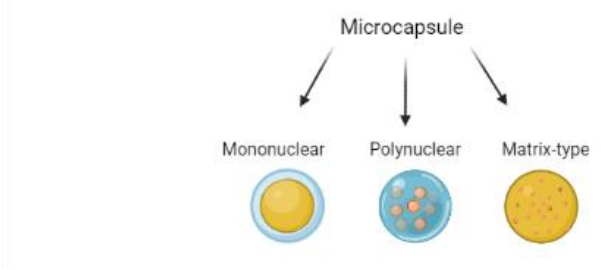


Figure 2. Morphology of microcapsules. Created with BioRender.com. Adapted from Ref.[16].

2.3 Microencapsulation processes

Generally, microencapsulation is consisted by three fundamental stages: core material entrapment, production of microcapsules and harden of microcapsules shell. There are three distinguished groups of microencapsulation technologies differentiated on microcapsules' formation mechanism: mechanical, chemical, and physicochemical processes[1]. The microencapsulation technique selected is related to the desired characteristics of the final product. Table 2 discloses some examples of microencapsulation techniques.

Table 2. Microencapsulation techniques: mechanical, chemical and physicochemical[3].

Mechanical	Chemical	Physicochemical
Spray drying	Interfacial polymerization	Simple coacervation
Spray cooling	In situ polymerization	Complex coacervation
Pan coating	Emulsion crosslinking	Molecular inclusion

Mechanical processes are known by the previous polymeric reaction and, only afterwards, the introduction of the core material. Spray drying, spray cooling, fluidised bed coating, pan coating and solvent evaporation are some examples of mechanical techniques[3, 17, 18].

Microparticles must have an adequate size to be impregnated on textile substrates. Besides microparticles with a mean size between 20-40 μm are acceptable, the ones presenting mean diameter of 1-10 μm are recommended. As mechanical processes tend to produce microcapsules with high mean diameters, textile field is more focused on chemical and physicochemical methods[1].

Chemical processes, as interfacial polymerization and in situ polymerization, are based on polymeric reaction occurring on the interface with the core material[3, 17].

Physicochemical processes are related to conditions that may modify solubility of polymers, leading to their precipitation. Phase coacervation is a physicochemical technology that can be divided in two categories: simple coacervation and complex coacervation[3, 17]. It can be described as the separation of a homogeneous macromolecular solution into two immiscible liquid phases: a dense coacervate phase constituted mostly on polymer and a dilute equilibrium phase which is polymer poor[1, 4, 18]. Coacervation can be induced in different manners, such as pH changes, temperature oscillations or addition of a substance[4]. For instances, simple coacervation occurs when only one polymer is induced to precipitate by changing pH, alteration on temperature or addition of a hydrophilic substance, causing the formation of the two phases aforementioned[4, 17, 19].

2.4 Complex coacervation

Complex coacervation can be characterized as the phase separation raised by the electrostatic attraction of two oppositely charged polymers, leading to the aggregation of the newly formed coacervates around the core material that is emulsified in the reaction media[3, 4, 12].

Microencapsulation by coacervation is a widely used technique to produce microcapsules, since it is noted for its capacity of loading significant amounts of the active principle and for its controlled release[20]. It may be used to design microencapsulation systems with a hydrophobic core to improve beneficial properties, as preservability, miscibility, and deliverability[12].

Additionally, complex coacervation makes microencapsulation more attractive to industries because of its non-necessity of organic solvents, easy operation, low cost, and easily applicable for large scale[3, 4, 6]. Nevertheless, complex coacervation presents the limitation of not being suitable for encapsulating hydrophilic core materials[4].

Given the applications' diversity in microencapsulation, the products obtained by complex coacervation are implemented in many fields, as can be seen in the Figure 3.

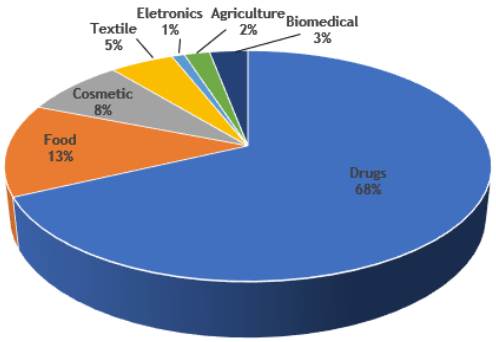


Figure 3. Diagram illustrating statistical distribution of the use of microencapsulation in different industries. Adapted from Ref.[12].

Microencapsulation by complex coacervation is graphically summarized in the Figure 4. First, biopolymers are mixed with the core material and the emulsifier. Thus, droplets of the core material are dispersed on polymer solution. Once adjustments in pH or temperature occur, the polymers present maximum opposite charges, promoting the electrostatic attraction between them and, consequently, coacervates formation. As so, phase separation takes place and the coacervates tend to concentrate around oily droplets of the core material. Thereafter, the microcapsule shell is formed and hardened by means of a hardening agent[3, 4, 19].

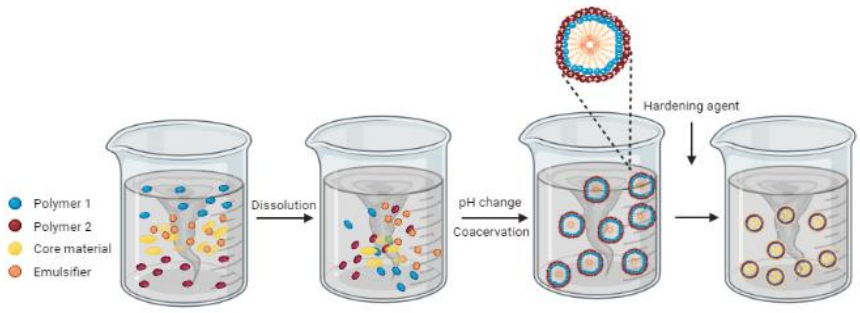


Figure 4. Schematic diagram of complex coacervation. Created with BioRender.com. Adapted from Ref.[6].

Studies regarding complex coacervation with the use of natural polymers have been growing continuously. Proteins and polysaccharides are polymers that promote natural-origin systems for complex coacervation. Gelatin, as a product obtained by collagen hydrolysis, has been studied exhaustively with a variety of other polysaccharides, such as Gum Arabic (GA), Pectin, Chitosan (CH) and so on[20, 21]. Generally, food industry employs gelatin as a shell material on microencapsulation by complex coacervation[12]. However, this primary protein is very viscous[4]. Hence, the need to study new pairs of natural materials to encapsulate microcapsules was continually increasing. Chitosan, as a positively charged polyelectrolyte, and Gum Arabic, as a negatively charged polyelectrolyte, have emerged as an efficient pair. Sharkawy et al. produced microcapsules with the referred pair in small-scale with limonene and vanillin as the active principle[4]. The procedure followed in the previous study was used as a guidance throughout this project. Figure 5 summarizes the steps taken by Sharkawy et al.

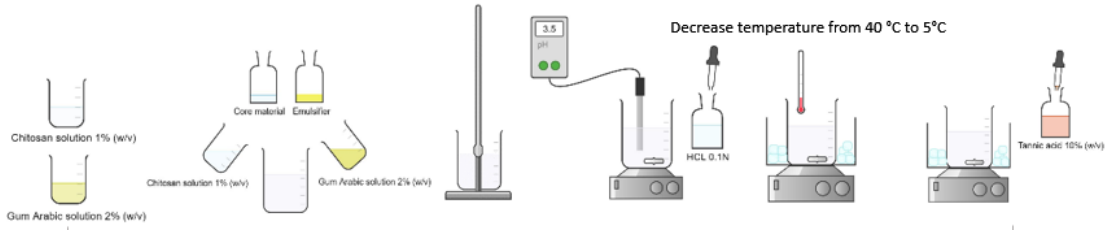


Figure 5. Schematic representation of Sharkawy et al. procedure [4].

Firstly, biopolymers solutions were prepared. Whereas chitosan was dissolved in acetic acid (AA) and left under mechanical stirring for 15 h, gum Arabic was dissolved in deionized water at 45 ºC for 2 h. Afterwards, the referred solutions were mixed, and the active principle and emulsifier were added. The emulsification then occurred with the use of a laboratory homogenizer at 40 ºC and 8000 rpm for 1 min. Subsequently, pH value was lowered to 3.5 with the use of a hydrochloric solution 0.2 N and the speed rate to 400 rpm. Thereafter, temperature was lowered from 40 ºC to 5 ºC with the help of an ice bath. As the solution mixture achieved 5 ºC, hardening agent was used to toughen the microcapsules shell. At last, microcapsules were separated by decantation and recovered[4].

The polymers ionization is triggered when the pH of the solution is calibrated to an approximate value of the pKa range of both polymers. Chitosan and gum Arabic underline specific reactive sites on their chemical structures at a low pH. The electrostatic attraction is stablished between the amino groups of chitosan and the

carboxylic groups of gum Arabic, thus, promoting the formation of polyelectrolytic complexes[3].

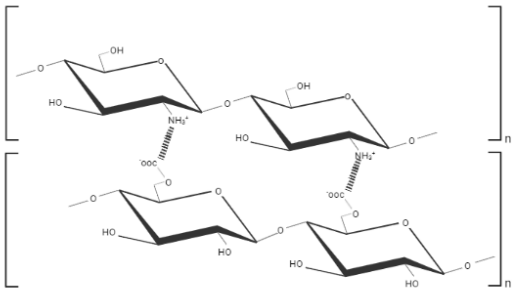


Figure 6. Electrostatic attraction between NH3+ of chitosan and COO- of an anionic polymer. Created with BioRender.com. Adapted from Ref. [3].

In addition to the importance of biopolymers on the dispersed systems, emulsifiers are crucial to microencapsulation procedure. Emulsifiers surround the oil droplets and as they positioned between the two immiscible phases, the emulsion is stabilized. Thus, they can protect hydrophobic materials and increase their solubility in aqueous solutions[6]. These surfactants molecules contain both hydrophobic and hydrophilic parts. As so, emulsifiers are known to have an amphiphilic nature. For this reason, they diminish surface tension when are present at an interface, such as oil-in-water. Hydrophilic-lipophilic balance, also known as HLB, is a measure value that relates the ratio of hydrophilicity/lipophilicity capacity of a certain surfactant. It is an important factor since it influences the capture of oil droplets into coacervates and the later tends to secure hydrophobic substances[6]. Stability, texture, and mechanical properties of microcapsules depend on the emulsifier utilized[12]. Among other surfactants, polyglycerol polyricinoleate (PGPR) is commonly used in the production of microcapsules[6].

The hardening agent is also responsible for improving microcapsules' mechanical properties. Toxic aldehydes, such as formaldehyde, are mentioned in literature as traditional cross-linking agents for the shell materials since they are known to have good thermal stabilities. However, apart from not being recyclable, these polymers are hazardous to human health[22]. Thereby, hardening agents that are eco-friendly and benign, such as tannic acid (TA), are continuously required. Tannic acid is a natural polyphenol belonging to the hydrolysable tannin category detected in most of aerial plant tissues[3, 23]. It has the capability of stablishing bonds to polymers through hydrogen bonding and hydrophobic interactions. Essentially, the hydroxyl groups

present in the chemical structure of tannic acid form hydrogen bonding with the amino groups of chitosan[3, 4].

2.5 Biodegradable polymers as coating material

Research and development regarding natural, eco-friendly, and inexpensive polymeric coating materials for microencapsulation has increased prominently. Biodegradable polymers used as shell material to microcapsules can be either natural or synthetic. Nonetheless, natural polymers are of more interest at the economic and environmental point of view, given that synthetic polymers are difficult to biodegrade[20].

2.5.1 Chitosan

Followed by cellulose, chitin is the second most plentiful polysaccharide in nature and can be found on exoskeletons of insects, shells of crustaceans and cell walls of fungi. Chitin may undergo alkaline treatment, which promotes deacetylation. This process refers to the removal of N-acetyl group from chitin and replacement by reactive amino groups to obtain chitosan[4, 20, 24].

Chitosan is a natural cationic polysaccharide constituted of ß-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine[25]. It has been of great relevance to research areas of alternatives to traditional polymeric wall materials, due to its nontoxicity, biodegradability, and relative abundance in nature[20, 24]. The degree of deacetylation (DDA), which dictates the molar fraction of free amino groups in chitosan, may affect its properties[25]. A higher DDA stands for a higher charge density and, consequently, a better complexation potential[4].

Within acidic solutions, polysaccharides are generally neutral or anionic. However, chitosan exhibits positive charge under those conditions, since its amine groups are mainly positive at pH below 6.5. Thereby, when combined with anionic or neutral polymers, pH is a crucial factor to promote electrostatic attraction between polymers, and consequently, complexes formation[3, 25]. Besides, chitosan solubility is affected not only for its DDA but also for environmental pH[3, 4].

Despite not recognizing the certain mechanism that undergoes between chitosan and bacteria, chitosan has been reported as antibacterial. Some theories proposed its positive charge may be seen as a starting point for fully explanation[25]. Once more, DDA and pH are related to the charge density of chitosan, and therefore on its antibacterial activity. At low pH, the amino groups of chitosan exhibit positive charge, stimulating the attachment to the negatively charged components on the bacterial cell

wall. Therefore, an impermeable layer is formed, hindering vital substances transport into the cell, ending up causing its death. Gram-negative bacteria introduced a higher negative charge on the cell surface than gram-positive bacteria, resulting in a more effective antibacterial activity towards gram-negative bacteria[25].

An important factor to consider on textile industries is the linkage between the polymeric material of the microcapsules and the fabrics used. Notwithstanding chitosan solution has not a strong chemical bond with textiles, chitosan microcapsules overcome it by conferring a large surface area, and consequently, promote a strong contact with fabrics. Additionally, chitosan nanoparticles have shown an effective antibacterial activity against *E.coli*, *S. choleraesis*, *S. typhimurium* and *S. aureus* in textiles[24].

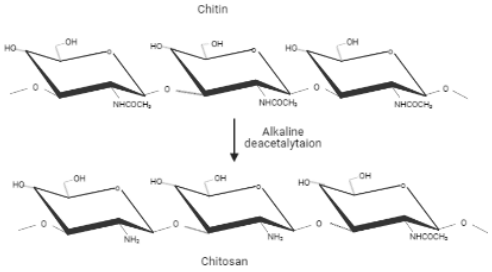


Figure 7. Chitin and chitosan chemical structures. Created with BioRender.com. Adapted from Ref. [26].

2.5.2 Gum Arabic

Gum Arabic is the oldest and most popular gum among all-natural ones, presenting itself as a dried gum-like exudate of the trunks and branches of *Acacia senegal* and *Acacia seyal* trees, Family Leguminosae. This branched polysaccharide is constituted of a complex mixture of polysaccharidic acids, such as calcium, magnesium and potassium. Structurally, gum Arabic is composed of L-arabinose, L-rhamnose, D-glucuronic acid and 1,3-linked ß-D-galactopyranosyl units[3, 4, 27, 28].

The main branch of gum Arabic consists of 1,3-linked ß-D-galactopyranosyl units, whereas the side chains have two to five 1,3-linked ß-D-galactopyranosyl units connected to the main branch by 1,6-linkages[28].

Gum Arabic is a negatively charged polyelectrolyte and is commonly used in industry given its microencapsulation properties, low viscosity, high solubility in water and high ability to preserve volatile compounds[3, 19]. When encountered at acidic conditions,

hydroxyl groups of gum Arabic enhance environmental hydrophilicity, which increases its emulsifying ability[3]. Also, gum Arabic extracts manifests antimicrobial activity[28].

Due to the referred properties, a great range of industries are interested in using gum Arabic, whether for food, pharmaceutical or cosmetic products. Textile industry may resort gum Arabic to stiffen the printing pastes used in the coloration of cellulose fabrics[4].



Figure 8. Gum Arabic exudate. Adapted from Ref. [27].

2.6 Core materials: Essential oils

There has been an increasing interest in researching essential oils and their properties to produce natural and biodegradable products for human health. Essential oils are susceptible to volatilization in the presence of light, heat, and oxygen. Therefore, essential oils do not have a long shelf-life. To counteract such a fact, microencapsulation rises as an alternative, providing essential oils resistance to different environmental conditions. It has been reported that microencapsulation resulted in an effective process to stabilize essential oils, thus increasing their storage life. Essential oils microencapsulation is increasingly relevant to textile industries[12, 20].

2.6.1 Peppermint oil

Peppermint, *Mentha piperita L.*, belonging to the family Lamiaceae is a hybrid mint of *Mentha aquatica L.* and *Mentha spicata L.* [29]. *Mentha piperita L.* is located on many regions across the globe for its ability to adapt to rather distinct climatic conditions[29, 30].

Peppermint oil (PO) is extracted from the undersides of Mentha piperita L. leaves by steam distillation[31]. It is composed of approximately ten compounds: limonene (1.0-5.0 %), menthofuran (1.0-9.0 %), cineole (3.5-14.0 %), isomenthone (1.5-10.0 %), menthyl acetate (2.8-10.0 %), isopulegol (maximum 0.2 %), pulegone (maximum

4.0 %), carvone (maximum 1.0 %), being the main constituents menthone (14.0-32.0 %) and menthol (30.0-55.0 %)[31].

This essential oil is widely used due to its odor, flavor and pharmaceutical properties, being one of the most fabricated essential oils[29]. Even though peppermint oil is known as a non-toxic essential oil, it may damage skin and mucus membranes[31].

2.6.2 Thyme oil

Thyme, *Thymus vulgaris*, is a blooming plant belonging to the family Lamiaceae that grows in southern Europe[32].

Thyme oil (TO) is the essential oil extracted from the leaves of *Thymus vulgaris* by steam distillation, steam diffusion or hydro-distillation processes. Sadjia et al. reported that steam distillation and steam diffusion were profitable in terms of extraction time[33]. Six compounds are identified on the present essential oil: thymol (40.5 %), p-cymene (23.6 %), carvacrol (3.2 %), linalool (5.4 %), ß-caryphyllene (2.6 %), and terpinen-4-ol (0.7 %)[34].

Thyme oil is acknowledged for its plentiful medicinal properties: antiseptic, diuretic, disinfectant, bactericidal, antioxidant, antibacterial and antifungal. The later three may be explained by presence of thymol and carvacrol on its composition since these phenolic compounds are studied for emphasizing those properties[32, 33].

2.7 Microcapsules application: Textile industry

Microcapsules application on textile substrates is the main part of the final stage for obtaining functional products and can be concluded through different processes, such as padding, spraying, dipping, grafting. Chemicals and substrates established are responsible for dictating the chosen method. In the case of sharing great affinity, batch processes resorting exhaustion after dyeing are appropriate. However, when this is not the case, continuous processes are preferred by textile immersion on the finishing chemical or even appealing mechanical manners[6]. Furthermore, the selection of microcapsule impregnation technique affects not only the release of microcapsules content over time, but also the finish resistance on the fabric after significant amount of washing steps[3, 35].

Padding is the most well-known technique employed to incorporate finishing materials onto textile substrates. This method involves dipping the material in a solution and pressing it in rollers. After padding operation, the substrate must release water by passing through a drying process using heat convection, microwaves, or vacuum.

Subsequently, it is cured by temperature increase higher than the one required in drying process[6].

Whereas in curing physical forces sustain the connection to the substrate, in grafting the microcapsules are linked chemically to the textile. In this method, the monomers are bonded to the polymer chain by covalent bonds[6].

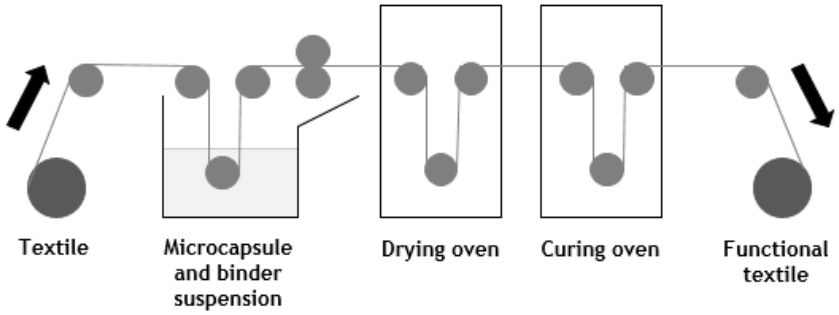


Figure 9. Schematic representation of impregnating microcapsules to textile substrates. Adapted from Ref.[35].

In all the referred techniques, it may be required a binder agent to use upon textiles without compromising the color or touch of the product. Generally, those additives are crosslinking agents, binders, fillers, or surfactants[35]. Binders, being polymeric resins, are suggested to use on microencapsulation formulations for an efficient fixation of the finish material on the fabrics[3, 4]. However, binders may restrict the microcapsules content release[4]. Thereby, crosslinking of microcapsules to textile substrates is noteworthy. Crosslinking comprises a chemical reaction which is focused on allowing the connection between reactive sites on both polymer chain and crosslinking agents[6]. In the example of using cotton cellulose fabrics, crosslinking is dictated by an esterification reaction occurred between the present carboxyl groups on the crosslinking agent and the hydroxyl groups of the cellulose or the polymeric material[4]. Apart from dictating which type of bond is established, the crosslinker affects important properties: permeability, thermal and chemical stability, and the microcapsule content release[6].

Citric acid is a nontoxic organic acid found on citrus fruits. It has been studied to react with polysaccharides, such as cellulose, for enhancing their characteristics on textile applications. Also, is highlighted in literature as a crosslinking agent in proteins and polysaccharides[6]. As a non-formaldehyde based cross-linker, citric acid is reported as a safe and eco-friendly choice over formaldehyde-based ones[4].

3 Materials and methods

3.1 Materials

The microencapsulation production process required numerous reagents that are present on Table 3. Additionally, the reagents that were used to prepare the peppermint oil standard solutions to evaluate the encapsulation efficiency are disclosed. The respective function of each substance is also identified.

Table 3. Reagents used and the respective function in the microcapsule production.

Compound	Function	Supplier
Chitosan	Shell material	BioLog Biotechnologie Und Logistik
Gum Arabic	Shell material	Sigma Aldrich®
Acetic Acid 0.1 N	Polymer solvent	Alfa Aesar
Lactic Acid	Polymer solvent	Sigma Aldrich®
Peppermint Oil	Core material	*HeiQ*
Thyme Oil	Core material	*HeiQ*
PGPR 4150	Emulsifier	Palsgaard
Brij C2 MBAL-SO-(SG)	Emulsifier	Sigma Aldrich®
Hydrochloric acid 0.1 N	pH adjustment	Merck
Hydrochloric acid 0.2 N	pH adjustment	Sigma Aldrich®
Tannic acid	Hardening agent	Merck
n-hexane	Standard solutions	Merck
Sodium chloride	Standard solutions	Sigma Aldrich®
Anhydrous sodium sulphate	Standard solutions	José Manuel Gomes Santos, Lda
Citric acid	Crosslinking agent	*HeiQ*

The fabrics that were used for the application of the produced microcapsules are listed on Table 4. The dimensions were approximately of a A4 paper (21.0 cm x 29.7 cm).

Table 4. Fabrics used and their respective compositions.

Fabric	Color	Composition
American Fleece	Off White	50 % Organic cotton and 50 % Recycled cotton
Knit	Beige	100 % Recycled polyester

3.2 Production process of microcapsules

The production process of microcapsules was based into three fundamental stages: emulsification, induction of complex coacervation and hardening of the produced microcapsules.

Numerous formulations were developed to evaluate parameters that influence the microcapsules properties. The production of microcapsules was initially made on small-scale and subsequently on large-scale. The formulations of both scales are considered throughout this section. Although with some modifications, the experimental method used to produce microcapsules was adapted from procedures disclosed in preceding reports[3, 4]. In addition to the production of the microcapsules, these were applied to textile fabrics. Thus, their application is described, and the characterization of such textile fabrics is also disclosed in this section.

3.2.1 Laboratory scale microcapsules formation

The procedure explained in this section refers to a specific formulation, whereby the chemical system of each formulation is disclosed on Table 6. First, chitosan solution 1 % (w/v) was prepared by adding 0.5 g of chitosan to 50 mL of a solvent solution, acetic acid or lactic acid (LA), and left under continuous stirring overnight. Likewise, Gum Arabic solution 2 % (w/v) was obtained by adding 1 g of gum Arabic to 50 mL of deionized water and left under stirring at 45 ºC for 2 h. The biopolymers were mixed at approximately 40 ºC and a known amount of the core material and emulsifier were added. The emulsification occurred when the solution was subjected 1 min to the Ultra-turrax IKA DI 25 Basic at 8000 rpm. Complex coacervation was then triggered by lowering the pH to 3.5 with the use of hydrochloric acid solution, 0.1 N or 0.2 N, under magnetic stirring at 400 rpm. When the desired pH was achieved, the solution continued to be stirred for 30 min. To complete the complex coacervation induction, the temperature solution must decrease to 5 ºC with the help of an ice bath. The hardening step was done by adding dropwise tannic acid solution to the mixture at 5 ºC. Finally, the solution was left under magnetic stirring at 400 rpm and 5 ºC for3 h. It is important to refer that, unlike the base studies, in the present project no phase separation was carried out.

3.2.2 Large-scale microcapsules formation

The experimental procedure was identical to the one described previously until the induction of complex coacervation. Soon the pH reached the set value, the mixture

was placed on the jacketed batch reactor IKA LR-2.ST at 40 ºC and 100 rpm for 30 min. Afterwards, the temperature was decreased to 5 ºC with the help of a thermostatic bath and at that temperature a certain amount of tannic acid solution was added dropwise to the mixture. Lastly, the solution was left under stirring at 100 rpm and 5 ºC for 3 h. Table 6 shows the volumes of the mixture in the reactor for every assay.

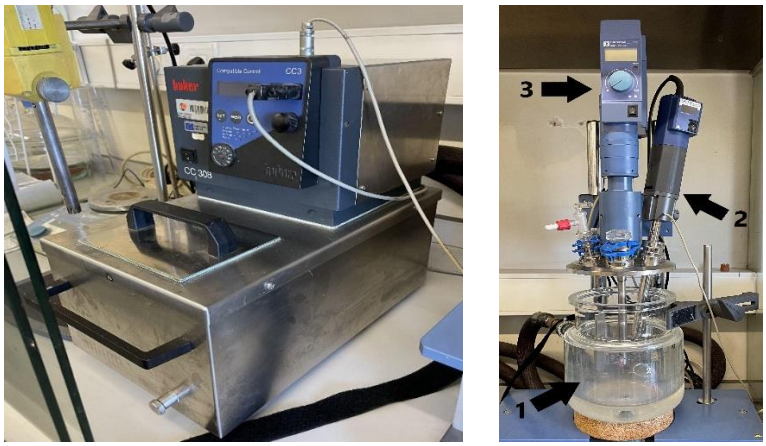


Figure 10. Thermostatic bath, Hubber CC308 with compatible control CC3 (left) Jacketed batch reactor, IKA LR-2.ST (right): (1) reactor vessel; (2) ultraturrax; (3) overhead stirring drive.

The stages done at large-scale were based on the experimental procedure reported by Gil et al.[3]. The experimental system used for the large-scale trials is composed of a jacketed batch reactor IKA LR-2.ST with the software labworldsoft 4.5 and a thermostatic bath Hubber CC308 with compatible control CC3. On the other hand, the experimental system used on *HeiQ* laboratory to replicate a specific formulation is presented on Figure 11.

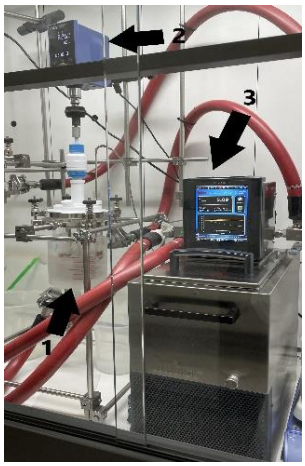


Figure 11. Experimental set-up used at HeiQ: (1) reactor vessel; (2) overhead stirring drive; (3) thermostatic bath.

3.3 Characterization methods

3.3.1 Optical microscopy (OM)

Optical microscopy is a common analysis technique used to observe a certain sample at different magnifications. At the end of the microcapsules production process, their morphology was observed by optical microscopy at magnifications of 100 x, 200 x and 1000 x, using the optical microscope ZEISS Axiotech 100 HD equipped with the microscope camera Axiocam 105 color and assisted by the software ZEN 2.3 (blue edition).



Figure 12. Optical microscope, ZEISS Axiotech 100 HD.

3.3.2 Solid content

Solid content was measured in triplicate after the microcapsules production process according to the European Standard EN 827. First, it was necessary to rigorously weight 1 g of the microcapsule solution to a watch glass. Afterwards, the sample was placed on an oven for 30 min to promote water evaporation. Then, the sample was placed in a desiccator for 15 min and the residual mass was weighted. The drying step was repeated until the difference between the initial mass (solution initial) and the final mass (solution after water evaporation) did not exceed 2 mg between two consecutive weightings. Therefore, the solid content was determined by the following equation:

$$solid\ content\ (\%) = \frac{final\ mass}{initial\ mass} \times 100 \qquad (1)$$

3.3.3 Particle size evaluation

Microcapsules size distribution were obtained in terms of number and volume by Beckman Coulter Laser Diffraction Particle Size Analyzer LS 230. Coulter LS 200 Series operates with reverse Fourier lens optics integrated in a binocular lens system to optimize light scattering through a wide size range in only one scan[36].



Figure 13. Laser diffraction particle size analyzer, Beckman Coulter LS 230[36].

3.3.4 Scanning electron microscopy (SEM)

The morphologies of the produced microcapsules were examined by scanning electron microscopy with Phenom ProX Desktop SEM at magnifications 500 x, 1000 x and 2000 x. The samples were prepared the day before by putting a tinny drop of the microcapsule's solution on a carbon fiber above a pin and left it to dry on the hotte.



Figure 14. Scanning electron microscope, Phenom ProX Desktop [37].

3.3.5 Gas chromatography (GC/MS)

Encapsulation efficiency (EE) of the active substance on the produced microcapsules was evaluated by GC/MS using Shimadzu GCMS-TQ8040 NX Triple Quadrupole equipped with a splitless injector and a cross bonded fused column (30 m x 0.25 mm, 0.25 µm film thickness).

The oven temperature was programmed so that sequential temperature ramps are followed. Initially the oven temperature was kept isothermal at 40 ºC for 1 min, then increased to 200 ºC for 2 min with a rate of 7 ºC/min, followed by another gradual increase to 250 ºC for 2 min at 15 ºC/min and, at last, to 280 ºC for 1 min at 20 ºC/min. The injector temperature was defined at 290 ºC and a sample volume of 1 µL was injected. The carrier gas was helium with a flow rate of 1 mL/min. The analysis was carried out with ion and interface temperature at 250 ºC and 260 ºC, respectively.



Figure 15. Shimadzu GCMS-TQ8040 (left) Himac CT15 Series Tabletop High Speed Micro Centrifuge (right).

The samples for injection were prepared by withdrawing 1.5 mL from the microcapsules solution and subjecting them to centrifugation on a Himac CT15 Series Tabletop High Speed Micro Centrifuge at 3000 rpm for 5 min. The collected supernatant was placed on a vial in the presence of sodium chloride to weaken the emulsion and 1 mL of n-hexane was added. Afterwards, 750 µL of the previous solution and 1 mL of n-hexane were set on another vial with pure anhydrous sodium sulphate. This whole reported procedure is due to the fact that it is necessary to remove water from the samples for injection, as it is recommended to avoid samples that contain water when using GC/MS equipment. The last-mentioned solution was filtered through PES filters of 0.45 µm diameter to obtain merely the outer medium of solutions. Thereby, the peaks given on the chromatograms represent the compounds that were not encapsulated, providing the unencapsulated mass of a certain core material compound. The encapsulation efficiency was calculated by Equation 2.

$$Encapsulation\ Efficiency_i(\%) = \frac{m_{total,i} - m_{unencapsulated,i}}{m_{total,i}} \times 100 \qquad (2)$$

in which $m_{total,i}$ is the total mass of a certain compound of the core material used in the process and $m_{unencapsulated,i}$ represents the mass of a specific unencapsulated compound of the core material.

3.4 Application of microcapsules onto fabrics

The wet pick-up (WPU) is the measured quantity of absorbed liquid by a substrate after it has been dipped and padded. Thereby, WPU was determined from the measurement of the mass of a certain substrate before and after it has been dipped on water and padded. Thus, the following equation was used:

$$WPU\ (\%) = \frac{m_{fabric\ after\ immersion} - m_{dry\ fabric}}{m_{dry\ fabric}} \times 100 \qquad (3)$$

The WPU percentage leads to knowledge the amount of microcapsules solution and water that must be added to the immersive solution, considering the percentage of weight of fabric (WOF). Table 5 lists the respective WPU values for each fabric.

Table 5. Fabrics used and their respective WPU (%).

Fabric	Color	WPU (%)
American Fleece	Off White	67
Knit	Beige	86

The substrates were initially subjected to an immersion bath containing 10% WOF microcapsules solution to allow the infiltration of the microcapsules. Subsequently, the fabrics were subjected through a foulard to remove the excess of liquor. Finally, the samples were dried on a mechanical convection oven. Further on, a different formulation containing the same percentage of the microcapsule's solution was tested with citric acid.

The padding step was made by a Rapid foulard, model P-A1. The padding pressure was 3 bar and the speed at 2.5 rpm. The drying process was carried out on a mechanical convection oven Xutemp XT5118-IO140 at 120 ºC for a specified time. Once the impregnation of the microcapsules onto the fabrics was completed, one sample of each substrate was washed twenty times according to ISO 6330 on a FOM71 CLS wascator from Electrolux. Thereafter, the samples were dried on a XT5118-IO140 Xutemp mechanical convection oven at approximately 105 ºC.



Figure 16. Foulard, Rapid model P-A1 (left) Mechanical convection oven, Xutemp XT5118-IO140 (middle) Wascator, Eletrolux FOM71 CLS (right).

At last, the samples were distributed in order to evaluate their properties.

3.5 Characterization of fabrics impregnated with microcapsules

3.5.1 Scanning electron microscopy (SEM)

After the application of the microcapsules on the textiles, scanning electron microscopy was carried out on 3B's Research Group to analyze the existence of impregnated microcapsules and their morphologies. Formulation 8 was observed on two different substrates.

3.5.2 Antimicrobial activity

The antimicrobial activity was examined according to ASTM E2149 with the aim of determining the antimicrobial capacity of the microcapsules produced, mainly chitosan since it is reported its antimicrobial properties[24, 25]. The ASTM E2149 method is performed through dynamic contact between antimicrobial surfaces and a microbially contaminated solution.[38]

Staphylococcus aureus was cultivated in sterile tryptic soy broth media at 35 ºC for 18 h to have a liquid culture. Afterwards, the culture was diluted into the sterile buffer solution until a final concentration of 1.5-3.0x10^5 CFU/mL was achieved and used as the working bacterial suspension. Two sterile glass jars were needed: On one sterile glass jar was placed only the bacterial suspension (inoculum) and on another was deposited the treated fabric. The direct contact was provided by a wrist action shaker for 24 h and afterwards it was possible to determine the microbial concentration on each glass jar by incubating all Petri dishes at 37 ºC for 24 h and then the accounting of colonies was performed. Consequently, the microbial concentration on the glass jar with the treated fabric was compared to the inoculum jar and it was concluded the antimicrobial capacity of the fabric if it evidenced a significant reduction regarding the other glass jar[38]. The bacterial reduction (%) was determined by Equation 4.

$$Reduction, \% \ (CFU/mL) = \frac{B-A}{B} \times 100 \quad (4)$$

where A is the CFU/mL of the flask with the treated substrate after the contact time of 24 h and B is the CFU/mL of the flask containing only the inoculum after the same contact time.

4 Results and discussion

4.1 Microcapsules production

This section presents and discuss the results obtained throughout the project. After the microcapsules production, their characteristics were studied. It was observed the morphologies, determined the mean diameter and the solid content. Additionally, the encapsulation efficiency of the active substance was calculated for two assays. At last, the fabrics with the impregnated microcapsules were also evaluated through scanning electron microscopy. The antimicrobial tests results are also presented. An additional study was carried out on CeNTI to evaluate the reduction rate of odor of fabric *Knit Beige* treated with a specific formulation. Such evaluation is presented on Appendix F.

The first stage of the project was dedicated to the production of the microcapsules. Accordingly, different parameters were studied to understand their direct influence on the properties of the microcapsules.

1. Effect of biopolymers concentration
2. Effect of increasing lactic acid concentration
3. Effect of the speed and time on the homogenization step
4. Effect of the emulsifier
5. Effect of the proportions of the core material

The different formulations employed for the microcapsules production are listed on Table 6. A total of ten assays were executed.

Table 6. Formulations for the different assays for the production of the microcapsules.

Assay	Total volume (mL)	CH solvent/ concentration (%, w/v)	CH solution (%, w/v)	GA solution (%, w/v)	Core material/volume (mL)	Emulsifier/mass (g)	Homogenization step (velocity/time)	TA concentration (%, w/v)	TA solution volume (mL)
1	100	AA/0.6	1	2	PO/1.2	PGPR 4150/0.35	8000 rpm/1 min	10	2
2	100	LA/1	2	2	PO/2.5	PGPR 4150/0.7	8000 rpm/1 min	10	4
3	500	LA/1	2	4	PO/12.5	PGPR 4150/3.5	8000 rpm/2 min	10	20
4	500	LA/1.5	2	4	PO/12.5	PGPR 4150/3.5	13500 rpm/2 min	10	10
5	500	LA/1.5	2	4	PO/12.5	PGPR 4150/3.5	9500 rpm/4 min	10	10
6	500	LA/1.5	2	4	PO/12.5	Brij C2/3.5	9500 rpm/4 min	10	10
7	500	LA/1.5	2	4	PO/10 + TO/2.5	PGPR 4150/3.5	9500 rpm/4 min	10	10
8	500	LA/1.5	2	4	PO/10 + TO/2.5	PGPR 4150/3.5	9400 rpm/4 min	10	10
9	500	LA/1.5	2	4	PO/2.5 + TO/10	PGPR 4150/3.5	9500 rpm/4 min	10	10
10	2000	LA/1.5	2	4	PO/40 + TO/10	PGPR 4150/14	12200 rpm/4 min	10	40

4.1.1 Effect of biopolymers concentration

The influence of the concentration of the biopolymers and the respective amounts of the other reagents on the structure of the microcapsules was evaluated on formulations 1 and 2.

The first assay was carried out with the same conditions as the optimized formulation developed by Sharkawy et al[4]. However, the replacement of acetic acid as a chitosan solvent was proposed since acetic acid has a strong odor and can directly interfere with the odor of the fabrics containing the microcapsules suspension. Lactic acid was reported as a potential substitute of acetic acid to dissolve chitosan[39]. Thereby, the remaining formulations used lactic acid. According to Goñi et al.[39] lactic acid was efficient to dissolve chitosan at 20 g/L with concentrations higher than 0.8 % (w/v). Thus, on the second formulation chitosan was dissolved on lactic acid 1 % (w/v) and its amount was doubled compared to the first one, as the other raw materials, excepting gum Arabic. The OM observations of the microcapsules at the end of the process are shown on Figure 17 with the magnification 100 x. The additional OM images at magnifications 200 x and 1000 x are presented on Appendix A.

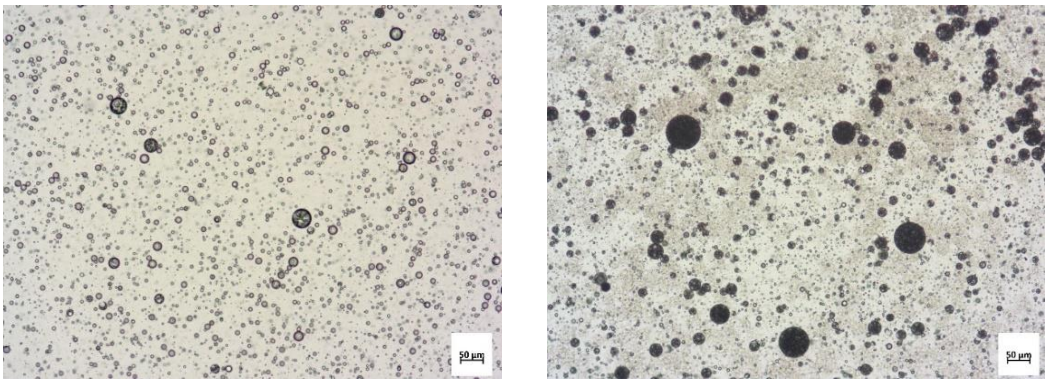


Figure 17. Optical Microscopy Images of assay 1 (left) assay 2 (right) at magnification 100 x.

Through the observation of Figure 17, it is noticeable the existence of spherical shape microcapsules on the two assays. The emulsifier used throughout the present project was PGPR, also used in preceding reports[3, 4]. It has been reported that PGPR stimulates the production of polynuclear microcapsules, which are capable of conferring protection for the core material[4]. Figure 17 and the OM images on Appendix A confirms this by showing the polynuclear morphology of the produced microcapsules.

Formulation 2 produced bigger and darker microcapsules than the ones formed on formulation 1. It can be seen some darker spots that may indicate the precipitation of the polymer or the gelation between the excess of chitosan and tannic acid[4]. Moreover, there is a visible aggregation in this specific formulation. As it is revealed on Table 6, from formulation 1 to formulation 2 the amounts of the reagents were doubled, excepting gum Arabic. This biopolymer has been disclosed in the literature as a potential stabilizer in oil-in-water

emulsions[4]. Thereby, the poor dispersion can be explained by the low amount of gum Arabic in relation to other reagents, especially chitosan, which consequently resulted in an unstable formulation. Additionally, on formulation 3 the amount of gum Arabic was doubled in relation to formulation 2 and it is noticeable, through observation of Figure 18, the decrease of aggregation observed.

4.1.2 Effect of increasing lactic acid concentration

At the end of the synthesis process to produce the microcapsules, it was observed some residues with an orangish color at the bottom of the flask on assay 2 and, mostly, on assay 3.

Formulation 3 was the first one carried out on large scale, and therefore all the quantities of the raw materials were calculated in proportion with the previous assay, including the volume of tannic acid. Tannic acid, as a hardening agent, stablishes intermolecular interactions with the wall material of the microcapsules, namely chitosan. The hydroxyl groups of tannic acid form hydrogen bonds with the reactive amino groups of chitosan[3, 4]. Thus, as the color of the sediments observed on the mentioned formulations was similar to tannic acid powder color, it was assumed that beyond the chitosan solution was not stable, there was an excess of tannic acid that did not stablished the referred intermolecular interactions with chitosan. Therefore, at the same time the influence of the concentration of the lactic acid to dissolve chitosan was studied, the volume of tannic acid was decreased.

As mentioned on the previous section, the minimal concentration of lactic acid to attain a stable chitosan solution is 0.8 % (w/v)[39]. The previous formulation used lactic acid 1 % (w/v) to dissolve chitosan. Therefore, it was proposed to increase its concentration to 1.5 % (w/v) on the next assays. The OM observations of the microcapsules at the end of the process are shown on Figure 18 with the magnification 100 x. The other OM images at magnifications 200 x and 1000 x are disclosed on Appendix A.

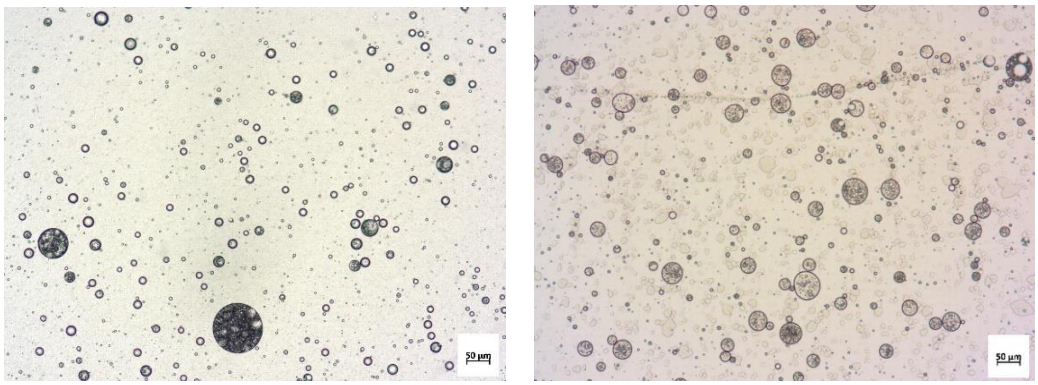


Figure 18. Optical Microscopy Images of assay 3 (left) assay 4 (right) at magnification 100 x.

In addition to the increase in lactic acid concentration and decrease of tannic acid volume applied, from formulation 3 to formulation 4, the speed used in the homogenization step was increased, which could provoke modifications on the morphology of the microcapsules. Comparing the images of formulation 3 and formulation 4 on Figure 18, the later exhibits more homogeneous microcapsules in size, but they do not seem perfectly rounded and well dispersed. Thus, the next subsection is dedicated to the study of the speed and time on the homogenization step.

At last, it is important to refer that at the end of the process of formulation 4 there were no residues at the bottom of the flask, which indicates that the increase of lactic acid concentration and the decrease of tannic acid volume were beneficial. Therefore, the subsequent assays were carried out with both same concentration of lactic acid and tannic acid volume as formulation 4.

4.1.3 Effect of speed and time on the homogenization step

After the tests have been carried out, the samples were stored at room temperature. The day after the experimental realization, formulation 4 presented some surface stains. The image is presented on Appendix B. The visual surface stains and the irregular microcapsules shape observed on Figure 18 could be an indicative that the emulsion was not stable. Thus, to promote a better stability of the emulsion, it was proposed to study the influence of the agitation speed in the homogenization step and the time at which the mixture was subjected to it[40]. Hence, the agitation speed in the homogenization step was decreased and the time was increased from formulation 4 to 5, as Table 6 discloses. The OM observations of the microcapsules at the end of the process are shown on Figure 19 with the magnifications 100 x and 1000 x.

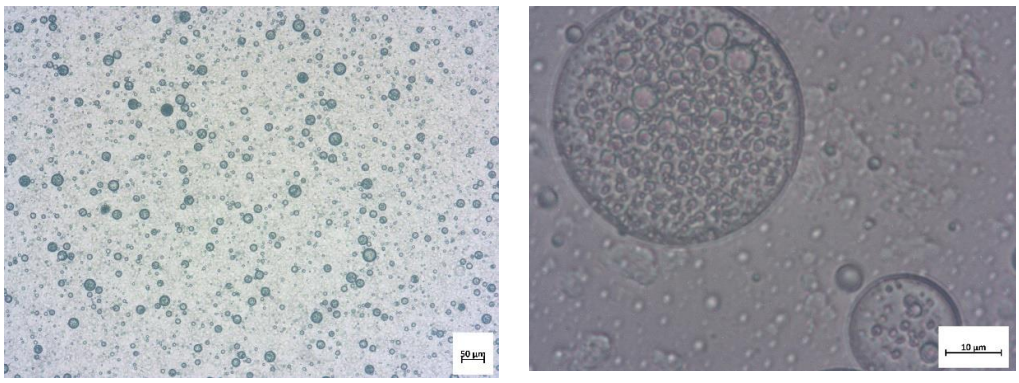


Figure 19. Optical Microscopy Images of assay 5: magnification 100 x (left) and magnification 1000 x (right).

The microcapsules produced by formulation 5 are visually more homogeneous than the ones from formulation 4. Furthermore, the former produced microcapsules smaller and rounder in shape. As the homogenization speed increases, the higher shear forces will be, which causes

the reduction of droplets size, and consequently, improves emulsion stability[40-42]. However, the further increase of speed might promote osmotic swelling, reducing the stability of the emulsion[41]. Therefore, it is assumed that the homogenization speed used on formulation 4 was too high, which could cause the appearance of surface stains. On the other hand, Kamarudin et al. reported that with the increase of homogenization time, the size of the emulsion droplets decreases, resulting in a more stable emulsion[41]. With that being said, it can be supposed that the increase of homogenization time promoted a stable emulsion on formulation 5. Additionally, there were no surface stains observed on formulation 5 the day after the experimental realization. Such image is presented on Appendix B.

4.1.4 Effect of the emulsifier

Emulsifiers are capable to stabilize the emulsion, which is crucial to the microencapsulation procedure[6]. Hence, the influence of the emulsifier used in the microcapsules production was studied.

Whereas PGPR was implemented in mostly all trials, including formulation 5, a different emulsifier named Brij C2 MBAL-SO-(SG) was used on formulation 6. As shown in Table 6, the formulations 5 and 6 have equal synthesis conditions, except the type of emulsifier applied. Thereby, it is possible to relate the stability of the emulsion to the type of emulsifier used. The OM observations of the microcapsules at the end of the process are shown on Figure 20 with the magnifications 100 x and 1000 x.

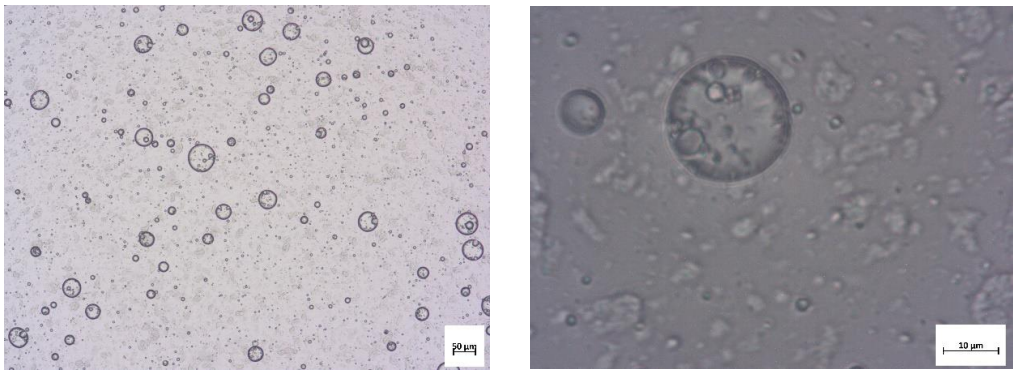


Figure 20. Optical Microscopy Images of assay 6: magnification 100 x (left) and magnification 1000 x (right).

The produced microcapsules from formulations 5 and 6 reveal quite differences. Optical microscopy image of formulation 6 on Figure 20 exhibits a worst dispersion, which resulted on bigger microcapsules, when compared to the image of formulation 5 on Figure 19. Concerning the morphology of the microcapsules, the optical microscopy image at magnification 1000 x on Figure 20 confirms that they are polynuclear.

The emulsifiers PGPR 4150 and Brij C2 MBAL-SO-(SG) have low HLB values. PGPR has a HLB value range of 2-4 [4] and Brij C2 MBAL-SO-(SG) of 5[43]. Emulsifiers with high values of HLB are preferred to oil-in-water emulsions because of its hydrophilic character. However, the capability of the emulsifier to reduce the interfacial tension does not depend exceptionally on HLB, but also on its molecular weight and the location of the hydrophilic and hydrophobic groups[44]. Emulsifiers with low HLB values are generally used in water-in-oil emulsions. Nevertheless, it has been reported the use of emulsifiers with low HLB values in the development of oil-in-water emulsions followed by complex coacervation. Sharkawy et al. stated that the use of emulsifiers with low HLB values promoted higher values of encapsulation efficiency, which indicates the tendency of the encapsulation of hydrophobic substances for these emulsifiers[4].

4.1.5 Effect of the proportions of the core material

Thyme oil was suggested to be applied together with peppermint oil as the core material since it is known for its antibacterial properties[32, 33]. Therefore, formulations 7 and 9 were carried out with equal synthesis conditions but with different proportions of the essential oils. Whereas formulation 7 was developed using 80 % of peppermint oil and 20 % of thyme oil, formulation 9 applied 20 % peppermint oil and 80 % thyme oil. The two essential oils were well mixed before the introduction to the system. The OM observations of the microcapsules of both formulations at the end of the process are shown on Figure 21 with the magnifications 100 x and 1000 x.

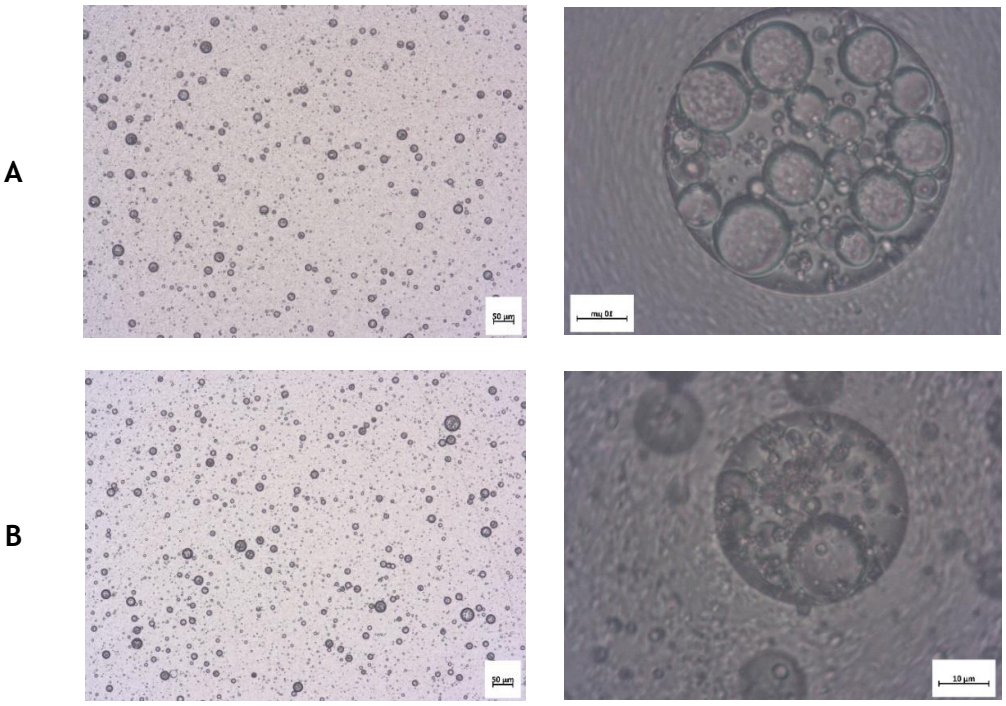


Figure 21. Optical Microscopy Images of (A) assay 7 at magnifications 100 x (left) and 1000 x (right) (B) assay 9 at magnifications 100 x (left) and 100 0x (right).

Figure 21 shows that the produced microcapsules of the two formulations are identical. It seems that the properties of the essential oils utilized as the core material do not influence the microcapsules.

Moreover, formulation 8 was carried out on *HeiQ* as a replication of formulation 7. It should be noted that the materials used in the *HeiQ* laboratory are different from those used in the LSRE laboratory, as explained in the materials and methods section.

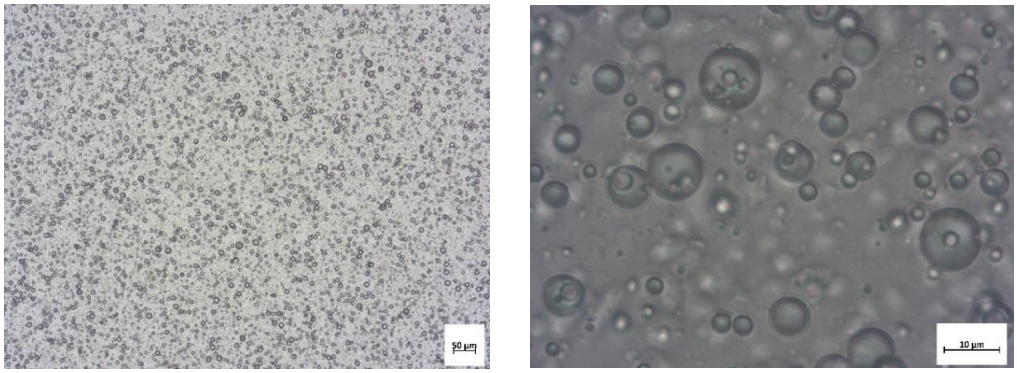


Figure 22. Optical Microscopy Images of formulation 8 at magnifications 100 x (left) and 1000 x (right).

Comparing the images observed on optical microscopy of formulations 7 and 8, on Figures 21 and 22, the produced microcapsules are not visually identical. Formulation 8 produced smaller microcapsules than the ones by formulation 7, which can be explained by the change of experimental set-up used, mainly at the homogenization step. Due to the high accelerations of the homogenizer on the solution, strong shear and thrust forces were produced[45]. Regarding the morphology of the microcapsules, both formulations produced round shaped microcapsules with polynuclear morphology, as it can be seen on Figure 22.

Still to finalize the production of the microcapsules in this project, a 2 L industrial trial was performed at LSRE. Formulation 10 was identical to the formulation 7, with all the quantities in proportion, but as the total volume of the mixture was higher, the mixture was placed on the jacketed batch reactor at the homogenization step to subject it to an appropriate speed.

It can also be examined if there were significant changes from the test of formulation 7 to the test of formulation 10 on optical microscopy.

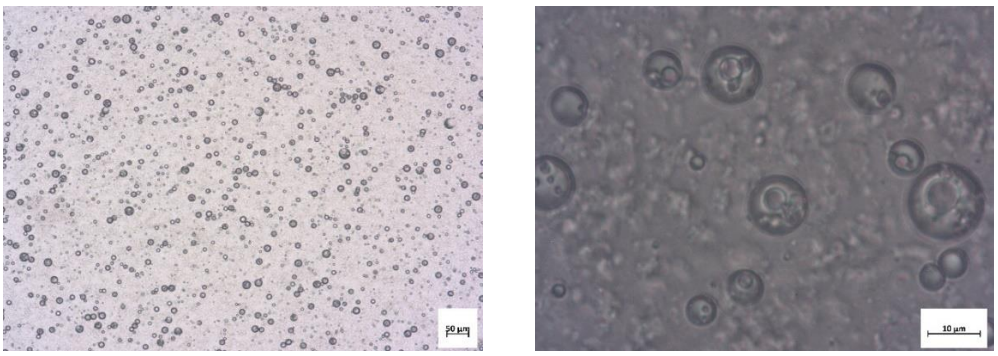


Figure 23. Optical Microscopy Images of assay 10 at magnifications 100 x (left) and 1000 x (right).

The optical microscopy images of formulation 7 and formulation 10 on Figures 21 and 23, respectively, show that the produced microcapsules are relatively identical. They are both rounded with polynuclear morphology. However, formulation 10 generated smaller microcapsules. That can be explained by the increase on the speed at the homogenization step inside the jacketed batch reactor[41, 42].

4.2 Microcapsules characterization

4.2.1 Particle size evaluation

The mean diameter of the produced microcapsules was determined for the appropriate assays approximately 24 h after the synthesis by laser diffraction, being the results obtained disclosed in Table 7. Formulation 1 was not included because it was only an experimental replication[4].

Table 7. Mean diameters and the standard deviations of the produced microcapsules.

Assay	Mean diameter (µm)	St. Dev. ± (µm)
2	28.6	0.6
3	14.7	0.2
5	22.3	0.5
6	23.3	4.3
7	10.7	0.6
8	28.6	2.9
9	17.5	0.1
10	42.6	3.4

Table 7 demonstrates the mean particle size and the respective standard deviation for every assay performed. It shows that the microcapsules have a mean size range from 10.7 to 42.6 µm. Literature reports that microcapsules produced by complex coacervation comprise an extensive variation (1 and 500 µm)[3]. With that being stated, the range values presented on Table 7 agrees with the reported literature. It is important to refer that the mean particle size of formulation 4 was not determined, since visually it did not seem stable.

As stated previously, the amount of gum Arabic applied interferes directly with the stabilization of the emulsion[4]. Therefore, the increase of its concentration promotes a better dispersion and, consequently, less agglomeration occurs. Formulation 3 implemented a higher concentration of this biopolymer than formulation 2, which resulted on microcapsules more dispersed, as seen on Figures 17 and 18. Hence, the mean diameter of formulation 2 is expected to have a higher value and a higher standard deviation, which is confirmed on Table 7.

The value obtained for formulation 5, 22.3 µm, corresponds to what is observed on Figure 19. Even more, the standard deviation is quite low, indicating a good homogenization of the solution, as clearly visible on Figure 19.

The emulsifier used also has an enormous impact on the particle size. The formulation that used PGPR as emulsifier, formulation 5, exhibits a lower mean diameter value when compared to the one produced with Brij C2 MBAL-SO-(SG). This can be clearly visible on the optical microscopy images obtained on Figures 19 and 20. Brij C2 MBAL-SO-(SG) did not improve the emulsion on formulation 6, resulting on microcapsules not well dispersed. Hence, beyond the mean diameter of the produced microcapsules with this emulsifier is a bit higher, the standard deviation is extremely high when compared to the other obtained values.

As formulations 7 and 9 were carried out on equal synthesis conditions, excepting the proportions of the core material, it is perceptible the relatively close values of their mean diameters. Regarding formulation 8, that was developed on *HeiQ* laboratory, the mean diameter was higher than what expected, given not only the fact that it has the same synthesis conditions of formulation 7 but also because of the observation of the optical microscopy image on Figure 22. Thence, the fact that standard deviation is quite higher, specially, when compared to the standard deviation of formulation 7.

At last, the mean particle size of 42.6 µm is not in agreement with the optical microscopy image obtained for formulation 10. Additionally, the standard deviation is quite higher. Therefore, it can be assumed that the test sample was not fully homogenized during the study of the particle size evaluation.

The particle size distribution of the microcapsules in number and in volume are disclosed on Appendix C.

4.2.2 Encapsulation efficiency

Encapsulation efficiency was quantified for microcapsules produced at large-scale containing only peppermint oil as the core material: assays 5 and 6. Thereby, it was possible to study the encapsulation efficiency regarding the influence of the surfactant used.

To quantify the concentrations of each compound present on peppermint oil it was essential to consider the calibration curves to relate the areas of the peaks of the compounds to their concentrations. These referred calibration curves are disclosed on Appendix D. The major compounds identified are listed on Table 8 with their percentage on the peppermint oil and their respective retention time.

Table 8. Quantification of the compounds of peppermint oil and their retentions time.

Compound	Percentage of the compound (%)	Retention time, t_r (min)
Eucalyptol	5.33	7.68
Menthone	36.30	10.32
Menthofuran	5.41	10.51
Menthol	2.12	10.64
iso-Menthol	45.34	10.80
Menthyl acetate	5.50	13.06

Through the observation of the peak's areas of the peppermint oil compounds on the calibration curves, it was possible to obtain the respective concentrations. Thus, knowing the percentage of each compound in peppermint oil and its initial volume, it was possible to calculate the encapsulation efficiency for the two assays by the Equation 2. The obtained efficiency values and the respective standard deviations for formulations 5 and 6 and encapsulated and nonencapsulated oil data are represented on Table 9 and 10, respectively.

Table 9. Encapsulation efficiencies of formulation 5 and respective standard deviations.

Compound	m_{total} (g)	$m_{nonencapsulated}$ (g)	$m_{encapsulated}$ (g)	EE (%)	St. Dev. ± (%)
Eucalyptol	$5.98x10^{-1}$	$1.06x10^{-3}$	$5.97x10^{-1}$	99.8	3.2
Menthone	4.07	$3.46x10^{-3}$	4.07	99.9	0.8
Menthofuran	$6.08x10^{-1}$	$5.64x10^{-4}$	$6.07x10^{-1}$	99.9	1.9
Menthol	$2.38x10^{-1}$	$2.43x10^{-4}$	$2.38x10^{-1}$	99.9	1.3
iso-Menthol	5.09	$3.98x10^{-3}$	5.09	99.9	0.9
Menthyl acetate	$6.17x10^{-1}$	$1.90x10^{-4}$	$6.17x10^{-1}$	99.9	0.9
Total	$1.12x10^{1}$	$9.50x10^{-3}$	$1.12x10^{1}$	99.9	1.5

Table 9 reveals that the formulation 5 exhibits high encapsulation efficiencies for all the compounds of approximately 100 %. The global encapsulation efficiency is quite high with a value of (99.9±1.5) %.

Table 10. Encapsulation efficiencies of formulation 6 and respective standard deviations.

Compound	m_{total} (g)	$m_{nonencapsulated}$ (g)	$m_{encapsulated}$ (g)	EE (%)	St. Dev. ± (%)
Eucalyptol	$5.98x10^{-1}$	$1.13x10^{-3}$	$5.97x10^{-1}$	99.8	2.2
Menthone	4.07	$4.91x10^{-3}$	4.07	99.9	0.9
Menthofuran	$6.08x10^{-1}$	$5.75x10^{-4}$	$6.07x10^{-1}$	99.9	2.0
Menthol	$2.38x10^{-1}$	$2.93x10^{-4}$	$2.38x10^{-1}$	99.9	2.0
iso-Menthol	5.09	$5.82x10^{-3}$	5.08	99.9	2.8
Menthyl acetate	$6.17x10^{-1}$	$5.11x10^{-4}$	$6.17x10^{-1}$	99.9	1.8
Total	$1.12x10^{1}$	$1.32x10^{-2}$	$1.12x10^{1}$	99.9	1.9

From formulation 5 to formulation 6 the emulsifier used was changed. The type of emulsifier may influence the total encapsulation efficiency and, as so, it is important to study the effect of the emulsifier. Surfactants are characterized by the HLB value and, therefore, it is important to highlight that the HLB value of PGPR 4150 is between 2-4, whereas for Brij C2 MBAL-SO-(SG)

is 5. As the values are close to one another, the EE should not be disparate between the two assays. It is notorious that the EE values of the two formulations are similar, as formulation 6 presents an EE value of (99.9±1.9) %.

Through the EE values obtained it is possible to notice that for all compounds of the two examined samples a high EE was reached of approximately 100 %. The values obtained greater than 90 % are expected and coincide with the literature[3, 4]. Sharkawy et al. obtained an EE of 90.4 % for a specific formulation of the chitosan-gum Arabic produced microcapsules with limonene as core material and PGPR as the emulsifier[4]. Gil et al. also achieved an EE of about 99 % with a similar formulation as the one reported previously[3]. A similar study whereas chitosan-gum Arabic was carried out by complex coacervation with Miglyol 812N as the active substance, reported an EE of 95.2 %. Whereas this value was obtained for a biopolymer mass ratio, $R_{CH/GA}$, of 0.25 (chitosan/gum Arabic) and NaTPP as the hardening agent, the current project was carried out for a $R_{CH/GA}$ of 0.5 and tannic acid as the hardening agent[46].

4.2.3 Solid content

The solid content of the produced microcapsules was determined at the end of the synthesis and the results are presented on the Table 11. Formulation 1 was not included because it was only an experimental replication[4].

Table 11. Solid content of the produced microcapsules.

Assay	Solid content (%, w/w)	St. Dev. ± (%)
2	3.90	0.12
3	4.04	0.04
5	4.36	0.02
6	4.13	0.02
7	4.04	0.04
8	5.02	0.09
9	4.15	0.07
10	4.11	0.04

Table 11 shows that the values of the solid content range from 3.90 % to 5.02 % to the formulations that did seem stable. The low solid content percentage of all assays can be explained by the fact that this process is bio-based, with all the formulations having almost 100 % of water. The percentage of the compounds for each assay is disclosed on Appendix E.

Additionally, the determined values presented on Table 11 are in accordance with the results obtained by Gil et al. for a similar formulation before the washing step, with the value of 4.35 % (w/w), except the use of limonene as the core material, its correspondent amount and the tannic acid volume applied. That is indicative that the increase of the oil amount and a minor volume of tannic acid did not favor the production of the microcapsules.[3] However, the solid content value determined by Sharkawy et al. was higher before the washing step than the ones achieved in the present study, 27.8 % (w/w)[4]. It is important to refer that a phase separation was carried out after the microcapsules synthesis on the mentioned reports[3, 4]. That can explain the higher value obtained by Sharkawy et al. since the solid content was evaluated only to the microcapsules suspension, whereas in the present project no phase separation was performed.

4.3 Fabric with impregnated microcapsules characterization

4.3.1 Simple coating method: pad-drying

The fabrics studied were immersed on a bath containing the microcapsules solution and thereafter submitted to a mechanical convection oven at 120 ºC. As the microcapsules were not subjected to any chemical reaction, the ones that are fixed onto the fabrics would not be sufficiently resistant to wash. Figure 24 illustrates the SEM images observed of the untreated fabrics: *American Fleece Off White* and *Knit Beige*.

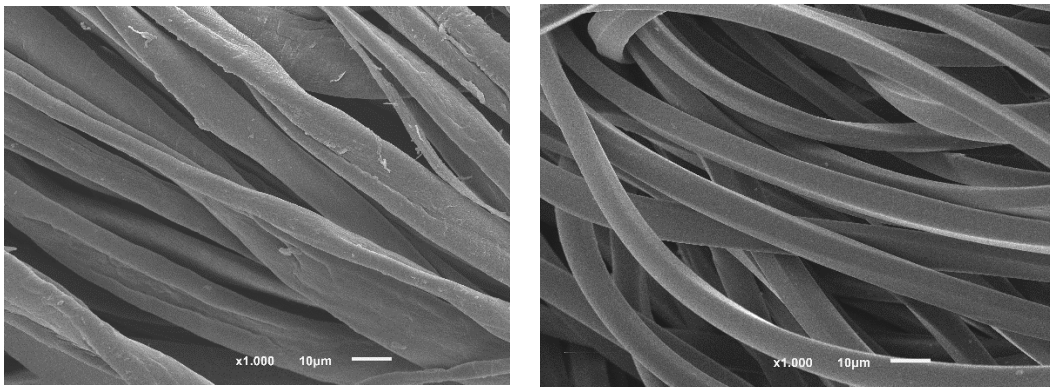


Figure 24. Scanning electron microscopy images of the fabrics at magnification 1000 x: Untreated American Fleece Off White (left) Untreated Knit Beige (right).

American Fleece Off White and *Knit Beige* fabrics were treated with the test carried out at HeiQ laboratory, formulation 8.

The immersive bath contained 10 % WOF of the microcapsule's solution. The influence of the washability was studied by washing the fabric twenty times according to ISO 6330.

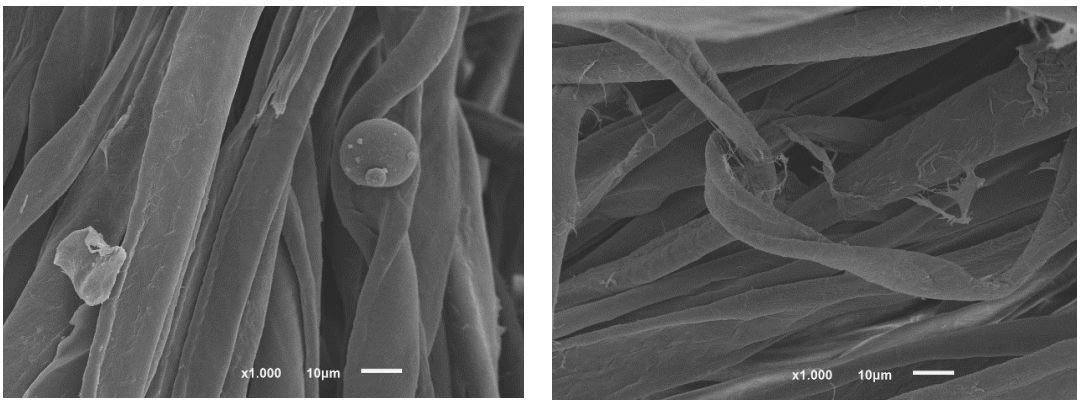


Figure 25. Scanning electron microscopy images of the fabrics treated with formulation 8 at magnification 1000 x: American Fleece Off White Unwashed (left) American Fleece Off White Washed 20 x (right).

Figure 25 demonstrates the presence of a rounded shaped microcapsule with approximately 20 µm attached to the cotton fabric. This specific particle size is expected, since the mean diameter obtained on Table 7 for formulation 8 is close enough, 28.6 µm.

The solid content of formulation 8 is 5.02 %, which is a quite low value. Hence, it is difficult to notice the microcapsules attached to the fabrics on scanning electron microscopy. Moreover, the microcapsules tend to be located in the spaces between the fabric fibers, which makes them harder to be found[4].

The effect of the washability can be clearly seen on Figure 25. There are neither microcapsules nor its fragments on the washed fabric, with indicates that the washability destroyed them.

This specific formulation was also applied on the *Knit Beige* fabric with the exact conditions as the other formulations.

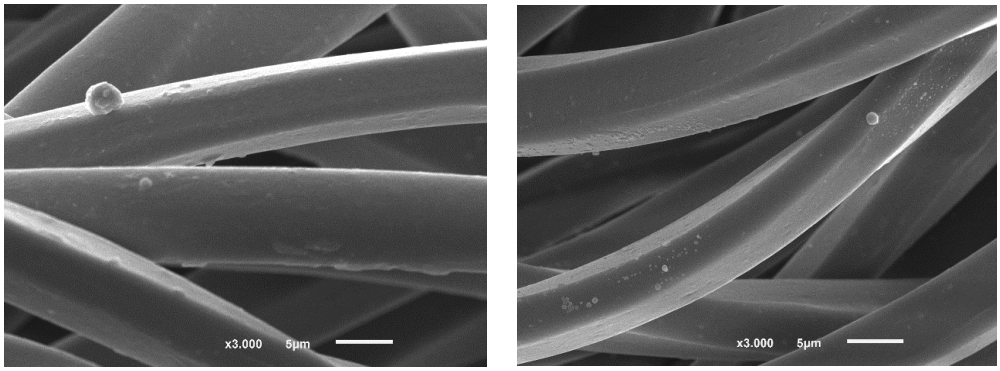


Figure 26. Scanning electron microscopy images of the fabrics treated with formulation 8 at magnification 3000 x: Knit Beige Unwashed (left) Knit Beige Washed 20 x (right).

Even though the particle displayed on Figure 26 is not perfectly rounded and its size is approximately 2.5 µm, it might be one small microcapsule. This might indicate that the smaller microcapsules have enough resistance to hold onto the fabrics. Furthermore, *Knit Beige* fabric is constituted of 100 % polyester. Polyester fabric, polyethylene terephthalate (PET), is a polymer which has hydrophobic properties[47]. The hydrophobicity of such polymer can be an obstacle to applications, such as dyeing and finishing. Therefore, it has been reported several modifications of polyester surface to improve its hydrophilicity and, consequently, promote microcapsules adhesion[47, 48].

Regarding the durability to wash, a microcapsule with about 1 µm is noticed on the washed fabric. Once again, smaller particles seem to be capable to resist to high temperatures on the dry stage and washing.

4.3.2 Coating method with citric acid: pad-drying

The microcapsules of formulation 8 were impregnated to *American Fleece Off White* fabric with the aid of a cross-linker, citric acid. The immersive bath contained 10 % WOF of the microcapsule's solution and three drops of citric acid. The wash step was carried out identically to the tests stated above.

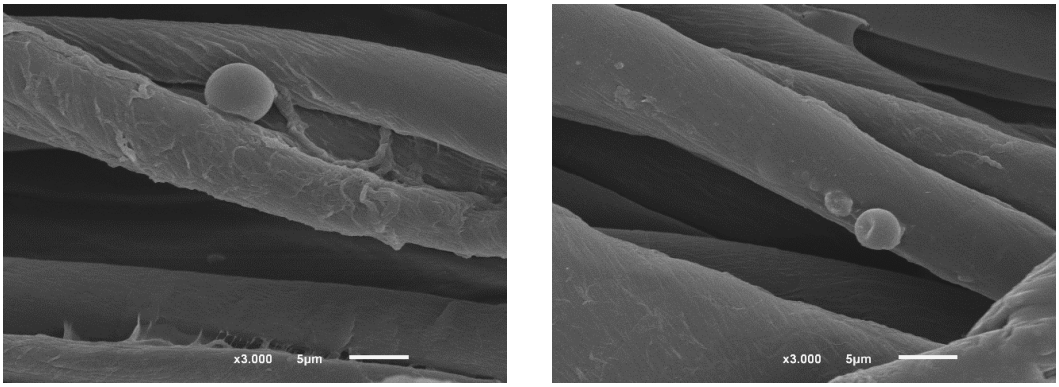


Figure 27. Scanning electron microscopy images of American Fleece Off White fabric treated with formulation 8 and citric acid at magnification 3000 x.

Citric acid was studied as a crosslinker to promote a better adhesion of the microcapsules onto the fabrics[6]. Both scanning electron microscopy images on Figure 27 illustrate perfectly rounded shaped microcapsules with about 5 and 4 µm, respectively. This suggests that the procedure did not improve the adhesion of bigger microcapsules. Nevertheless, it is also fundamental to highlight that the observation of the impregnated microcapsules was easier with the fabrics that applied citric acid than the fabrics that did not.

4.3.3 Antimicrobial activity

The antimicrobial activity of the fabrics treated with microcapsules of formulation 8 was evaluated. The assessment was carried out after washing the samples as described above.

Table 12. Bacterial reduction (%) of the tested fabrics with formulation 8.

Sample	T0	T24		Bacterial reduction (%)
	Reference (CFU/mL)	Reference (CFU/mL)	Sample (CFU/mL)	
American Fleece Off White	$2.90x10^5$	$1.90x10^7$	$3.62x10^6$	80.90
Knit Beige			$6.64x10^4$	99.70

The achievement of a reduction percentage between 90 % and 99 % of the total bacterial count (CFU/mL) in the original inoculum leads to the consideration that the functional fabric has bacteriostatic activity[4]. Through the analysis of Table 12, it can be verified that the fabric *Knit Beige* exhibited antibacterial activity against *Staphylococcus aureus*, whereby the fabric exhibited a bacterial reduction of 99.70 % after 24 h of dynamic contact. Regarding the fabric *American Fleece Off White*, the bacterial reduction obtained was inferior when compared to the value achieved for the *Knit Beige* fabric, with a value of 80.90%.

It can be concluded that the produced microcapsules attached to *Knit Beige* fabric, containing chitosan, peppermint oil and thyme oil exhibited the antimicrobial properties of such raw materials, against a gram-positive bacteria, *Staphylococcus aureus*.

4.3.4 Reduction rate of odor

The analysis of the reduction rate of odor of an applied substrate was conducted on CeNTI according to an internal method adapted from ISO 17299-3:2014 standard. Two samples of *Knit Beige* were examined: an unwashed sample and a washed sample. The representative odoriferous marker of sweat odor studied on CeNTI was isovaleric acid (IVA). The results obtained for this specific marker are presented on Appendix F. It was possible to conclude that the *Knit Beige* fabric, containing the microcapsules from formulation 8, cannot be considered to have deodorant properties.

5 Conclusion

The production of aroma-loaded microcapsules with biopolymers, chitosan and gum Arabic, as the coating material containing essential oils, peppermint oil and thyme oil, was performed by complex coacervation. The procedure followed was based on preceding reports [3, 4] with the purpose to increase its production scale. The produced microcapsules were applied to textile substrates. Both the properties of the microcapsules and their impregnation on fabrics were evaluated.

Several formulations were developed to study the influence of different parameters until the achievement of a stable microcapsules solution. Regarding the influence of the biopolymers concentration, the biopolymers mass ratio, $R_{GA/Chi}$, of 2 proved to decrease the aggregation observed on the optical microscopy images obtained when an $R_{GA/Chi}$ of 1 was used. It can be concluded that gum Arabic improved the stability of the emulsion, as reported on literature. The increase of lactic acid concentration to 1.5 % (w/v) and the decrease of tannic acid volume to 10 mL promoted the disappearance of the orangish residues observed on the bottom of the flask containing the microcapsules solution. The evaluation of speed and time on the homogenization step, indicated that a better emulsion was attained with a homogenization speed of 9500 rpm with a time of 4 min, since the surface stains observed on assay 4 were not visualized on assay 5. A different emulsifier was also studied, and it was concluded that formulations that applied PGPR 4150 achieved better results on terms of dispersion observed on the optical microscopy images. The proportion of the essential oils used as core material was changed and the optical microscopy results did not differ from one another. The assay carried out with a total volume of 2 L presented microcapsules with similar morphology as the assay with equal synthesis conditions and a volume of 500 mL. The produced microcapsules exhibited a spherical and polynuclear morphology on all formulations, including the one that applied Brij C2 as the emulsifier.

Regarding the microcapsules size, the mean size ranged from 10.7 to 42.6 µm. In addition to this range of values agreeing with the values reported on literature to complex coacervation, it is also suitable for the impregnation onto textiles. As expected, the formulations that exhibited on the optical microscopy a greater microcapsules aggregation, had both a higher particle size and a high standard deviation, which indicates that those formulations are not well dispersed.

The encapsulation efficiency of the produced microcapsules in formulations 5 and 6 were (99.9±1.5) % and (99.9±1.9) %, respectively. As the two emulsifiers have approximate HLB values, it was expected that the encapsulation efficiencies were close to one another. These

high values are in accordance with the literature for the complex coacervation method on the production of microcapsules.

The produced microcapsules exhibited a percentage of solid content between 3.90 % and 5.02 %. The low values can be explained by the fact that the major constituent of the microcapsules, almost 100 % of the solution, is water.

Pad-drying method was used to impregnate the microcapsules from formulation 8 to the textile substrates. Such treated fabrics exhibited small microcapsules, ranging from 2.5 to 20 μm, which is an indicative that only the smaller microcapsules were resistant enough to the pressure applied on the foulard and the temperature of the oven. The durability to wash was merely observed on the *Knit Beige* fabric. Another study was carried out with the same method containing microcapsules from formulation 8 and citric acid and it was concluded that the adhesion of the microcapsules onto the textile was improved.

At last, the antimicrobial activity was determined for the two fabrics treated with formulation 8. While on *American Fleece Off White* fabric the bacterial reduction percentage of *Staphylococcus aureus* was 80.90 %, on *Knit Beige* was 99.70 %. As the bacterial reduction percentage on the last-mentioned fabric was above 90 %, it can be concluded that such treated fabric has bacteriostatic activity.

6 Assessment of the work done

6.1 Objectives achieved

Several objectives were achieved during the realization of this project. The large-scale production of sustainable microcapsules by complex coacervation method with two essential oils was successfully accomplished since its viability was studied through the evaluation of their properties. The viability achieved was centralized on the characterization of the microcapsules morphology through optical microscopy and scanning electron microscopy, in the particle size evaluation and in the determination of the encapsulation efficiency of both the main oil compounds and the global encapsulation efficiencies. The produced microcapsules were impregnated onto textile substrates and its examination was carried out on scanning electron microscopy. At last, the antimicrobial activity of the treated fabrics was studied, achieving positive results.

6.2 Limitations and future work

Further optimization of the microencapsulation process could not be carried out due to time limitation. It was not possible to study the long-term stability of the microcapsules solutions either. However, these two continuous studies can be approached for future work. Additionally, a new approach for future work regarding the microcapsules impregnation would be the use of a crosslinker to improve their adhesion to the fabrics and, consequently, promote an efficient resistance to washing.

6.3 Final assessment

My assessment of the present work is quite positive. The main imposed objectives were achieved successfully, and additional work was also developed. The lack of time hindered the discovery of answers to some problems that have arisen, and consequently, the optimization of the formulation. Nevertheless, given the results obtained throughout the project, the assessment of about five months of work is quite positive.

7 References

1. Salaün, F., *Microencapsulation technology for smart textile coatings*, in *Active Coatings for Smart Textiles*. 2016. p. 179-220.
2. Marinkovic, S.S., D. Bezbradica, and P. Skundric, *Microencapsulation in the Textile Industry*. 2005.
3. Gil, G.A., M.F. Barreiro, C.C. Sipoli, and I.P. Fernandes, *Development of functional biobased microencapsulation systems for textile applications*. 2020, Universidade Tecnológica Federal do Paraná and Instituto Politécnico de Bragança p. 96.
4. Sharkawy, A., I.P. Fernandes, M.F. Barreiro, A.E. Rodrigues, and T. Shoeib, *Aroma Encapsulation for Eco-friendly Textile Application*. 2016, The American University In Cairo p. 166.
5. Vieira, R.V., *Líder em inovação têxtil*, G. Empresarial, Editor. 2021: Gestão Empresarial
6. Valle, J.A.B., R.d.C.S.C. Valle, A.C.K. Bierhalz, F.M. Bezerra, A.L. Hernandez, and M.J. Lis Arias, *Chitosan microcapsules: Methods of the production and use in the textile finishing*. Journal of Applied Polymer Science, 2021. **138**(21).
7. Scopus, *Number of published articles over the last thirty years* 2021: Scopus.
8. Bohringer, B.W., DE), Klemund, Michael (Düsseldorf, DE),, *Functional textile material provided with microcapsules containing an active ingredient and use thereof*. 2010, Blucher GmbH (Erkrath, DE): United States.
9. Copete Vidal, T.S.C.d.V., ES), Pi Subirana, Rafael (Granollers, ES), Tacies Capdevila, Anna (Barcelona, ES),, *Finished fibers and textiles*. 2011, Cognis IP Management GmbH (Duesseldorf, DE): United States.
10. Viladot Petit, J.L.B., ES), Delgado Gonzalez, Raquel (Gava Barcelona, ES), Fernández Botello, Alfonso (Malaga, ES),, *Process of treatment of fibers and/or textile materials*. 2017, Lipotec, S.A. (Gava, ES): United States.
11. Vandendaele, P.R., BE), Temmerman, Robin (Lokeren, BE), *Microcapsules containing microorganisms*. 2019, Devan Chemicals NV (Ronse-Renaix, BE): United States.
12. Aloys, H., S. A. Korma, T.M. Alice, N. Chantal, A. H. Ali, S. M. Abed, and H. Ildephonse, *Microencapsulation by Complex Coacervation: Methods, Techniques, Benefits, and Applications - A Review*. American Journal of Food Science and Nutrition Research 2016.
13. Shrimali, K. and E.M. Dedhia, *Microencapsulation for Textile Finishing*. Journal of Polymer and Textile Engineering 2015. **2**(2): p. 01-04.
14. Lengyel, M., N. Kállai-Szabó, V. Antal, A.J. Laki, and I. Antal, *Microparticles, Microspheres, and Microcapsules for Advanced Drug Delivery*. Scientia Pharmaceutica, 2019. **87**(3).
15. Das, S.K., S.R.N. David, R. Rajabalaya, H.K. Mukhopadhyay, T. Halder, M. Palanisamy, J. Khanam, and A. Nanda, *Microencapsulation Techniques and Its Practice*. Pharma Sci Tech 2011. **6**(2).
16. Almadhoni, K. and S. Khan, *Using Metallic Foams with Macro-encapsulated Paraffin to Enhance the Charging and DIS-charging Processes*. Journal of Mechanical and Civil Engineering 2015. **12**(4): p. 09-22.
17. Carmona, C.G., *Desarrollo de microcápsulas rígidas de fragancia y de técnicas que permitan la cuantificación de la eficiencia de encapsulación*, in *Institut d'Investigació Téxtil* 2019, Universitat Politècnica de Catalunya.

18. Jyothi, N.V., P.M. Prasanna, S.N. Sakarkar, K.S. Prabha, P.S. Ramaiah, and G.Y. Srawan, *Microencapsulation techniques, factors influencing encapsulation efficiency.* J Microencapsul, 2010. **27**(3): p. 187-97.

19. Andrews, H.E., J.G.B. González, F.C. Sosa, and E.J.V. Carter, *Gum Arabic-Chitosan Complex Coacervation.* Biomacromolecules 2007. **8**(4): p. 1313-1318.

20. Ocak, B., *Complex coacervation of collagen hydrolysate extracted from leather solid wastes and chitosan for controlled release of lavender oil.* J Environ Manage, 2012. **100**: p. 22-8.

21. Brito de Souza, V., M. Thomazini, I.E. Chaves, R. Ferro-Furtado, and C.S. Favaro-Trindade, *Microencapsulation by complex coacervation as a tool to protect bioactive compounds and to reduce astringency and strong flavor of vegetable extracts.* Food Hydrocolloids, 2020. **98**.

22. Sharkawy, A., I.P. Fernandes, M.F. Barreiro, A.E. Rodrigues, and T. Shoeib, *Aroma-Loaded Microcapsules with Antibacterial Activity for Eco-Friendly Textile Application: Synthesis, Characterization, Release, and Green Grafting.* Industrial & Engineering Chemistry Research, 2017. **56**(19): p. 5516-5526.

23. Robles, H., *Tannic Acid*, in *Encyclopedia of Toxicology*, P. Wexler, et al., Editors. 2014. p. 2.

24. Liu, J., C. Liu, Y. Liu, M. Chen, Y. Hu, and Z. Yang, *Study on the grafting of chitosan-gelatin microcapsules onto cotton fabrics and its antibacterial effect.* Colloids Surf B Biointerfaces, 2013. **109**: p. 103-8.

25. Cheung, R.C., T.B. Ng, J.H. Wong, and W.Y. Chan, *Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications.* Mar Drugs, 2015. **13**(8): p. 5156-86.

26. Kandile, N.G., H.T. Zaky, M.I. Mohamed, A.S. Nasr, and Y.G. Ali, *Extraction and Characterization of Chitosan from Shrimp Shells.* Open Journal of Organic Polymer Materials, 2018. **08**(03): p. 33-42.

27. Ltda., D.B., *Os Tipos de Gomas E Suas Aplicações Na Indústria* D.B. Ltda., Editor. p. 30-39.

28. Dave, P.N. and A. Gor, *Natural Polysaccharide-Based Hydrogels and Nanomaterials*, in *Handbook of Nanomaterials for Industrial Applications*. 2018. p. 36-66.

29. Iscan, G., N. Kirimer, M. Kurkcuoglu, K.H.C. Baser, and F. Demirci, *Antimicrobial Screening of Mentha piperita Essential Oils.* Journal of Agricultural and Food Chemistry, 2002. **50**(14): p. 3943-3946.

30. Rajkumar, V., C. Gunasekaran, C.A. Paul, and J. Dharmaraj, *Development of encapsulated peppermint essential oil in chitosan nanoparticles: characterization and biological efficacy against stored-grain pest control.* Pestic Biochem Physiol, 2020. **170**: p. 104679.

31. Alankar, S., *A Review On Peppermint Oil.* Asian Journal of Pharmaceutical and Clinical Research 2009. **2**(2): p. 187-193.

32. Martins, I.M., S.N. Rodrigues, M.F. Barreiro, and A.E. Rodrigues, *Polylactide-Based Thyme Oil Microcapsules Production Evaluation of Surfactants.* Industrial & Engineering Chemistry Research, 2011. **50**(2): p. 898-904.

33. Sadjia, B., S. Naima, and B. Chahrazed, *Extraction of Thyme (Thymus pallecensde Noé) Essential Oil by Steam-Distillation, Steam-Diffusion and Hydro-Distillation Processes: Optimization of Operating Conditions and Antioxidant Activity.* Journal of Essential Oil Bearing Plants, 2012. **15**(2): p. 336-347.

34. Allahverdiyev, A.M., M. Bagirova, S. Yaman, Rabia C. Koc, E.S. Abamor, S.C. Ates, Serap Y. Baydar, S. Elcicek, and O.N. Oztel, *Development of New Antiherpetic Drugs*

Based on Plant Compounds, in *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components*. 2013. p. 245-259.

35. Bojana, B.P. and S. Marica, *Microencapsulation technology and applications in added-value functional textiles.* Physical Sciences Reviews, 2016. **1**(1).

36. Powerlab. *Beckman Coulter* 2007 11 June 2021]; Available from: http://e-powerlab.co.kr/powerlab/sub_02_01_view.php?seq=93&page=1&depth1=&depth2=78&depth3=&PHPSESSID=949e57877686919b30290711f7972320.

37. Scientific, T. *SCANNING ELECTRON MICROSCOPES Phenom ProX Desktop SEM*. 11 June 2021]; Available from: https://www.thermofisher.com/pt/en/home/electron-microscopy/products/desktop-scanning-electron-microscopes/phenom-prox.html.

38. ASTM, *Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions*, in *E2149-13a*. 2014.

39. Goni, M.G., B. Tomadoni, S.I. Roura, and M.D.R. Moreira, *Lactic acid as potential substitute of acetic acid for dissolution of chitosan: preharvest application to Butterhead lettuce*. J Food Sci Technol, 2017. **54**(3): p. 620-626.

40. Lima, F.F.d., C.H. Lescano, E.F.R.d. Oliveira, F.M. Fakhouri, I.C.F. Moraes, C.A.L. Kassuya, and E.J. Sanjinez-Argandoña, *Characterization and optimization of oil microcapsules from Attalea phalerata Mart. for the preservation of bioactive compounds.* Brazilian Journal of Pharmaceutical Sciences, 2019. **55**.

41. Kamarudin, K.S.N., I. Bhatti, S.N. Suahadah, U.N. Hamzah, and Z.F. MohdFaiz, *Removal of Carbon Dioxide Using Water-in-Oil Emulsion Liquid Membrane Containing Triethanolamine.* Journal of Applied Sciences Research 2010. **6**(12): p. 2251-2256.

42. Kheradmandnia, S., E. Vasheghani-Farahani, and M. Nosrati, *The Effect of Process Variables on the Properties of Ketoprofen Loaded Solid Lipid Nanoparticles of Beeswax and Carnauba Wax.* Iranian Journal of Chemistry and Chemical Engineering 2010. **29**(4): p. 181-187.

43. Sigma-Aldrich. *SP Brij® C2 MBAL-SO-(SG)*. 2021 20 June 2021]; Available from: https://www.sigmaaldrich.com/PT/en/product/aldrich/388831.

44. McClements, D.J. and S.M. Jafari, *Improving emulsion formation, stability and performance using mixed emulsifiers: A review.* Adv Colloid Interface Sci, 2018. **251**: p. 55-79.

45. IKA, *IKA ULTRA TURRAX T 25 digital*, imlab, Editor.

46. Butstraen, C. and F. Salaun, *Preparation of microcapsules by complex coacervation of gum Arabic and chitosan.* Carbohydr Polym, 2014. **99**: p. 608-16.

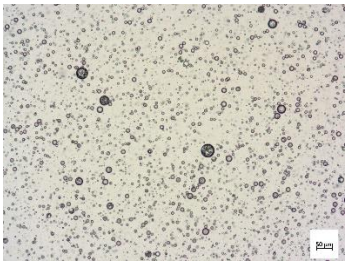
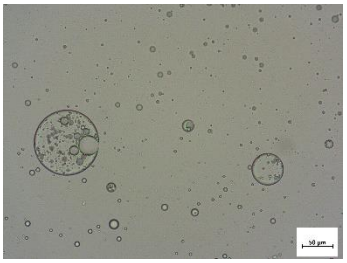
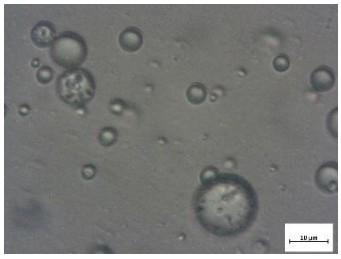
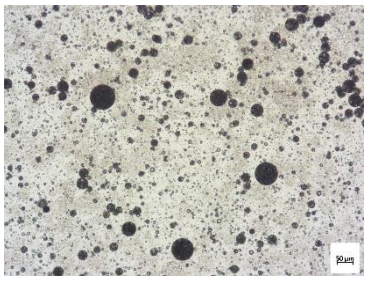
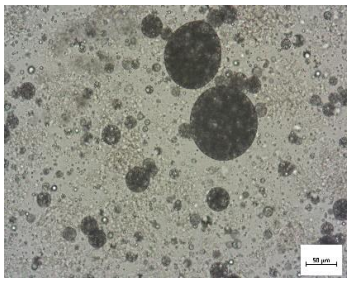
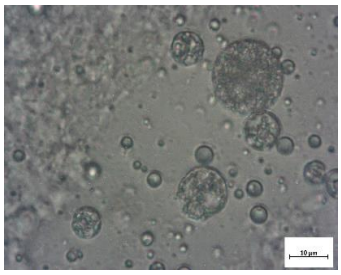
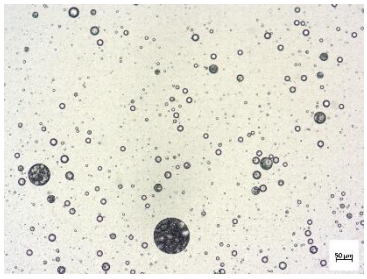
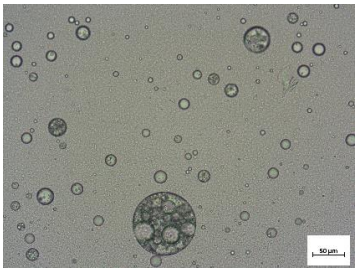
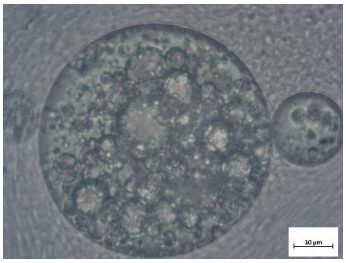
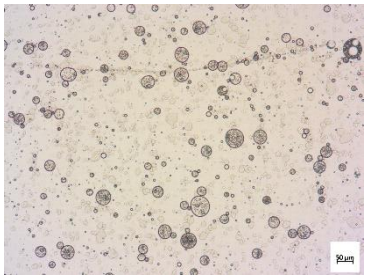
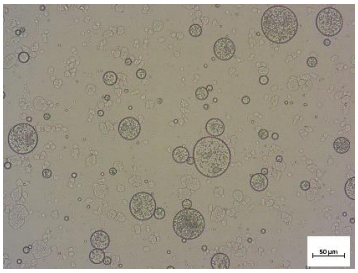
47. Samu, R., A. Moulee, and V.G. Kumar, *Effect of Charge and Hydrophobicity on Adsorption of Modified Starches on Polyester.* Journal of Colloid and Interface Science 1999. **220**: p. 260-268.

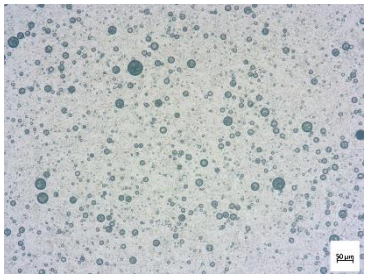
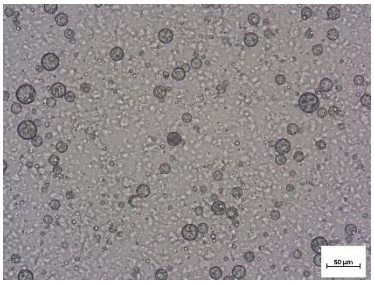
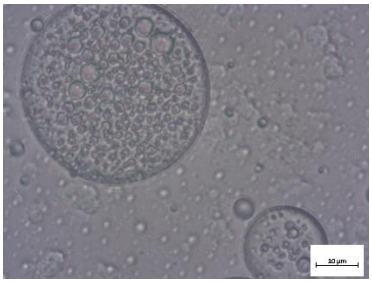
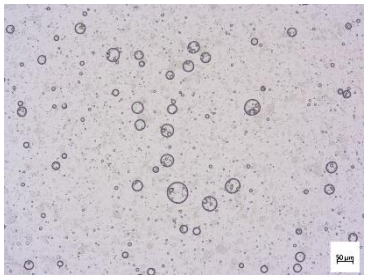
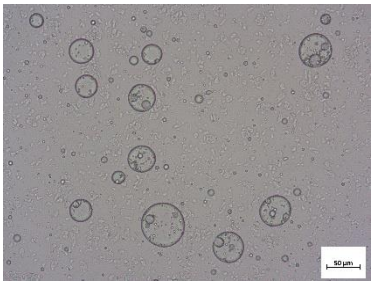
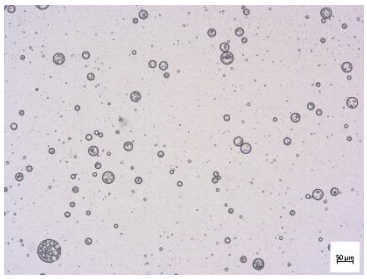
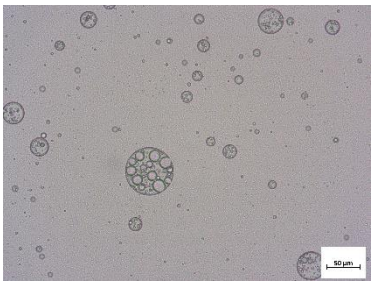
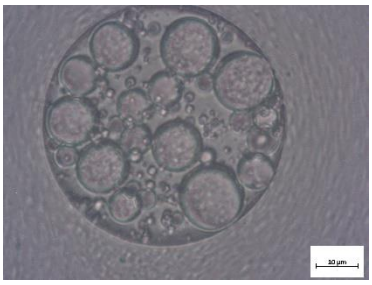
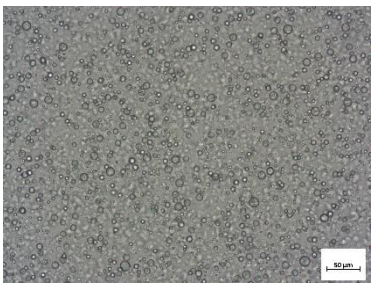
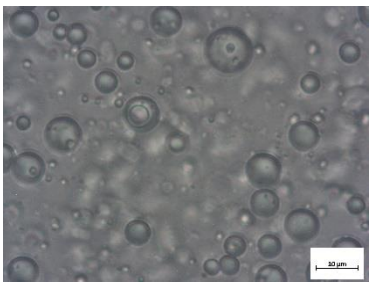
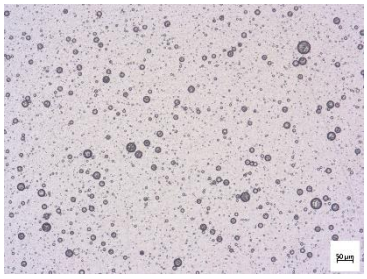
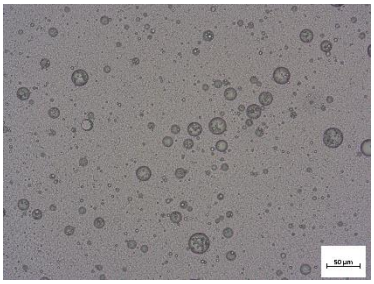
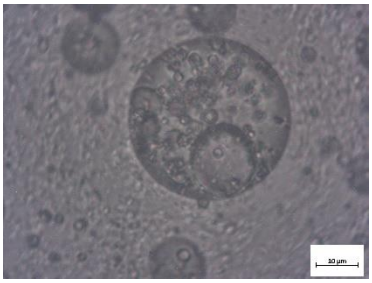
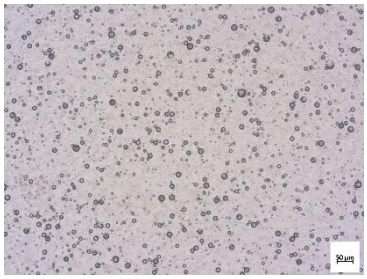
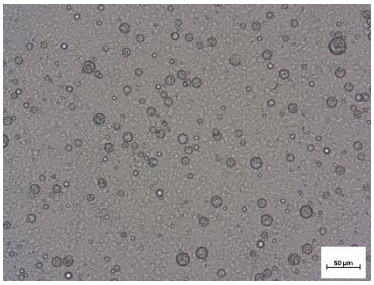
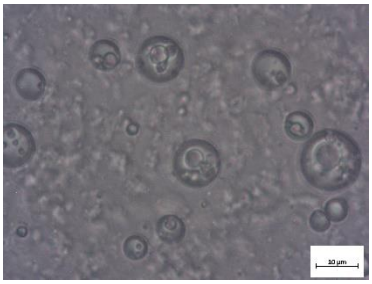
48. Chatterjee, S., F. Salaun, and C. Campagne, *Development of multilayer microcapsules by a phase coacervation method based on ionic interactions for textile applications.* Pharmaceutics, 2014. **6**(2): p. 281-97.

Appendix A - Optical and scanning electron microscopy images

A.1 – Optical microscopy images

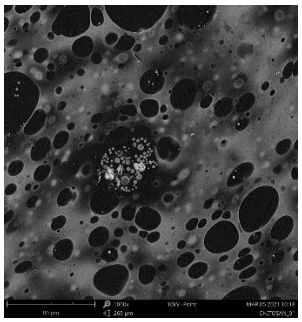
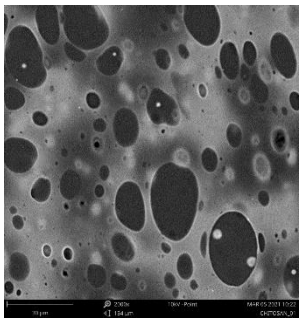
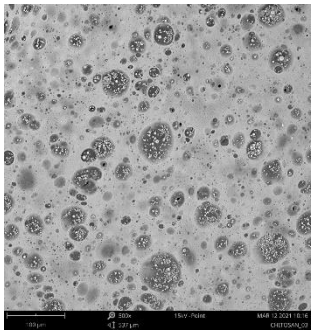
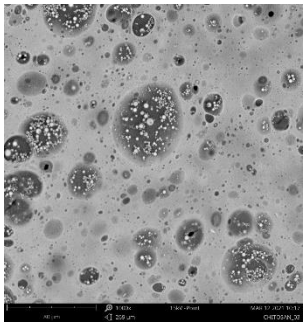
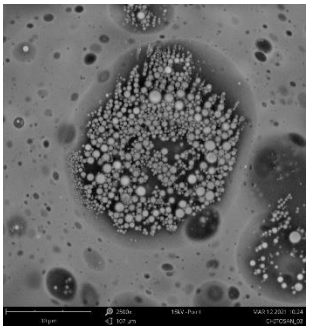
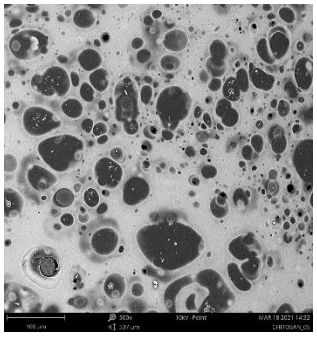
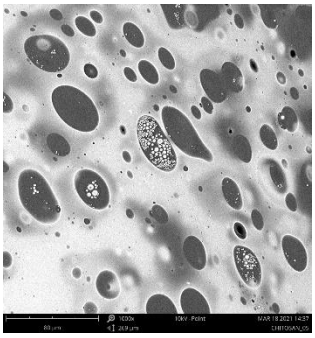
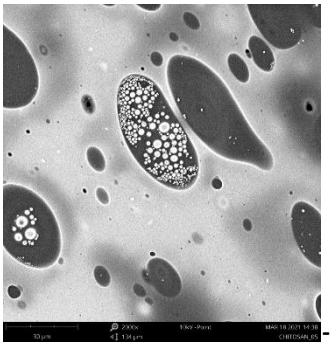
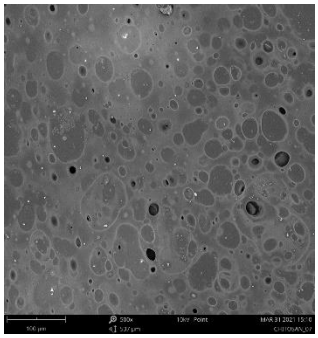
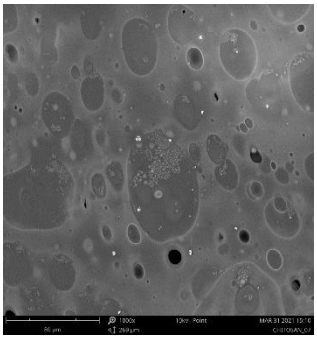
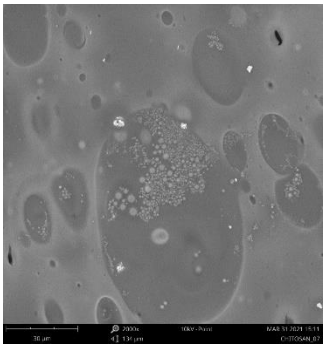
Table 13. Optical microscopy images of the microcapsules at the end of the process.

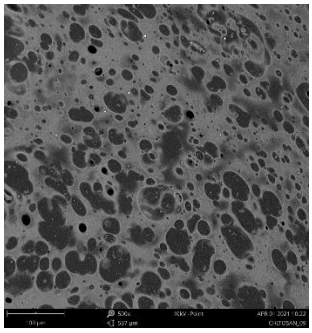
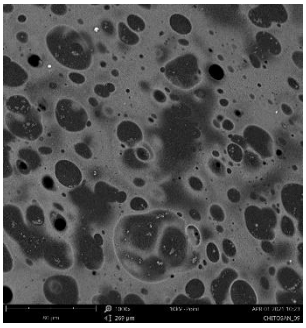
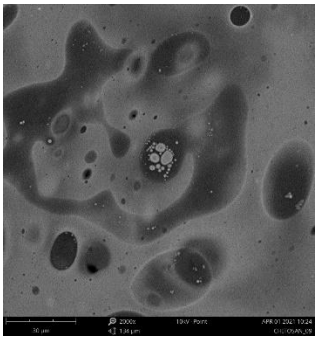
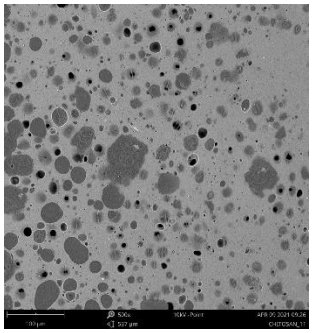
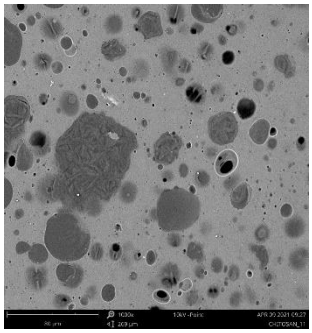
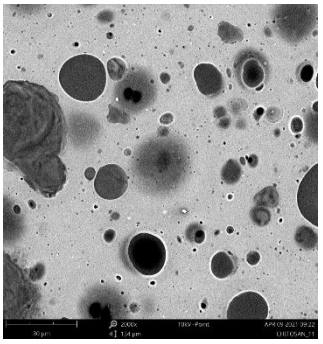
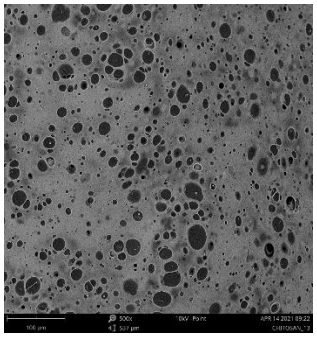
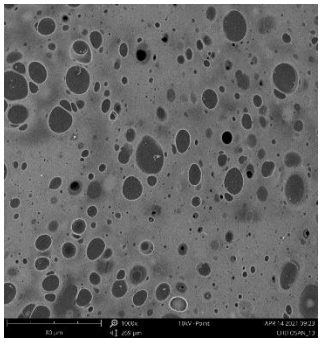
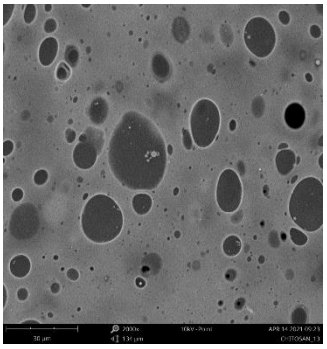
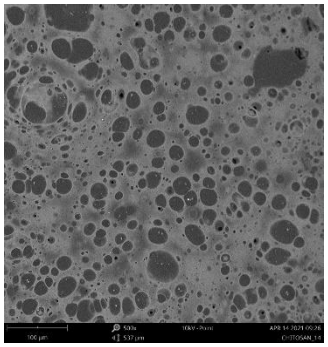
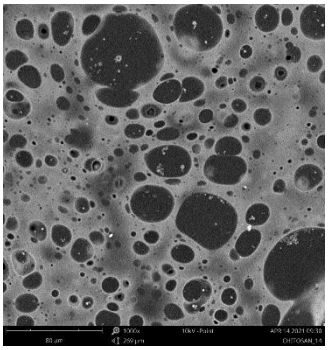
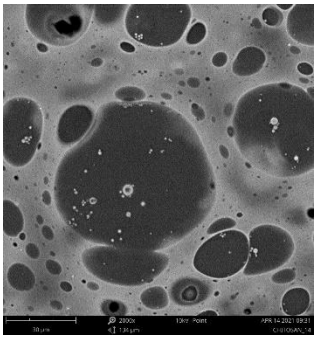
	Microcapsules sample at the end of the process		
Formulation	**100x magnification**	**200x magnification**	**1000x magnification**
1			
2			
3			
4			

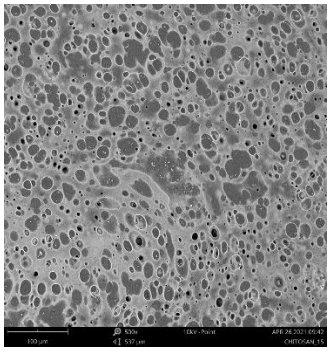
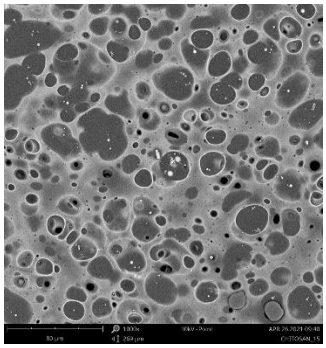
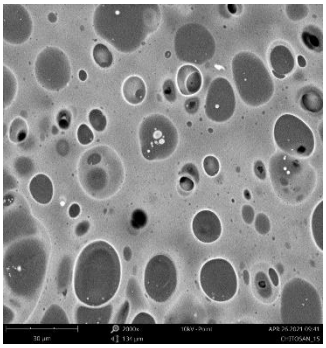
5			
6			
7			
8			
9			
10			

A.2 – Scanning electron microscopy images

Table 14. Scanning electron microscopy images of the microcapsules at the end of the process.

	Microcapsules sample at the end of the process		
Formulation	**500x magnification**	**1000x magnification**	**2000x or 2500x magnification**
1	-		
2			
3			
4			

5			
6			
7			
8	-	-	-
9			

10			

Appendix B – Effect of the speed and time at the homogenization step images

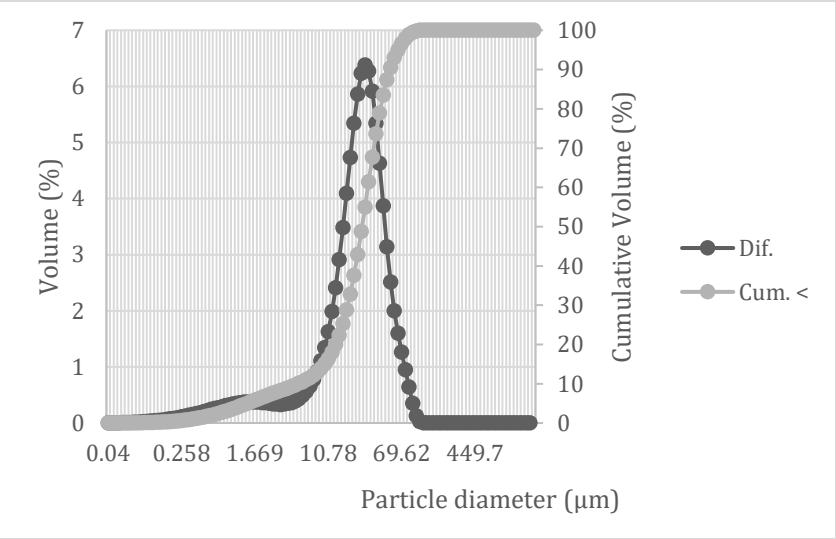
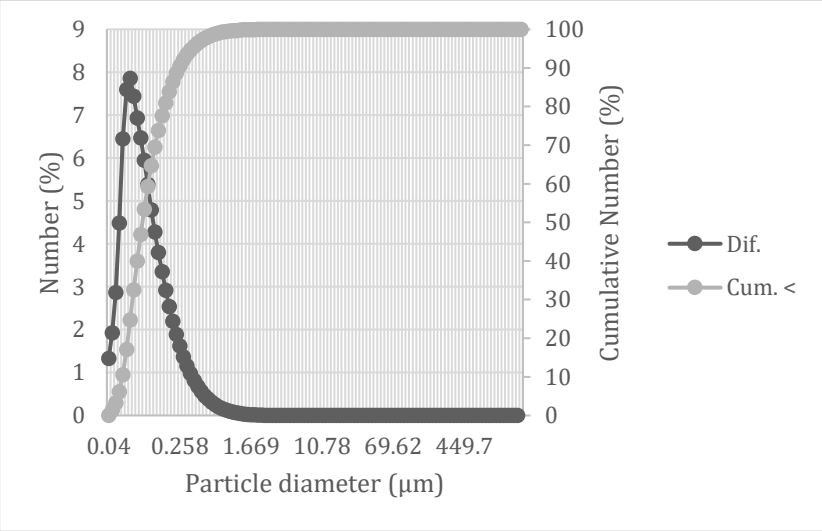


Figure 28. Images relating to the visual appearance of: formulation 4 (left) formulation 5 (right).

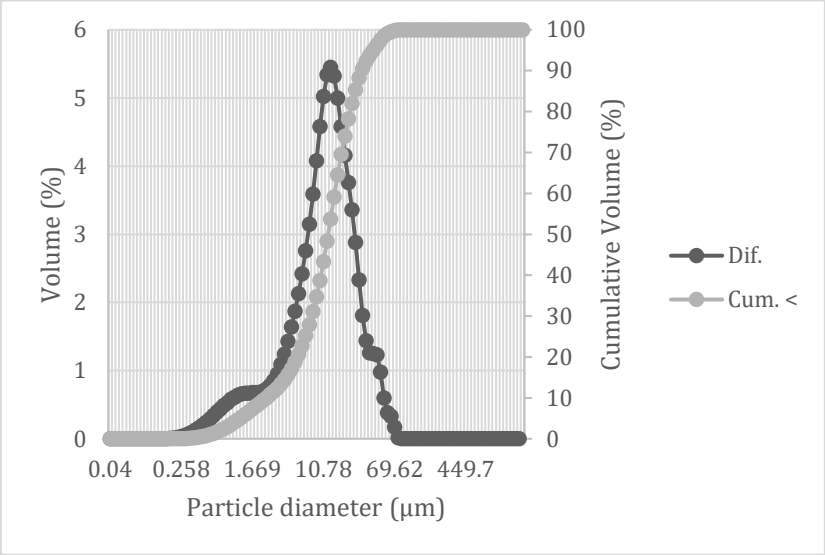
Figure 28 shows that the sample from formulation 4 presents surface stains, whereas formulation 5 does not.

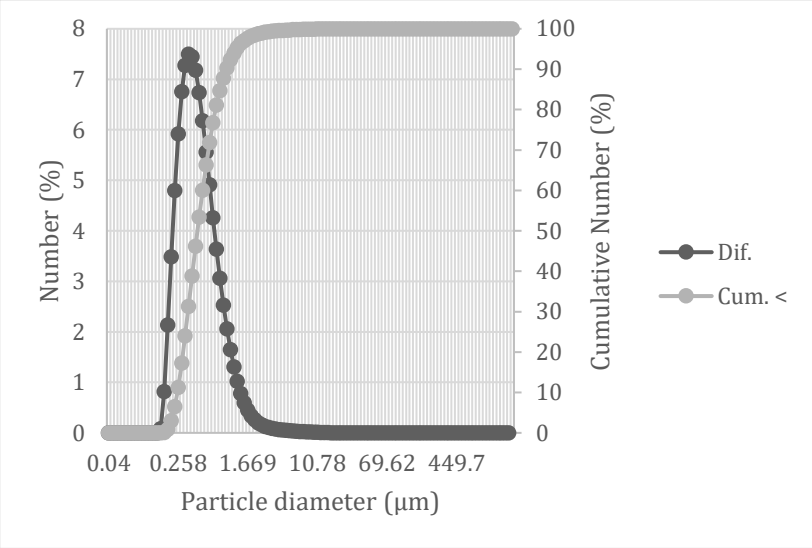
Appendix C - Particle size evaluation

Table 15. Particle size evaluation in volume and in number.

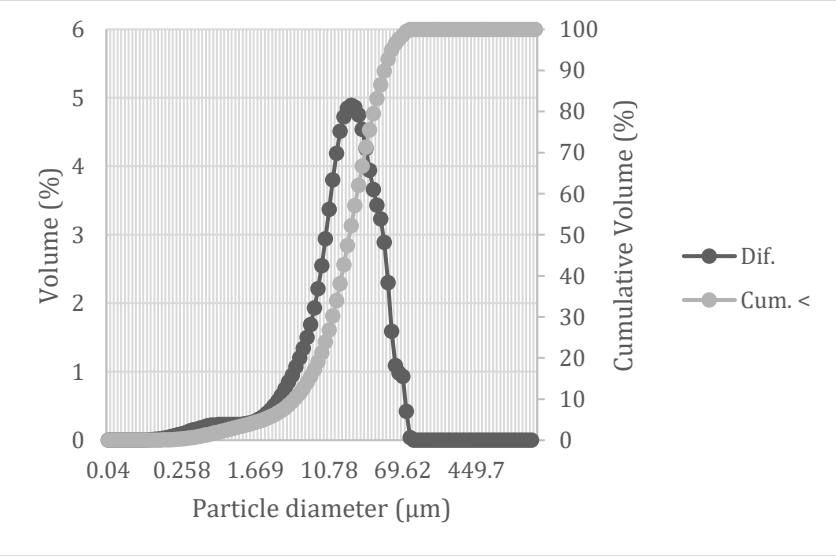
Assay	Volume	Number
2		

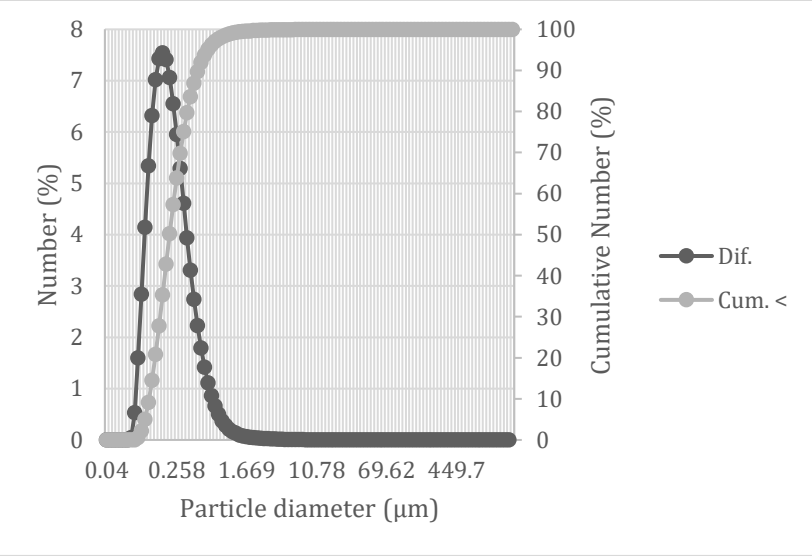
3


Volume (%)
Cumulative Volume (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

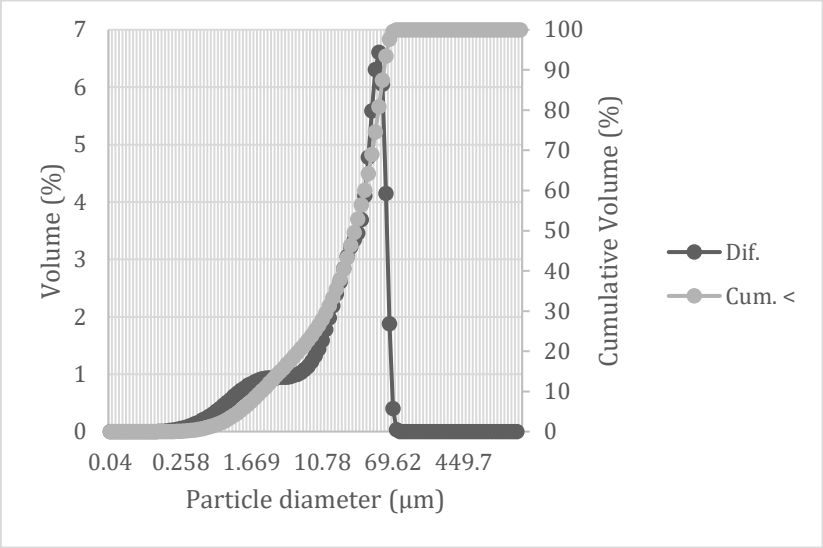

Number (%)
Cumulative Number (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

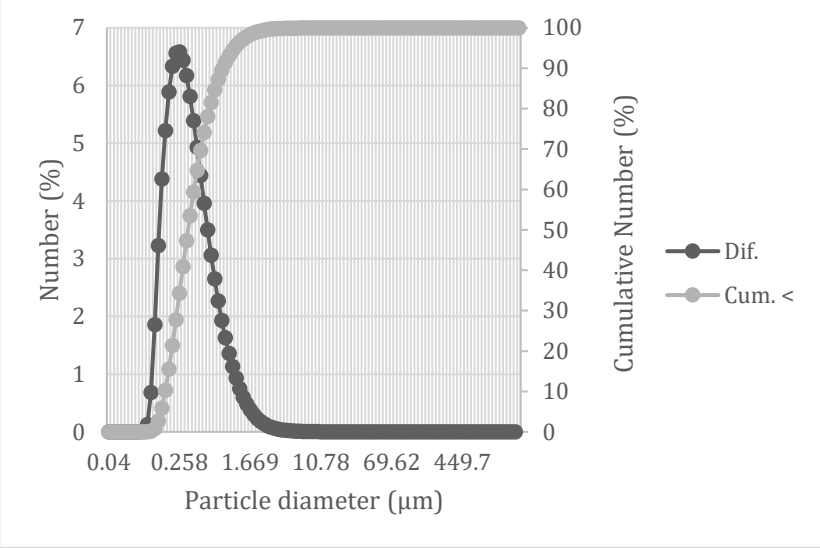
5


Volume (%)
Cumulative Volume (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

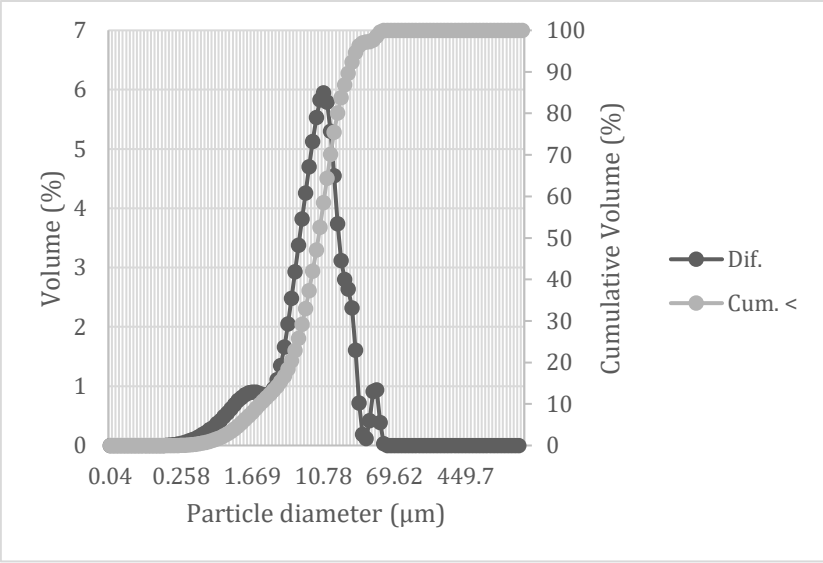

Number (%)
Cumulative Number (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

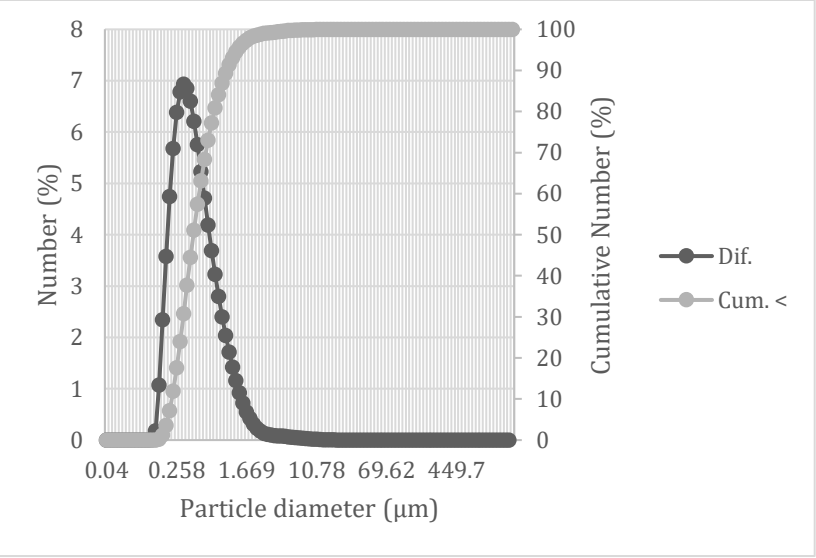
6


Volume (%)
Cumulative Volume (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

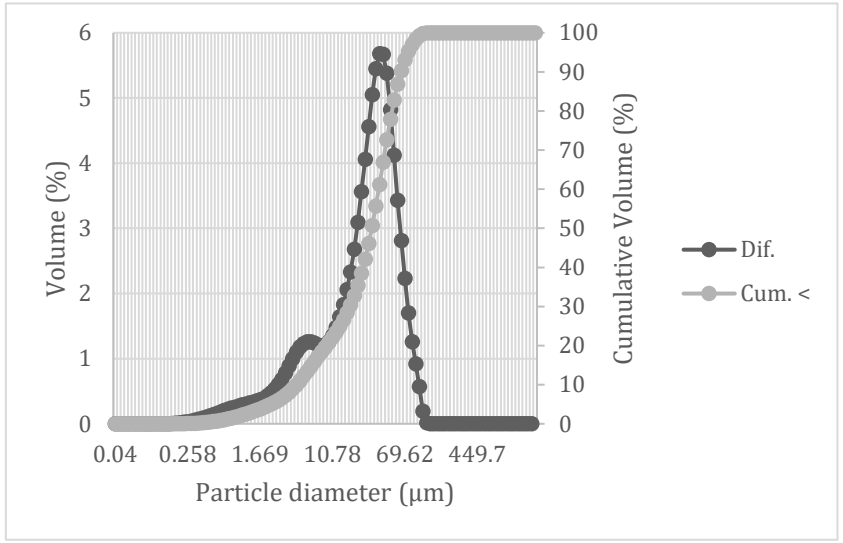

Number (%)
Cumulative Number (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

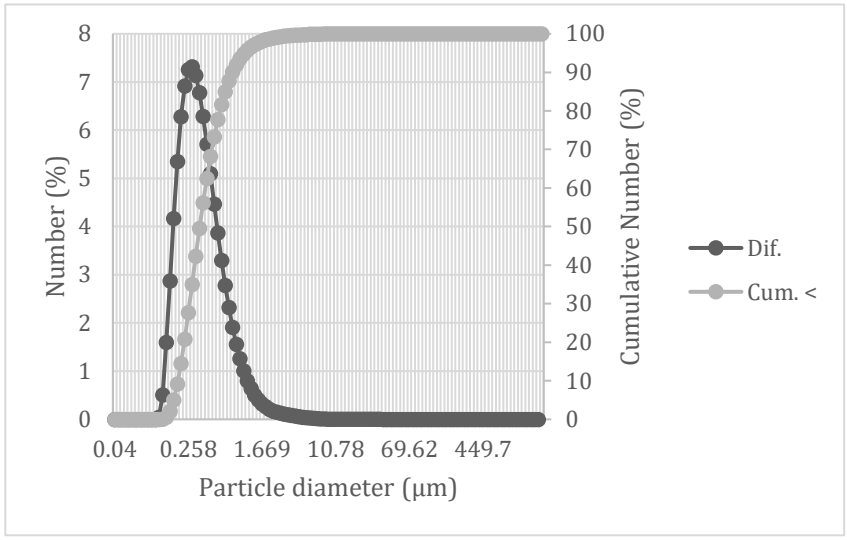
7


Volume (%)
Cumulative Volume (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

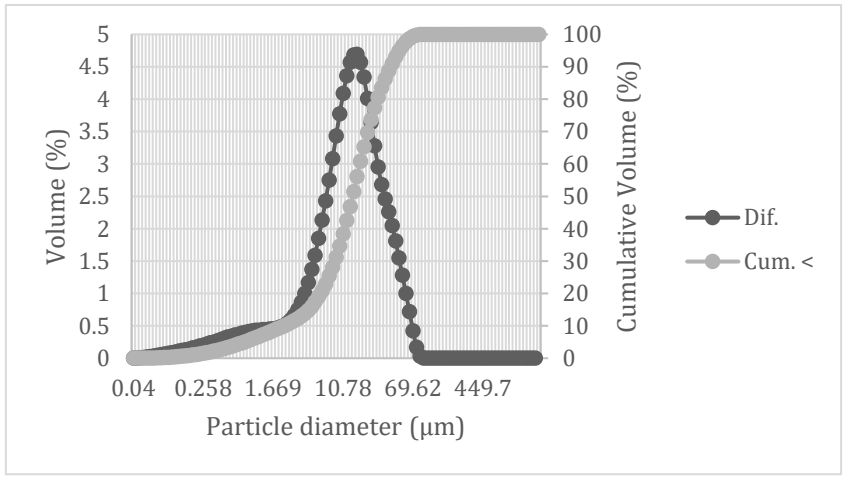

Number (%)
Cumulative Number (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

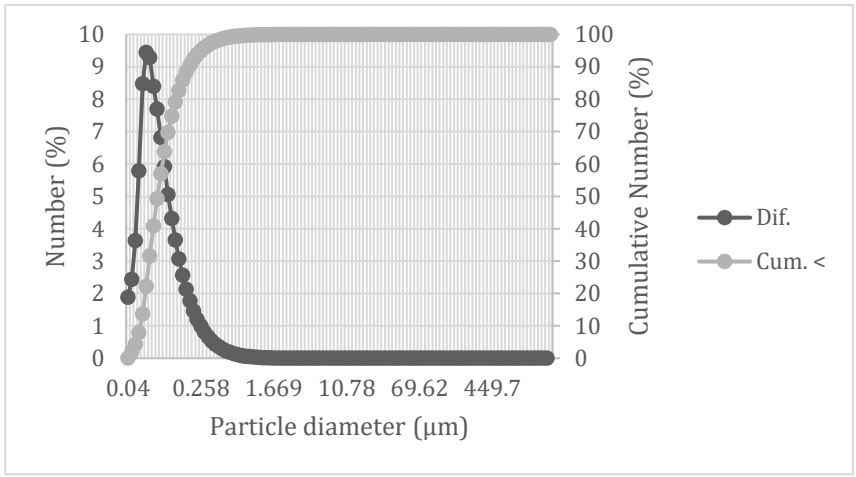
8


Volume (%)
Cumulative Volume (%)
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)
Dif.
Cum. <

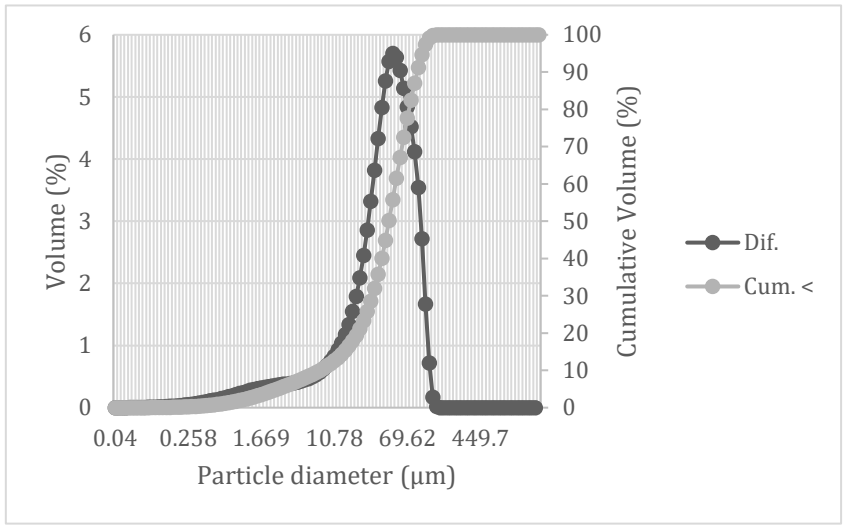

Number (%)
Cumulative Number (%)
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)
Dif.
Cum. <

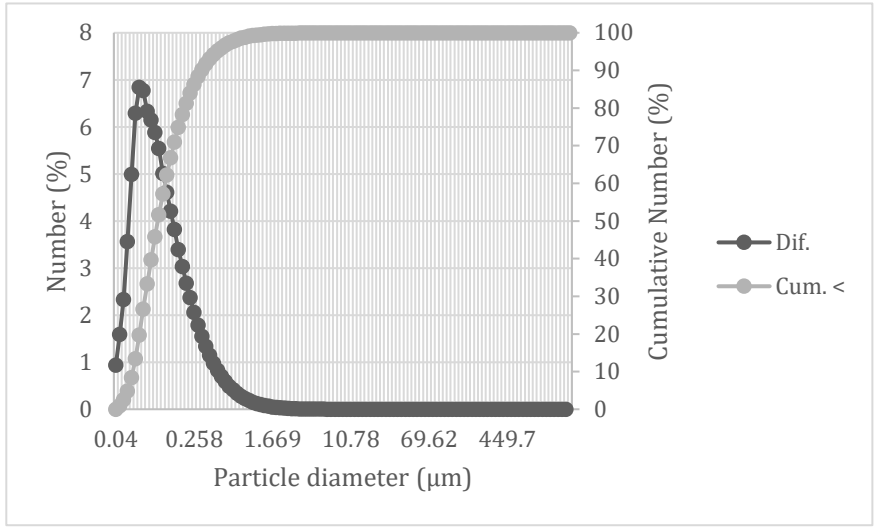
9


Volume (%)
Cumulative Volume (%)
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)
Dif.
Cum. <


Number (%)
Cumulative Number (%)
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)
Dif.
Cum. <

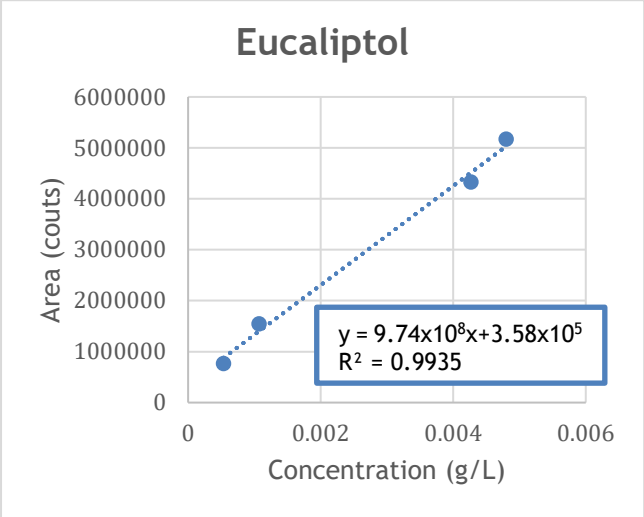
10

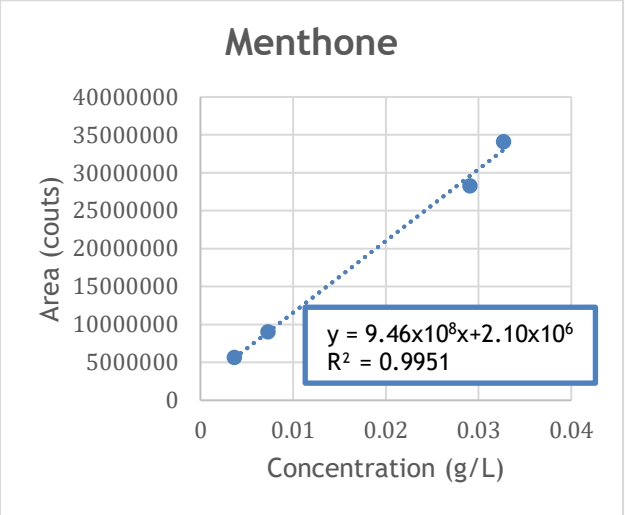

Volume (%)
Cumulative Volume (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

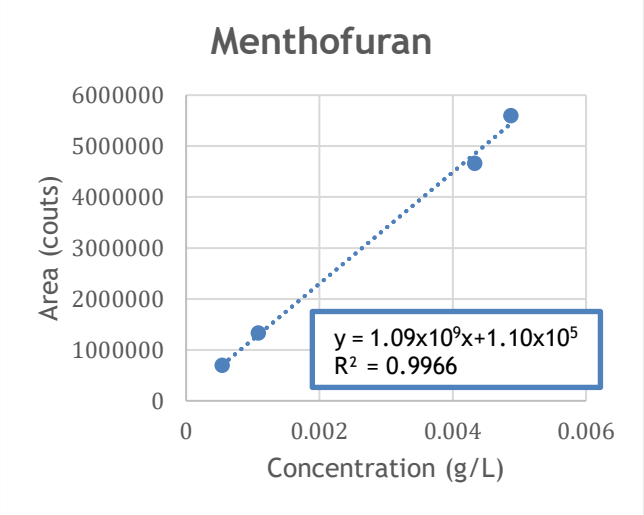

Number (%)
Cumulative Number (%)
Dif.
Cum. <
0.04 0.258 1.669 10.78 69.62 449.7
Particle diameter (µm)

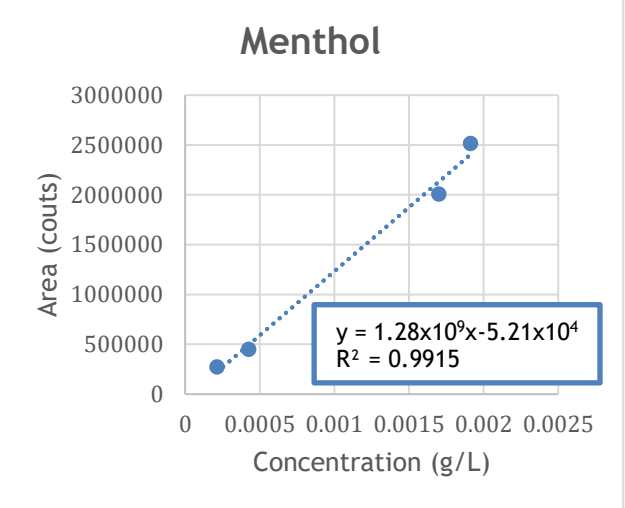
Appendix D - Calibration curves

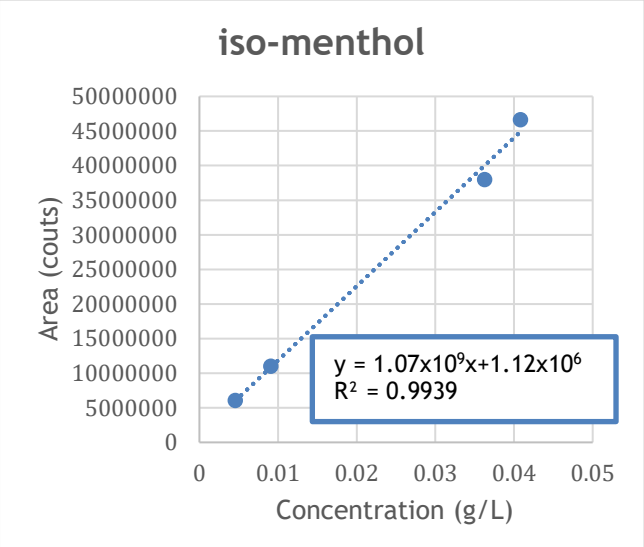
Table 16. Calibration curves for peppermint oil compounds.

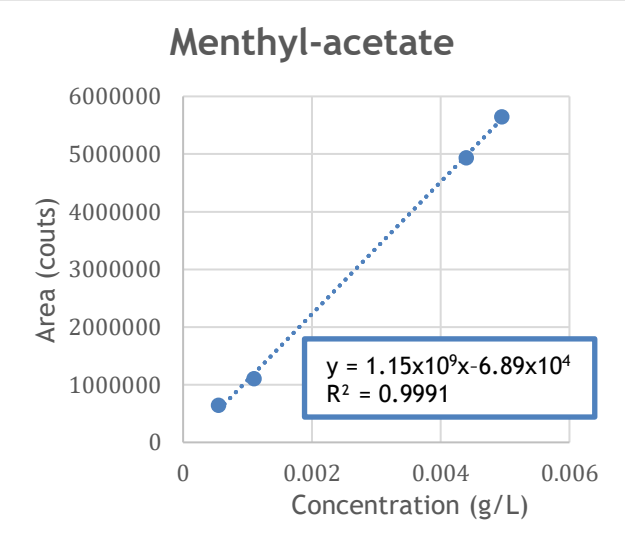












Appendix E - Percentage of each compound for all assays

Table 17. Percentage of each compound for all formulations.

Formulation	Water (%)	Acid (%)		Core material (%)		Emulsifier (%)		CH (%)	GA (%)	TA (%)
		AA	LA	PO	TO	PGPR	Brij C2			
1	45.59	47.87	-	0.98	-	0.32	-	0.46	0.91	3.87
2	87.74	-	0.49	1.97	-	0.61	-	0.88	0.88	7.44
3	86.97	-	0.48	1.95	-	0.61	-	0.87	1.74	7.38
4	90.08	-	0.75	2.02	-	0.63	-	0.90	1.80	3.82
5	90.08	-	0.75	2.02	-	0.63	-	0.90	1.80	3.82
6	90.08	-	0.75	2.02	-	-	0.63	0.90	1.80	3.82
7	90.06	-	0.75	1.62	0.42	0.63	-	0.90	1.80	3.82
8	90.06	-	0.75	1.62	0.42	0.63	-	0.90	1.80	3.82
9	90.01	-	0.75	0.40	1.69	0.63	-	0.90	1.80	3.82
10	90.06	-	0.75	1.62	0.42	0.63	-	0.90	1.80	3.82

Appendix F - Reduction rate of odor

According to ISO 17299-3:2014 standard, textile fabrics are considered to have deodorant properties if there is at least an 85% reduction for the IVA marker.

Table 18. Odor reduction rate (%) of the textile substrate studied with formulation 8.

Textile substrate	Odor reduction rate (%)
Knit Beige Unwashed	42
Knit Beige Washed	53

The results obtained might appear contradictory, since the unwashed treated fabric should exhibit a better result than the washed treated fabric. However, unwashed fabrics have the fibers all twisted together and when the microcapsules are impregnated, they have the tendency to not be exposed. Once the fabric is washed, the fibers start to untangle and, consequently, the microcapsules are more easily exposed. This can be verified by the presence of the small rounded shaped microcapsule on the washed fabric in Figure 26. Hence, in textile industry it is recurrent that the washed substrate achieves better results than the unwashed one.