Deliberate Chemical Contamination of Water Supply Systems – Carbofuran and Chlorfenvinphos as Case Studies

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Contents

Abstract

Water is one of the most important substances on earth, and is also indispensable for human living. The susceptibility of water supply systems to contaminations, deliberate or accidental, is a known fact. SecurEau was a European Union funded project that aimed to develop an early warning system to minimize the impact of a contamination event, deliberate or not, that could affect public water infrastructures. This work, which was an integrant part of the SecurEau project, aimed to develop analytical methodologies for the detection and quantification of previously selected model compounds (carbofuran and chlorfenvinphos) in water and deposits from drinking water distribution systems, and to study the interaction of the two pesticides with those deposits.

The first analytical method developed, consisting on the direct injection (DI) and analysis by liquid chromatography with diode array detection (LC-DAD), intended to be a rapid way of detecting a possible contamination, at high concentration levels, in water. Limits of detection (LODs) of 4 and 11 $\mu g.L^{-1}$ were obtained for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed at three spike levels (0.1, 1 and 10 $mg.L^{-1}$) in different water matrices, and average recoveries of 99% and 95% were obtained for carbofuran and chlorfenvinphos, respectively. A second method, involving the preconcentration of water samples by dispersive liquid-liquid microextraction (DLLME) and analysis by gas chromatography with mass spectrometric detection (GC-MS), was also developed. The main objective of this methodology was to enable the rapid and reliable identification and quantification of both pesticides, up to the levels required by European legislation. LODs of 0.04 and 0.02 $\mu g.L^{-1}$ were achieved for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed at two spike levels (50 and 200 $\mu g.L^{-1}$) in different aqueous matrices (river, tap, plain and sparkling water) and, except for the results obtained for the sparkling water, the recoveries for the extraction in the other matrices were in the range of 75-120% (average value of 98.2% was reached). Both methods showed wide linearity (DI-LC-DAD: 0.05-100 $mg.L^{-1}$; DLLME-GC-MS: 0.1-250 $\mu g.L^{-1}$ for carbofuran and 0.1-200 $\mu g.L^{-1}$ for

chlorfenvinphos), good repeatability and high recoveries in several matrices, within a short analysis period.

The possibility of contaminants being accumulated in the deposits, which exist in water distribution systems, should not be discarded. Thus, another developed methodology consisted in the ultrasonic extraction with acetonitrile of chlorfenvinphos from clay, and was intended to allow the detection and quantification of a possible contamination, in a fast, simple and reliable way. The obtained limits of detection and quantification were of 0.35 and 1.05 $mg.g^{-1}$, respectively. Also, high average recoveries were obtained for the three contamination levels tested, with values ranging from 101 to 106%. This method was also tested in the extraction of chlorfenvinphos from two other types of deposits: S3, a tubercle deposit (composed mainly of goethite), and S4, a white deposit (composed mainly of calcite) (nomenclature of the deposits results from SecurEau reports). Detection limits of 0.23 and 0.11 $mg.g^{-1}$ were obtained for the extraction of S3 and S4 deposits, respectively. Additionally, the method was tested for the extraction of carbofuran from clay, S3 and S4 deposits, and the obtained results indicate that it can be used to the quickly detect and quantify carbofuran in clay and S4 deposit. Thus, this simple and rapid extraction method enabled the detection and quantification of carbofuran and chlorfenvinphos in different types of deposits, whenever the appropriate extraction percentages are taken into account.

Kinetic and equilibrium experiments were performed, in order to study the adsorption of carbofuran and chlorfenvinphos onto S4 deposit. The kinetic study showed that pseudo-first and pseudo-second order kinetic models fitted well to the experimental results. Nevertheless, the kinetic study showed that the adsorption of carbofuran onto S4 deposit is best described by a pseudo-first order model, whereas the adsorption of chlorfenvinphos is best described by a pseudo-second order model. At equilibrium, the three isotherm models tested were able to successfully fit the experimental results, of each pesticide; yet, Langmuir model was slightly better overall. Maximum adsorption capacities (estimated by Langmuir model) of 1.4 and 1.6 $mg.g^{-1}$ were obtained for carbofuran and chlorfenvinphos, respectively, at 20 °C for S4 deposit.

The simultaneous adsorption of carbofuran and chlorfenvinphos onto S4 deposit was also studied and no significant competition effects between the two pesticides was

observed. Additionally, it was verified that, at equilibrium, the extended Freundlich model is able to satisfactorily describe the simultaneous adsorption of the pesticides onto the S4 deposit.

Therefore, it can be stated that the developed methods allow the detection and quantification of carbofuran and chlorfenvinphos in water and deposits, in a rapid and easy form, with low solvent consumption. Furthermore, interaction studies allow to conclude that, in a contamination event of drinking water distribution systems, little adsorption of the two pesticides onto white deposits should occur since, in the pipe networks, the contact times and the surface area available for adsorption ought to be much lower.

Finally, it can be affirmed that this work is a relevant contribution to the development of appropriate contamination warning strategies for drinking water distribution systems.

Resumo

A água é uma das substâncias mais importantes na Terra, e é também indispensável à vida humana. A suscetibilidade dos sistemas de abastecimento de água a contaminações, sejam elas deliberadas ou acidentais, é um facto conhecido. O projeto SecurEau, financiado pela União Europeia, tinha como objetivo o desenvolvimento de um sistema de alerta rápido que minimize o impacto de uma contaminação, deliberada ou não, que possa afetar infraestruturas públicas de abastecimento de água. Este trabalho, que foi parte integrante do projeto SecurEau, pretendeu desenvolver metodologias analíticas para a deteção e quantificação de carbofurão e clorfenvinfos em água e depósitos de sistemas de distribuição de água, e estudar a interação dos dois pesticidas com esses depósitos.

O primeiro método analítico desenvolvido, que consiste na injeção direta (DI) e análise por cromatografia líquida com deteção de díodos (LC-DAD), pretendeu ser uma forma rápida de detetar uma possível contaminação, a níveis de concentração elevados, em água. Alcançaram-se limites de deteção de 4 e 11 $\mu g.L^{-1}$ para o carbofurão e clorfenvinfos, respetivamente. Realizaram-se ensaios de recuperação a três níveis de fortificação (0.1, 1 and 10 $mg.L^{-1}$), e obtiveram-se recuperações médias de 99% e 95% para o carbofurão e clorfenvinfos, respetivamente. O segundo método desenvolvido consiste na pré-concentração de amostras de água por microextração líquido-líquido dispersiva (DLLME) e análise por cromatografia gasosa com deteção por espetrometria de massa (GC-MS). O objetivo principal desta metodologia era permitir a identificação e quantificação de ambos os pesticidas aos níveis de concentração requeridos pela legislação Europeia, de forma rápida e confiável. Alcançaram-se limites de deteção de 0.04 e 0.02 $\mu g.L^{-1}$ para o carbofurão e clorfenvinfos, respetivamente. Realizaram-se ensaios de recuperação a dois níveis de fortificação (50 and 200 $\mu g.L^{-1}$) em diversas matrizes aquosas (água de rio, da torneira, simples e com gás) e, com exceção dos resultados obtidos para a água com gás, as recuperações para as extrações noutras matrizes variaram entre 75-120% (valor médio de 98.2%). Ambos os métodos revelaram ampla linearidade (DI-LC-DAD: 0.05-100 $mg.L^{-1}$; DLLME-GC-MS: 0.1-250 $\mu g.L^{-1}$ para o

carbofurão e 0.1-200 μg.L^{-1} para o clorfenvinfos), boa repetibilidade e recuperações elevadas em várias matrizes, para um tempo de análise curto.

A possibilidade dos contaminantes ficarem acumulados nos depósitos, os quais existem nos sistemas de distribuição de água, não deve ser descartada. Assim, foi desenvolvida uma outra metodologia que consiste na extração por ultrassons com acetonitrilo de clorfenvinfos de caulino, e que foi concebida para a deteção e quantificação de uma possível contaminação, de uma forma rápida, simples e fiável. Os limites de deteção e quantificação obtidos foram de 0.35 e 1.05 mg.g^{-1}, respetivamente. Obtiveram-se ainda recuperações elevadas para os três níveis de concentração testados, com valores entre os 101 e 106%. Este método foi também testado na extração de clorfenvinfos de dois outros tipos de depósitos: S3, um depósito do tipo "tubérculo" (composto principalmente por goethite) e S4, um depósito branco (composto principalmente por calcite) (a nomenclatura adotada para os depósitos é resultante dos relatórios do projeto SecurEau). Obtiveram-se limites de deteção de 0.23 e 0.11 mg.g^{-1}, para a extração dos depósitos S3 e S4, respetivamente. Adicionalmente, o método foi testado na extração de carbofurão de caulino, e dos depósitos S3 e S4, e os resultados obtidos indicaram que pode ser utilizado na rápida deteção e identificação de carbofurão em caulino e no depósito. Assim, verificou-se que este método simples e rápido permite a extração de carbofurão e clorfenvinfos de diferentes tipos de depósitos, sempre que se tiver em conta as percentagens de extração.

Realizaram-se ensaios de cinética e de equilíbrio, de forma a estudar a adsorção de carbofurão e clofenvinfos no depósito S4. O estudo cinético demonstrou que os modelos de pseudo-primeira e pseudo-segunda ordem ajustam os resultados experimentais. No entanto, considerando a generalidade dos resultados, concluiu-se que a adsorção do carbofurão segue o modelo de pseudo-primeira ordem, enquanto a adsorção do clorfenvinfos no depósito S4 é mais bem descrita pelo modelo de pseudo-segunda ordem. No equilíbrio, verificou-se que os três modelos testados descreviam de forma satisfatória os resultados experimentais, para cada pesticida. No entanto, concluiu-se que, de uma forma geral, o modelo de isotérmica de Langmuir revelou um ajuste ligeiramente melhor que os outros modelos. Obtiveram-se valores de capacidade

máxima de adsorção (estimadas pelo modelo de Langmuir) de 1.4 e 1.6 $mg.g^{-1}$ para o carbofurão e clofenvinfos, respetivamente, a 20 °C para o depósito S4.

A adsorção simultânea de carbofurão e clorfenvinfos no depósito S4 foi também testada, não se tendo verificado um efeito de competição significativo entre os dois pesticidas. Verificou-se ainda que, no equilíbrio, o modelo de Freundlich "estendido" descreveu satisfatoriamente a adsorção simultânea dos pesticidas no depósito S4.

Pode-se, então, dizer que os métodos desenvolvidos permitem a deteção e quantificação de carbofurão e clorfenvinfos em água e depósitos, de forma rápida e fácil, com baixo consumo de solventes. Os estudos de interação permitiram concluir que, no caso de uma contaminação de sistemas de distribuição de água, a adsorção dos dois pesticidas em depósitos brancos deverá ser mínima, para baixos tempos de contacto.

Finalmente, pode-se afirmar que este trabalho é uma contribuição relevante para o desenvolvimento de sistemas de alerta adequados para sistemas de distribuição de água potável.

Figure captions

Table captions

Nomenclature

Abbreviations

AC – acetone

AChE – acetylcholinesterase

ACN – acetonitrile

AMD – automated multiple development

ARE – average relative error

AU – arbitrary units

BChE – butyrylcholinesterase

BTEX – benzene, toluene, ethylbenzene and xylene

CB – chlorobenzene

CCD – central composite design

cf. – compare

CL – chemiluminescence

cont. – continuation

CW - carbowax

DAD – diode array detector

DF – degrees of freedom

DI – direct injection

DLLME – dispersive liquid-liquid microextraction

DoE – design of experiments

DVB - divinylbenzene

ECD – electron capture detector

EI – electronic ionization

EF – extended Freundlich model

e.g. – *exempli gratia*

EL – extended Langmuir model

ESI – electrospray ionization

EU – European Union

FID – flame ionization detector

FLD – fluorescence detector

FPD – flame photometric detector

FTD – flame thermionic detector

GC – gas chromatography

GC×GC - comprehensive two-dimensional gas chromatography

HF – hollow fiber

HPLC – high-performance liquid chromatography

HPTLC – high-performance thin layer chromatography

ICP – inductively coupled plasma

LC – liquid chromatography

LLE – liquid-liquid extraction

LOD – limit of detection

LOI – limit of identification

LOQ – limit of quantification

LPME – liquid-phase microextraction

LSE – liquid-solid extraction

McAb – monoclonal antibodies

MEKC - micellar electrokinetic chromatography

MeOH – methanol

MLS – method of least squares

MS – mass spectrometry

MSC – mathematic/model selection criterion

MS/MS – tandem mass spectrometry

MSPE – magnetic solid-phase extraction

n – number of replicates

N– number of calibration standards

n.d. – not determined

NPD – nitrogen phosphorus detector

OES – optical emission spectrometry

OPP – organophosphorus pesticide

OSP – organosulfur pesticide

PA - polyacrilate

PAH – polycyclic aromatic hydrocarbon

PBDE – polybrominated diphenyl ether

PCB – polychlorinated biphenyl

PDMS - polydimethylsiloxane

PE – phthalate ester

PLE – pressurized liquid extraction

PVC – polyvinyl chloride

QqQ – triple quadrupole

QuEChERS – Quick Easy Cheap Effective Rugged Safe

Rec – recovery

rpm – rotations per minute

RSD – relative standard deviation

RSM – response surface methodology

S – slope

SBSE – stir bar sorptive extraction

SD– standard deviation

SDS – sodium dodecyl sulphate

SIS – selected ion-storage

SPE – solid-phase extraction

SPME – solid-phase microextraction

ST – solvent terminated

TBA – tetrabutylammonium

TCC – carbon tetrachloride

TCE – tetrachloroethylene

TOF – time of flight

TSD – thermionic specific detector

XRD – X-ray diffraction

UASEME – ultrasound-assisted surfactant-enhanced emulsification microextraction

USA – United States of America

USE – ultrasound extraction

UHPLC – ultra-high-performance liquid chromatography

UV – ultraviolet

wt. - weight

Notation

A – Temkin isotherm constant ($L.mg^{-1}$)

b – Temkin constant related to heat of adsorption ($J.mol^{-1}$)

b_0 – interception term

b_i – influence of the variable i in the response

b_{ii} – parameter that determines the shape of the curve

b_{ij} – effect of the interaction among variables i and j

C – concentration ($\mu g.L^{-1}$ or $mg.L^{-1}$)

C_0 – initial concentration ($\mu g.L^{-1}$ or $mg.L^{-1}$)

C_e – equilibrium concentration ($\mu g.L^{-1}$ or $mg.L^{-1}$)

$\overline{d_p}$ – mean particle size (µm)

k_1 – apparent kinetic constant of pseudo-first order adsorption ($L.min^{-1}$)

k_2 – apparent kinetic constant of pseudo-second order adsorption ($g.mg^{-1}.min^{-1}$)

K_d – distribution (or partition) coefficient ($\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ or $mg.g^{-1}.(\mu g.mL^{-1})^{-1}$)

K_F – Freundlich adsorption (or distribution) coefficient ($mg.g^{-1}.(mg.L^{-1})^{-1/n}$)

$K_{F,1}$ – adsorption coefficient for component 1, estimated from mono component Freundlich isotherm model ($mg.g^{-1}.(mg.L^{-1})^{-1/n}$)

$K_{F,2}$ – adsorption coefficient for component 2, estimated from mono component Freundlich isotherm model ($mg.g^{-1}.(mg.L^{-1})^{-1/n}$)

k_{ip} – intraparticle constant ($mg.g^{-1}.h^{-1/2}$)

K_L – Langmuir equilibrium adsorption (or equilibrium constant) ($L.mg^{-1}$)

$K_{L,i}$ – equilibrium constant for component i, estimated by the individual Langmuir isotherm equation) ($L.mg^{-1}$)

K_{ow} – octanol-water partition coefficient

m – mass; number of experimental points (in MSC equation) (mg or g)

m/z – mass to charge ratio

n – measure of the adsorption density (Freundlich model)

N – number of components (in the extended Langmuir model)

n_1 – measure of adsorption intensity for component 1, estimated from mono component Freundlich isotherm model

n_2 – measure of adsorption intensity for component 2, estimated from mono component Freundlich isotherm model

p – number of fitting parameters (in MSC equation)

pH_{pzc} – pH at the point zero charge

q_e – adsorption capacity at equilibrium ($mg.g^{-1}$)

$q_{e\ bin}$ – adsorption capacity at equilibrium for the binary system ($mg.g^{-1}$)

$q_{e,i}$ – equilibrium adsorption capacity for component i in a multi component mixture ($mg.g^{-1}$)

$q_{e\ mono}$ – adsorption capacity at equilibrium for the mono component system ($mg.g^{-1}$)

$q_{experimental}$ – experimental adsorption capacity ($mg.g^{-1}$)

q_{max} – maximum adsorption capacity ($mg.g^{-1}$)

$q_{max,i}$ – maximum adsorption capacity for component i ($mg.g^{-1}$)

$q_{predicted}$ – predicted adsorption capacity ($mg.g^{-1}$)

q_t – adsorption capacity at time t ($mg.g^{-1}$)

$\overline{q_t}$ – mean of experimental concentration in the solid ($mg.g^{-1}$)

$q_{t_{calc}}$ – concentration in the solid, calculated by the model ($mg.g^{-1}$)

R – ideal gas constant ($J.mol^{-1}.K^{-1}$)

R_L – separation factor (or equilibrium parameter)

R^2– coefficient of determination

S_{BET} – BET (Brunauer, Emmet and Teller) surface area ($m^2.g^{-1}$)

S/N – signal to noise ratio

S_w – water solubility ($mg.L^{-1}$)

t – time (min or h)

T – temperature (°C or K)

U – global uncertainty

V – volume (µL, mL or L)

v/v – volume of the substance in the total volume of the solution

w/w – weight of the substance in the total weight of the solution

X_0 – value of variable i in the center of the domain (x_i=0)

x_1 – multi component Freundlich adsorption constantfor the component 1

x_2 – multi component Freundlich adsorption constantfor the component 2

x_i – dimensionless codified variable

X_i – natural variable

Y – process response

y_1 – multi component Freundlich adsorption constant for the component 1

y_2 – multi component Freundlich adsorption constant for the component 2

z_1 – multi component Freundlich adsorption constant for the component 1

z_2 – multi component Freundlich adsorption constant for the component 2

ΔX – difference of the variable between x_i=+1 and x_i=0

Chapter 1

1 General Introduction

1.1 Overview

Water is one of the most important substances on earth. Given that about 71% of the surface of the earth is covered with water and the human body consists of 75% of it, it is clear that water is one of the most essential elements for the existence of life and a fundamental need. At least 20 to 50 liters of clean, safe water a day are needed by a single person, in order to meet its basic needs (drinking, cooking, and hygiene) [1]. The crucial importance of water to so many aspects of human health, development and well-being, turns the supply of safe water into a subject of extreme relevance.

The susceptibility of water supply systems to contaminations, deliberate or accidental, is a known fact. Water distribution systems are spatially diverse, susceptible to intrusion, and contain many components, thus, they are inherently vulnerable to physical, chemical or biological threats. Usually, physical disruption of the water supply can result in economic costs and is an inconvenience, yet, a direct threat to human health is limited. Conversely, a chemical and/or biological contamination, is generally viewed as one of the most serious potential threats to water consumers. A review of two examples of waterborne disease outbreaks, resulting from accidental contamination of municipal drinking water systems, illustrates the potential consequences of an intentional act of water terrorism [2-4]. An example of the consequences arising from a waterborne disease is the outbreak that occurred in 1993, in Milwaukee, Wisconsin – United States of America. The cryptosporidiosis outbreak caused significant medical, public health and economic consequences in the community. This waterborne outbreak resulted, directly or indirectly, in the death of 54 residents [5]. In 2000, in Walkerton, Ontario – United States of America, the municipal water supply was contaminated with *Escherichia coli*, resulting in 7 deaths associated with the waterborne disease outbreak [6]. An extremely complete chronology on water conflicts was presented by Gleick and Ajami [7], in their biannual report. In the context of terrorism, numerous occurrences were reported, frequently regarding the disruption of the water supplies, but also some threats/attempts of water contamination by chemical

or biological agents [8]. There is, therefore, an increasing concern regarding these type of menaces to the general population. A quantitative review of the Web of Science database search showed that the term water security has increased, as pointed previously by Cook and Bakker [9]. A more recent search showed an interesting trend, as can be seen in Figure 1.1.

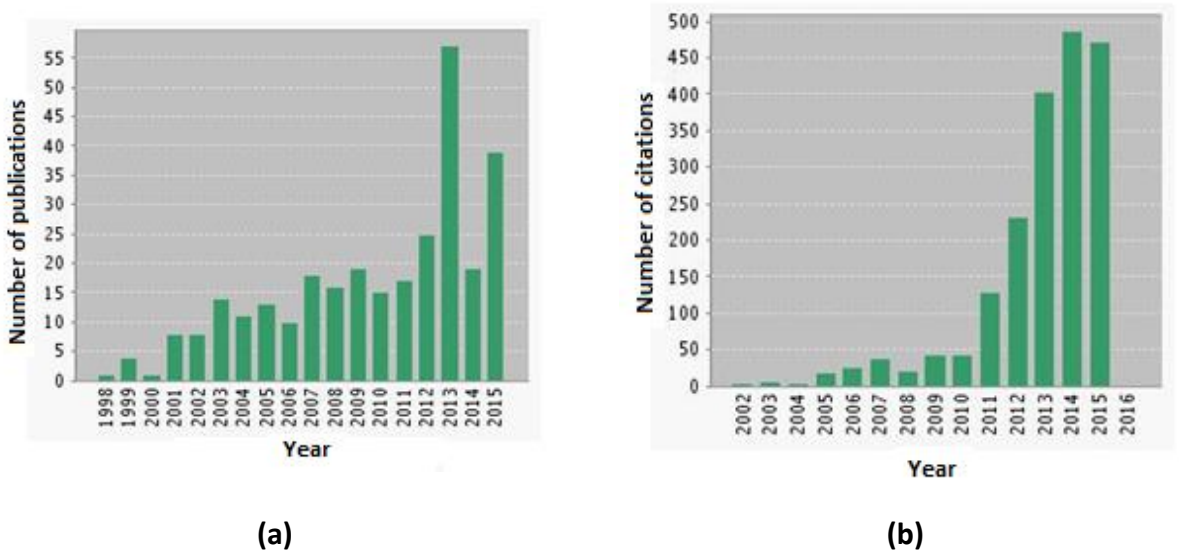


Figure 1.1 – (a) Number of publications including the term "water security" in the title; (b) number of citations including the term "water security". Source: Web of Science; accessed on November 4th, 2015.

It is clear that, there is an emerging preoccupation regarding the theme of the water security, as can be confirmed by the high number of publications and citations, regarding that subject.

Considering the vulnerabilities of the water distribution systems and the outcomes of a contamination, it is of paramount importance the awareness of the need of prevention and response to deliberate, or accidental, water contamination. It is impossible to prevent all contamination, whether accidental or the result of the deliberate introduction of a chemical, biological or radioactive agent. Nevertheless, an early detection of the contaminant would prevent its dissemination and significantly reduce human exposure. Considering this problematic, SecurEau, a European Union funded project was proposed [10]. Entitled "Security and decontamination of drinking

water distribution systems following a deliberate contamination", SecurEau project aimed at developing an early warning system to minimize the public impact of a terrorist attack, intended to affect public water infrastructures. Furthermore, the project intended to develop tools to quickly locate the source of contamination and its spreading, and methods to clean the water distribution system. The choice of the contaminants to be studied was made considering that commercial chemicals that are commonly produced, distributed and used throughout the world, are more likely to be used to contaminate water supplies. Thus, four organic (three pesticides and a flame retardant) and one inorganic agent (mercury) were chosen as model compounds, for this project. SecurEau had the duration of four years (from 01-02-2009 to 31-01-2013) and some of the work presented in this thesis was part of it.

In the case of a contamination event of water distribution systems, the foremost action to take is to confirm and quantify the foreign compound. Thus, in Chapter 2, different analytical methods are presented, which allow the detection and quantification of the two selected pesticides in waters, at different concentration levels. If a contamination occurs in a water distribution system it will have an impact, obviously, in the water, but it might also contaminate to some point the pipes and deposits and/or biofilms there attached. This fact is pertinent, because depending on the pesticide, there might exist affinity of the contaminant for the solid matrix, where accumulation might occur. Chapter 3 is related to the development of an extraction methodology of the pesticides, from real deposits of water distribution systems, for subsequent analysis. The interaction of the pesticides is further explored in Chapter 4, where an adsorption study is presented, which may help to understand the risk, or not, of contamination of the existing deposits in pipes and reservoirs. The main conclusions and the suggestions of future work are presented in Chapter 5.

Among the contaminants included in SecurEau project, carbofuran (a carbamate) and chlorfenvinphos (an organophosphorus pesticide) were selected to be the focus of the work developed in the scope of this thesis.

1.1.1 Target compounds

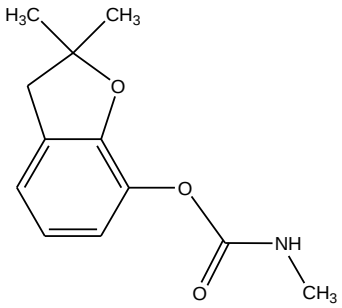
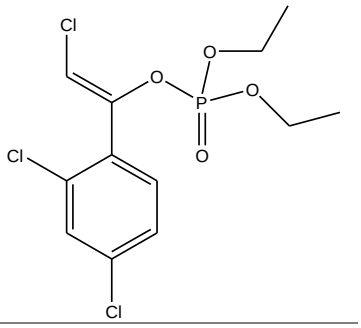
1.1.1.1 Carbofuran

Carbofuran was synthesized in the 1960s and patented in 1965 [11]. Carbofuran (trade name Furadan), a broad spectrum carbamate pesticide, is commonly used in agriculture and household as an insecticide, nematicide, and acaricide on contact or after ingestion. It is used against soil and foliar pests of field, fruits, vegetables and forest crops. Carbofuran exists either in the form of granules that are inserted into the soil, or in liquid form which is applied to the plants by watering or spraying. Due to its environmental toxicity, carbofuran use has been banned in many countries including the European Union [12], the United States of America [13] and Canada [14]. Carbofuran is highly toxic to humans and wildlife through the oral and inhalation routes of exposure, being a risk to those in immediate contact with it. As with other carbamate and organophosphorus pesticides, carbofuran has acetylcholinesterase-inhibiting effect, which is short term and reversible [15]. Complete recovery from an acute poisoning by carbofuran, with no long term health effect, is possible if exposure ceases and the victim has time to regain his normal level of cholinesterase and to recover from the symptoms [16]. To wildlife, however, the risk of death by poisoning is greater. Specially in the case of birds, there has been a number of poisoning and high mortality cases that have been reported in the United States of America, United Kingdom and Canada [17-20], but also in Kenya [21], Korea [22], Belgium, France, Greece and Spain [23, 24]. Furthermore, some cases were also described, regarding the poisoning of farm livestock, such as sheep and goats [25], and companion animals [24, 26, 27]. Even though some of the animal poisoning must be malicious [28], other cases exist that the poisoning of the animals is accidental [29]. It is, thus, imperative the rational use of such compounds, in order to protect humans, animals and the environment from the associated risks.

Some of the main physical-chemical properties of carbofuran are presented in Table 1.1. Carbofuran has a relatively short half-life in the environment (30 – 120 days in soil [30]). However, its relatively high water solubility along with relatively low adsorption onto soils and sediments, allows for its introduction into water matrices [31, 32],

through runoff and leaching [33, 34]. It is stable under neutral or acidic conditions but unstable in alkaline media, as can be confirmed by its value of the logarithm of acid dissociation constant, pk_a. Carbofuran is not expected to bioaccumulate to any great extent, due to its high water solubility and low logarithm of octanol-water partition coefficient, log K_{ow} [35]. Additionally, as most carbamate pesticides, carbofuran is a thermolabile compound [36]. Although it has been banned on some countries, carbofuran is still used and easily obtainable in many other countries. Thus, due to its properties, toxicity and availability it might be the chemical of choice in a deliberate contamination. Regarding legislation, the European Union has set regulations for the presence of pesticides in drinking water. The Drinking Water Directive determines the limit of 0.1 $\mu g.L^{-1}$ being the maximum permitted concentration for each individual pesticide, and 0.5 $\mu g.L^{-1}$ for the total pesticide concentration [37].

Table 1.1 – Physical and chemical properties of carbofuran and chlorfenvinphos [38].

Pesticides	**Carbofuran**	**Chlorfenvinphos**
Chemical structure		
CAS number	1563-66-2	470-90-6
Molecular form	$C_{12}H_{15}NO_3$	$C_{12}H_{14}Cl_3O_4P$
Molecular weight ($g.mol^{-1}$)	221.26	359.58
Solubility in water at 25 °C ($mg.L^{-1}$)	320	124
pK_a [39]	12.28	---
log K_{ow}	2.32	3.81
Melting point (°C)	92.80	85.90
Boiling point (°C)	311.41	397.78
Vapor pressure (mmHg)	5.54×10^{-5}	9.41×10^{-6}
Henry's constant ($atm.m^3.mol^{-1}$)	1.63×10^{-9}	5.17×10^{-8}

1.1.1.2 Chlorfenvinphos

Chlorfenvinphos is a broad spectrum organophosphorus contact type insecticide, synthesized in the early 1960s [40]. It is a non-flammable, yellow or amber liquid with a mild odor, which is used directly for treating soil as well as foliar spray. Chlorfenvinphos is commonly used to control household pest such as flies, fleas and mice. Like all organophosphorus pesticides, chlorfenvinphos acts on the nervous system of the parasites as inhibitor of acetylcholinesterase [41], by a mechanism virtually identical to that of carbamate pesticides [42]. Acetylcholine is a molecule involved in the transmission of nervous signals from nerves to muscles and between neurons and the brain. The enzyme acetylcholinesterase is responsible for the rapid hydrolytic degradation of acetylcholine into inactive products choline and acetic acid [42]. When acetylcholinesterase activity is inhibited, the termination of the nervous signals is prevented, keeping neurons in constant activity, greatly disturbing the normal movements of parasites and eventually leading them to death. The great difference between carbamates (e.g. carbofuran) and organophosphorus (e.g. chlorfenvinphos) induced inhibitory action is that carbamates bind reversibly to acetylcholinesterase [43]. Thus, due to its toxicity to mammals, birds, fish and possible cumulative effect on humans [44], chlorfenvinphos has been banned in several countries, including the European Union [45] and the United States of America.

Table 1.1 summarizes some of the main physical-chemical properties of chlorfenvinphos. Its solubility in water may allow for its leaching from hazardous waste sites through runoff after rainfall. Adsorption to particulate matter may also occur, transporting chlorfenvinphos from water to suspended solids and sediments [46]. As an organophosphorus insecticide, chlorfenvinphos is chemically reactive and not very stable either chemically or biochemically [47]. It is hydrolyzed slowly in neutral, acidic and slightly alkaline medium but quickly hydrolyzed in strong alkaline aqueous solution [41]. Chlorfenvinphos is still used, even in countries where restrictions have been established [48]. Therefore, regarding its characteristics, availability and low cost, its use should be limited and its occurrence should be controlled.

Furthermore, chlorfenvinphos is included in the list of priority substances, established by the European Union according to the Water Framework Directive [49-51]. The substances comprised in that list are considered to pose a significant risk to or via the aquatic environment. Directive 2013/39/EU [51] establishes Environmental Quality Standards for each priority substance in surface waters and, for chlorfenvinphos, an annual average value of 0.1 $\mu g.L^{-1}$ and a maximum allowable concentration of 0.3 $\mu g.L^{-1}$ should be met. Additionally, as for any pesticide, the Drinking Water Directive determines the limit of 0.1 $\mu g.L^{-1}$ being the maximum permitted concentration for each individual pesticide, and 0.5 $\mu g.L^{-1}$ for the total pesticide concentration [37].

1.1.2 Water distribution systems

Water distribution systems are complex infra-structures, whose basic function is to transport the water from the treatment facility to the costumer. The integrity of these systems is essential to ensure the safety of drinking water. Due to its inherent complexity and because they are large-scale and spatially extensive, water distribution systems are vulnerable to contamination, either accidental or deliberate. Drinking water supply systems, in particular, may be considered a potential target, for those aiming to affect a large number of people.

A distribution system is an intricate infra-structure that consists essentially of pipes, and other components such as pumps, valves, storage tanks, reservoirs, and other hydraulic apparatuses. Currently, pipes used in drinking water distribution systems can be divided into three main categories: metallic pipes, which includes steel, galvanized iron and cast iron pipes; cement pipes, comprising concrete cement and asbestos cement pipes; and plastic pipes, including plasticized polyvinyl chloride (PVC) and low-density polyethylene pipes [52].

The traditional pipe materials for the water supply industry have been quite varied. So, as an example, some information was collected in order to better understand what kind of materials are, at the moment, characteristic of the pipes of Oporto area. *Águas do Douro e Paiva* is the company that holds the concession of the water distribution system of the south metropolitan area. The water distribution is divided in three

subsystems: *Lever Norte, Lever Sul* and *Vale do Sousa e Baixo Tâmega*. Some characteristics of the water supply system are depicted in Table 1.2.

Table 1.2 – Constitution of the water supply network of the South Oporto metropolitan area, Portugal in 2013 (information suppied by *Águas do Douro e Paiva*; from [53]).

Subsystem	Municipalities	Pipe material	Number of pipelines	Pipeline length (m)
Lever Norte	Porto, Matosinhos, Gondomar, Valongo, Maia, Paredes oeste	Steel	1	5383
		Concrete	3	12106
		Cast iron	17	113557
Lever Sul	Vila Nova de Gaia, Santa Maria da Feira, Espinho, Ovar, São João da Madeira, Oliveira de Azeméis, Arouca, Vale de Cambra norte	Steel	3	18100
		Cast iron	30	148757
Vale do Sousa e Baixo Tâmega	Castelo de Paiva, Cinfães, Paredes este, Lousada, Felgueiras, Paços de Ferreira, Amarante oeste, Baião	Cast iron	40	191399

As can be seen, cast iron is the most commonly used pipeline material, and concrete pipelines are the least used. Actually, the pipelines made of this material are at the end of its life cycle, and are gradually being substituted. The pipelines made of steel and cast iron are more recent and were built in the last 15 years. Nevertheless, it should be pointed out that the water systems that distribute the drinking water from the local public facilities to the end point or user might be different.

Water distribution systems can be regarded as biological and chemical reactors that interact with the water being transported, and one of the outcomes of this interaction is the formation of unwanted deposits [54]. Additionally, in most drinking water distribution networks, the interface between water and pipe wall is a prime site for the accumulation of cells and organic matter, and for bacterial multiplication, originating biofilms [55]. The presence of deposits and/or biofilms on the distribution networks can influence water quality, by the release of corrosion products from iron alloys surfaces, by interacting with the biofilms, or in a chemical contamination event, by interacting with the contaminant. The development of unwanted deposits is unavoidable under standard conditions [56], therefore, the knowledge about the type of deposit and its composition is very important.

Through the analysis of unwanted deposits from a water distribution system of a tropical city, Echeverría, et al. [56] were able to identify the presence of three predominant type of deposits: brown, tubercle and white deposits. The studied pipeline network is primarily made of reinforced concrete (about 54%), ductile iron (about 27%) and steel (10%). The authors verified that brown deposits were formed everywhere in the system; tubercle deposits formed on steel and ductile iron pipes; and white deposits were found only in some places. Also, the authors concluded that brown deposits are mainly composed of aluminosilicates compounds, quartz and organic compounds (probably humic acids); and tubercles are composed of magnetite, goethite and lepidocrocite as main constituents. White deposits, which were most probably formed as a result of sedimentation of suspended particles or by local changes of physico-chemical conditions, are mainly composed of various mixtures of calcite, quartz and aluminosilicates [56]. Even though different conditions within the water distribution systems may lead to the formation of deposits with extremely diverse compositions, several authors have reported the finding of deposits with such characteristics that allow for its designation as brown or tubercle deposits [57-61]. Thus, it seems that the categorization of deposits, proposed by Echeverría, et al. [56] is suitable to describe drinking water distribution systems deposits.

Considering a contamination event in a water supply system, it is important to consider, not only the presence of the contaminant in the water, but also the possibility of interaction of the contaminant with the deposits and/or biofilms formed at the inner surface of the pipes, as well as suspended particles. As such, there is the possibility of contaminants being accumulated in the deposits (or particles in suspension) and biofilms, and later being released upon detachment of plaques of the deposits to the water or by slow migration of the contaminant to the aqueous phase. As mentioned, the existence of deposits in the conducts can strongly affect water quality [57] and, in the case of a contamination, their presence and influence in the whole occurrence should be considered.

Studies about the problematic of the interaction of chemical contaminants with suspended particles and attached inorganic deposits were already conducted at LEPABE/FEUP [62, 63]. A thorough study on the interaction of paraquat with real

deposits from drinking water distribution systems was presented by Santos, et al. [63]. The results obtained by the authors indicate that the extent of adsorption of the contaminant is dependent, among other factors, on the deposit characteristics. Also, as part of her doctorate thesis, Martins [62] studied the interaction of three pesticides (paraquat, carbofuran and chlorfenvinphos) in both suspended particles and deposits attached to flow cells. This last arrangement intended to simulate the formation of deposits and their interaction with a flow of contaminant. The author performed several adsorption experiments which allowed to conclude that there is, in fact, interaction between the pesticides with either the suspended particles and the deposits. Therefore, in the case of a contamination event, there is the possibility of contamination of, not only the water, but also of the deposit, due to possible interactions with the contaminant. In another study, Santos, et al. [64] addressed this possibility, and presented an analytical method which detects and quantifies paraquat in real deposits.

The eventuality of the contamination of deposits and their interaction with the two target compounds (carbofuran and chlorfenvinphos) was also addressed in this work. However, no studies were performed regarding the interaction between the two pesticides and biofilms. The results on the problematic of the deposits are presented in Chapters 3 and 4. The deposits used in the experiments were kindly supplied by Dr. Gabriela Schaule, from the IWW Water Centre in Germany, which was one of the members of the SecurEau project.

The main properties that characterize the adsorbents are presented in Table 1.3.

Table 1.3 - Physical-chemical properties of the real deposits and clay (from [[63, 65]).

	S3	**S4**	**Clay**
Deposit classification	Tubercle	White	n.d.
ICP-OES analysis (wt.% of the main elements at dry basis)	Fe: 97 P:1 Mn:1	Ca: 97 Fe: 1 Mg: 1	Al_2O_3: 34 SiO_2: 49
S_{BET} ($m^2.g^{-1}$)	36	1	n.d.
pH_{pzc}, 20 °C	6.1	9.9	
pH in water, 20 °C	7.2	9.0	
Main components identified by XRD	Goethite	Calcite ($CaCO_3$)	n.d.
Organic matter content (wt.%)	1.0	0.2	12

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Chapter 2

2 Carbofuran and chlorfenvinphos quantification in waters

2.1 Introduction

The intensive use of pesticides in agriculture may lead to contamination of surface and ground waters by runoff, drainage, drift and leaching. This contamination is dependent of several factors such as physical-chemical properties of the pesticides, soil characteristics, agricultural and environmental aspects. Thus, some of these pesticides might be bioaccumulative and, owing to their vertebrate and non-vertebrate toxicity, can affect non-target organisms [1]. Pesticide contamination has been well documented worldwide and a number of studies have reported the presence of pesticides in surface and ground waters of several countries, such as in Germany [2], Spain [3-5], Portugal [6, 7], France [8, 9], Greece [10], Pakistan [11] , Canada [12], United States of America [13] Costa Rica [14], Thailand [15] or Japan [16]. Konstantinou, et al. [10] studied the status of pesticide pollution in rivers and lakes in Greece and observed that carbofuran was one of the most detected compounds, with values up to 7300 $ng.L^{-1}$. Similarly, Chowdhury, et al. [17] studied the presence of organophosphorus and carbamate pesticides in surface waters in Bangladesh and a number of samples were found to contain, among others, carbofuran at concentrations ranging from 0 – 3.395 $\mu g.L^{-1}$. In a study about the pesticides in Portuguese surface and ground waters, Cerejeira, et al. [7] verified the presence of chlorfenvinphos, among other pesticides, in values up to 31.6 $\mu g.L^{-1}$. Terzopoulou, et al. [18] analyzed the samples of water from a Greek river and found thirty-nine compounds; chlorfenvinphos was one of the most detected compounds with concentrations up to 0.32 $\mu g.L^{-1}$.

Pesticide contamination of waters, due to its excessive use, is an issue that rises concern at local, regional, national and global scale [10]. This subject gets a whole new dimension if we consider a deliberate contamination of water with pesticides. It is, thus, of major importance to have ways of identifying, detecting and controlling the levels of pesticide contamination in water matrices.

2.1.1 Legislation

There has been an increasing concern about the contamination of water, due to the increasing reports of detection of pesticide in ground and surface waters, but also owing to the strict directives that are in place, in order to protect drinking water infra-structures.

As stated before on the characterization of each pesticide in study (topics 1.1.1.1 and 1.1.1.2), the European Union allows a maximum concentration of 0.1 $\mu g.L^{-1}$ of each individual pesticide and 0.5 $\mu g.L^{-1}$ of the sum of pesticides in drinking water [19]. Additionally, and because chlorfenvinphos is included in the list of priority substances, the Directive 2013/39/EU [20] establishes Environmental Quality Standards for each priority substance in surface waters and, in the case of chlorfenvinphos, an annual average value of 0.1 $\mu g.L^{-1}$ and a maximum allowable concentration of 0.3 $\mu g.L^{-1}$ should be met. Carbofuran is not covered by any additional legislation, regarding its detection in water matrices.

2.1.2 Analytical methods for detection and quantification of carbofuran and chlorfenvinphos in waters

There are numerous pesticides, with various end applications, and thus, with different physical-chemical properties. The specific characteristics of each pesticide have to be considered when aiming to detect and quantify a contaminant in aqueous matrices. Analytical methods for control of water quality are required to be highly specific, sensitive and reliable for the determination of very low amounts of contaminants. Environmental samples are complex, thus, there is the need to separate and concentrate the target compounds from the matrix components in order to achieve low limits of detection.

There are several analytical methodologies for the determination of pesticides, and specifically of carbofuran and chlorfenvinphos, in water matrices. As with pesticides in general, chromatography is still the most used instrumental technique for the detection

and quantification of carbofuran and chlorfenvinphos in water matrices, as can be seen in Figure 2.1.

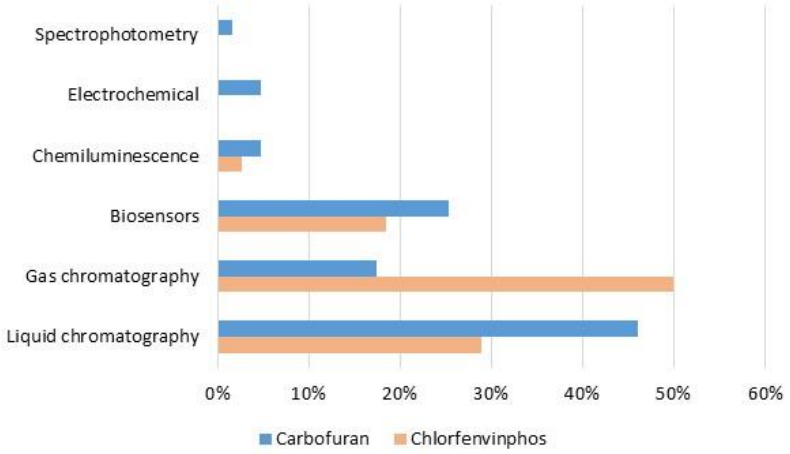


Figure 2.1 - Relative contribution of the instrumental methods of analysis found in literature for carbofuran (total of 63 studies) and chlorfenvinphos (total of 38 studies) in water (Search in Scopus database from 2000 to 2016).

As can be observed, most of the reports found, use chromatography, either gas or liquid, to analyze the target pesticides. Liquid chromatography is the most reported method for the analysis of carbofuran. This might be explained by the fact that some carbamates are thermally unstable, and tend to break down into their corresponding phenols and amines when analyzed by gas chromatography [21]. Nevertheless, under the appropriate conditions, the analysis of carbofuran by gas chromatography is possible, as proved by the several existing studies [22-25]. On the other hand, the preferred instrumental method of analysis of chlorfenvinphos in water matrices is gas chromatography, followed by liquid chromatography. The third most reported methodology for the analysis of both pesticides is through biosensors. Many pesticides are designed to inhibit various enzymes within insects or other pests. Considering this capability, these enzymes were incorporated into biosensors in order to detect pesticides in water or other matrices.

The most widely used methodologies for detection and quantification of carbofuran and chlorfenvinphos, chromatography and biosensors, are further explored below.

2.1.2.1 Liquid and gas chromatography methods

Chromatography is still the most common method of analysis of pesticides in environmental samples, and particularly in water matrices. With the aim of removing interferences and/or to pre-concentrate the sample, most of the reported studies use a preliminary extraction step, followed by the analysis itself. These extraction techniques are often used in conjunction with chromatographic analytical methods. Figure 2.2 represents the relative contribution of each extraction method for the number of publications found, regarding the analysis of carbofuran and chlorfenvinphos in water matrices by chromatography.

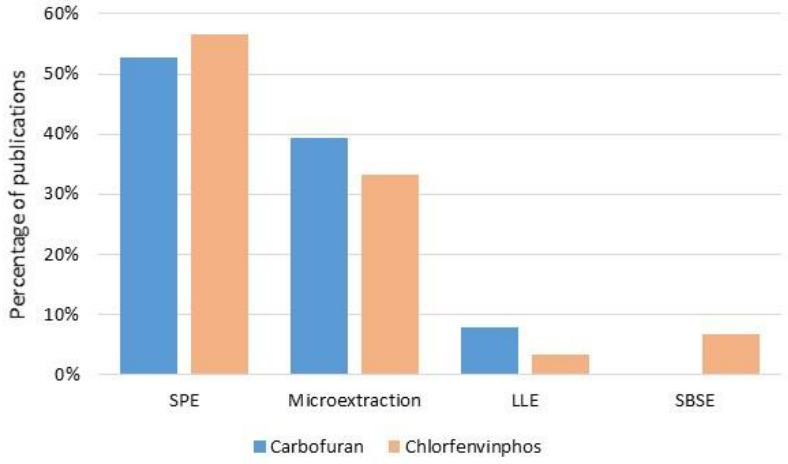


Figure 2.2 – Relative contribution of the extraction methods found in literature for the analysis of carbofuran (total of 38 studies) and chlorfenvinphos (total of 30 studies) in water, by chromatography (Search in Scopus database from 2000 to 2016).

It can be observed that, for both pesticides, the most used extraction method is the solid-phase extraction (SPE), followed by the microextraction techniques. From these, solid-phase microextraction (SPME) and dispersive liquid-liquid microextraction (DLLME) contribute with the higher number of publications. On the other hand, only two studies were found, reporting stir bar sorptive extraction (SBSE) as the preconcentration method in waters, for the pesticides considered. Liquid-liquid extraction (LLE), once one of the most used techniques, is now less utilized. Traditional extraction methodologies based on LLE have the disadvantages of being time consuming, using large amounts of

toxic organic solvents and not being easily automated [26]. Recently, there has been an increasing demand for simple, rapid and accurate separation methods that require lower amounts of organic solvents. These research efforts originated a number of new preparation methods, which are now extensively used.

The extraction methodology, as well as the instrumental method of analysis can have a relevant influence on the validation parameters of the analytical method, particularly in the limits of detection and on the accuracy and precision parameters. Table 2.1 and Table 2.2 present a summary of the main conditions of extraction techniques and instrumental methods, found in the literature, for the detection and quantification of carbofuran and chlorfenvinphos in water, respectively.

Comparing the limits of detection achieved by each method for carbofuran analysis, it can be observed that for SPE the LODs ranged from 0.0002 – 5.11 $\mu g.L^{-1}$, for LLE from 1.7 – 10 $\mu g.L^{-1}$, for SPME from 0.03 – 8.9 $\mu g.L^{-1}$, and for DLLME from 0.008 – 0.9 $\mu g.L^{-1}$.

The limits of detection of the methods for chlorfenvinphos ranged from 0.0001 – 0.032 $\mu g.L^{-1}$ for SPE, 0.002 – 0.024 $\mu g.L^{-1}$ for SPME, 0.0003 $\mu g.L^{-1}$ for LLE and 0.0019 $\mu g.L^{-1}$ for SBSE.

The pre-concentration by SPE yielded the lower limits of detection, for both pesticides. With SPE, pesticides present in the matrix can be isolated, concentrated and purified. Solid-phase extraction usually is a reliable method, however, when non-reproducible recoveries occur, they can be due to the interaction between the compound and the matrix, which leads to the unavailability of the analyte. This might be attributed to the adsorption of the compound onto solid suspended matter, thence, the importance of removing matrix interferents [27]. In solid-phase extraction, the water sample is passed through a short bed of packing material, which can contain functional groups of different polarity. The choice of the SPE sorbent is largely dependent on the physical-chemical characteristics of the pesticides. The most used SPE cartridges for the extraction of chlorfenvinphos and carbofuran are octadecyl-(C_{18}) bonded silica and hydrophilic-lipophilic balanced polymeric sorbents (Oasis HLB®).

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques.

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Ground and surface water	Thirty-five pesticides	On-line SPE-LC-ESI-MS/MS	Cartridge – C_{18} silica; Conditioning – 4 mL acetonitrile, 4 mL water; Load – 1330 µL; Washing – 4 mL water; Elution – 0.01% formic acid in acetonitrile-0.001% HCOOH in water gradient.	LOD – 4 $ng.L^{-1}$ Repeatability (RSD): 25 $ng.L^{-1}$ – 7%; 100 $ng.L^{-1}$ – 4%	[28] (2001)
Ground and surface water	Thirteen carbamate pesticides	On-line SPE-LC-DAD-UV	Cartridge – 5 µm Hypersil C_{18} silica; Conditioning – 10 mL methanol, 10 mL water; Elution – mobile phase (methanol-water gradient).	LOD – 5 $\mu g.L^{-1}$ % Recovery: HPLC water – 2.7% Tap water – 5.7%	[29] (2001)
Ultrapure water	Carbofuran	SPE-LC-DAD	Cartridge – Sep-Pak C_{18} silica; Conditioning – 5 mL methanol, 10 mL water; Load – 1 L; Drying – N_2 stream; Elution – 3 mL methanol; Evaporation – N_2 stream; Dissolution – 1 mL water.	Linearity range: 0.1 – 50 $\mu g.L^{-1}$ LOD – 0.06 $\mu g.L^{-1}$ LOQ – 0.08 $\mu g.L^{-1}$ % Recovery: 90% Repeatability (RSD): 3.2% Reproducibility (RSD): 7.0%	[30] (2002)
Drinking water	Six carbamate pesticides	SPE-LC-ESI-MS	Cartridge – Zorbax C_{18} silica; Conditioning – 3 mL methanol-acetonitrile 50:50 (v/v), 3 mL methanol, 2 × 3 mL water; Load – 50 mL; Washing – 3 mL water; Elution – 3 × 1 mL methanol-acetonitrile 50:50 (v/v); Evaporation – N_2 stream; Dissolution – 200 µL water.	LC-ESI-MS (without extraction): Linearity range: 1 – 50 $\mu g.L^{-1}$ LOD – 0.10 $\mu g.L^{-1}$ Repeatability (RSD): 7.8% SPE-LC-ESI-MS: LODs : 0.5 – 3 $ng.L^{-1}$ % Recovery: 0.03 $\mu g.L^{-1}$ – 85.3% RSD: 13.8%	[31] (2003)
Tap and raw water	Ten carbamate pesticides	SPE-LC-ESI-MS	Cartridge – Oasis HLB; Conditioning – 5 mL acetonitrile, 5 mL water; Load – 500 mL; Drying – centrifugation 3000 rpm, 3 min; Elution – 5 mL acetonitrile; Evaporation – N_2 stream until 0.2 mL acetonitrile; Dissolution – addition of 0.8 mL water.	LC-ESI-MS (without extraction): Linearity range: 0.5 – 1000 $\mu g.L^{-1}$ LOD – 0.4 $\mu g.L^{-1}$ LOQ – 0.7 $\mu g.L^{-1}$ SPE-LC-ESI-MS: % Recovery: 100% Reproducibility (RSD): 1 – 13%	[32] (2003)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Drinking and surface water	Twelve pesticides	SPE-LC-ESI-MS	Cartridge – LC-18; Conditioning – 5 mL acetonitrile, 5 mL water; Load – 500 mL; Drying – centrifugation 3000 rpm, 3 min; Elution – 5 mL acetonitrile; Evaporation – N_2 stream; Dissolution – 0.2 mL water.	LC-ESI-MS (without extraction): Linearity range: 1 – 50 $\mu g.L^{-1}$ LOD – 0.1 $\mu g.L^{-1}$ SPE-LC-ESI-MS: LODs: 0.5 – 3 $ng.L^{-1}$ Ultra-pure water: % Recovery: 0.10 $\mu g.L^{-1}$ – 76.3% Reproducibility (RSD): 8.1% Spike level of 0.10 $\mu g.L^{-1}$: Ultra-pure water: % Recovery – 90.5% Drinking water: % Recovery – 83.3% Surface water: % Recovery – 78.9%	[33] (2004)
Drinking, ground and surface water	Thirty-seven pesticides and ten transformation products	On-line SPE-LC-MS/MS	Cartridge – polymeric phase Hamilton PRP-1; Conditioning – 2 mL acetonitrile, 2 mL water; Load – 1330 µL of acidified water sample; Washing – 4 mL water; Elution – 0.01% formic acid in acetonitrile-0.001% HCOOH in water gradient.	Linearity range: 0 – 1000 $ng.L^{-1}$ LOD: 0.4 $ng.L^{-1}$ Repeatability (RSD): 25 $ng.L^{-1}$ – 8%; 100 $ng.L^{-1}$ – 4% 500 $ng.L^{-1}$ – 3% Spike level of 25 $ng.L^{-1}$: Drinking water: % Recovery – 95.8% Ground water: % Recovery – 104.5% Surface water: % Recovery – 94.8% Spike level of 100 $ng.L^{-1}$: Drinking water: % Recovery – 100.4% Ground water: % Recovery – 95.2% Surface water: % Recovery – 103.4% Spike level of 500 $ng.L^{-1}$: Drinking water: % Recovery – 95.3% Ground water: % Recovery – 94.4% Surface water: % Recovery – 98.4%	[34] (2004)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Ground water	Two pesticides and three metabolites	SPE-MEKC-DAD	Cartridge – Home-made cartridges with 500 mg graphite carbon Carbopack™; Conditioning – 10 mL ethyl acetate, 15 mL-acetonitrile, 10 mL water; Load – 250 mL; Elution – 2 mL acetonitrile; Evaporation – N_2 stream; Dissolution – 140 mM sodium dodecyl sulfate and 20 mM buffer borate solution.	LOD – 5.11 $\mu g.L^{-1}$ LOQ – 17.06 $\mu g.L^{-1}$ Repeatability (RSD): 5.27% Spike level of 30 $ng.mL^{-1}$: % Recovery – 80.81%	[35] (2004)
Surface water	Five pesticides	SPE-LC-MS/MS	Water samples preparation – filtration and addition of a surrogate in methanol (0.5 mL of 0.5 $mg.L^{-1}$ acetochlor) Cartridge – Oasis HLB; Conditioning – 5 mL methanol, 2 × 4 mL water; Load – 500 mL; Elution – 2 × 2 mL methanol; Evaporation – N_2 stream Dissolution – 0.5 mL acetonitrile.	LC-MS/MS (without extraction): Linearity range: 50 – 1000 $\mu g.L^{-1}$ Minimum detectable quantity – 0.1 ng SPE-LC-MS/MS: LODs: 0.5 – 3 $ng.L^{-1}$ Ultra-pure water: % Recovery: Spike 0.5 $\mu g.L^{-1}$ – 104.2% Spike 0.1 $\mu g.L^{-1}$ – 74.2% Spike 0.05 $\mu g.L^{-1}$ – 73.2%	[36] (2006)
Tap and river water	Twelve pesticides	SPE-GC-MS	Cartridge – 0.1 g of multi-walled carbon nanotubes; Conditioning – 5 mL methanol, 3 mL water; Load – 500 mL; Washing – 10 mL water; Elution – 5 mL acetone-*n*-hexane 1:1 (v/v); Evaporation – N_2 stream at 40 °C; Dissolution – 1 mL acetone.	Linearity range: 0.04 – 4 $\mu g.L^{-1}$ LOD: 0.02 $\mu g.L^{-1}$ Spike level of 0.1 $\mu g.L^{-1}$: Milli-Q water: % Recovery – 97.0% Tap water: % Recovery – 94.3% Spike level of 0.5 $\mu g.L^{-1}$: Milli-Q water: % Recovery – 96.7% Tap water: % Recovery – 94.8% Spike level of 1.0 $\mu g.L^{-1}$: Milli-Q water: % Recovery – 92.5% Tap water: % Recovery – 98.1% River water: % Recovery – 90.7%	[37] (2007)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface water	Ten pesticides	SPE-GC-MS	Cartridge – CarboPrep-90, graphitized carbon based SPE cartridges; Conditioning – 5 mL dichloromethane-methanol 8:2 (v/v), 2 mL methanol, 10 mL water containing 10 $mg.mL^{-1}$ ascorbic acid; Load – 1000 mL; Washing – 7 mL water; air dry 10 min; 1 mL methanol- water 1:1 (v/v), air dry; Elution –1 mL methanol and 1 mL dichloromethane-methanol 8:2 (v/v); Evaporation of the combined eluates – N_2 stream until 0.1 mL; Dissolution – 2 mL isooctane and evaporation to a final volume of 1 mL.	LOD: Isothermic injector temperature – 5 $ng.mL^{-1}$ Temperature-programmed injector – 0.5 $ng.mL^{-1}$ % Recovery: 84.1 – 109.1% Repeatability (RSD): 8.2 – 19.9%	[38] (2007)
Drinking water	Twenty-eight pesticides	SPE-LC-MS/MS	Drinking water samples preparation – pro-treatment with sodium thiosulphate (final concentration 10 $mg.L^{-1}$); Cartridge – Oasis HLB; Conditioning – 3 mL methanol, 3 mL water; Load – 500 mL; Elution –6 mL methanol; Evaporation – N_2 stream; Dissolution – 0.5 mL mobile phase: 90% ammonium acetate 5 $mmol.L^{-1}$ in water containing 10% methanol.	Linearity range: 0.025 – 0.150 $\mu g.L^{-1}$ LOD: 0.0064 $\mu g.L^{-1}$ LOQ: 0.025 $\mu g.L^{-1}$ % Recovery: Drinking water preserved with 10 $mg.L^{-1}$ $Na_2S_2O_3$ – 72% Drinking water preserved with 0.5 $g.L^{-1}$ ascorbic acid – 63%	[39] (2008)
Surface water	Sixty-six pesticides and seven degradates	SPE-GC-MS	Cartridge – Oasis HLB; Conditioning – 10 mL ethyl acetate, 10 mL methanol, 5 mL water; Load – 1000 mL; Drying – CO_2 for 1 h; Elution – 12 mL ethyl acetate; Drying of the Cartridge – CO_2 stream; After the extraction – 1 g sodium sulphate is added to the sample bottle and the bottle was rinsed with 3 × 4 mL dichloromethane. The bottle rinse is reduced by N_2 stream to 1 mL, and is added to the ethyl acetate fraction. The sample is reduced to 200 µL under a N_2 stream.	River water: % Recovery – 93%; RSD – 10%; LOD – 3.1 $ng.L^{-1}$ Drainage canal % Recovery – 108%; RSD – 13%; LOD – 8.8 $ng.L^{-1}$	[40] (2008)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface water	Three *N*-methylcarbamates	SPE-LC-UV-CL	Cartridge – Oasis HLB; Conditioning – 5 mL ethyl acetate, 5 mL methanol, 10 mL water; Load – 1500 mL; Washing – 5 mL water; air dry 15 min; Elution – 4 mL acetone; Evaporation – N_2 stream; Dissolution – 100 µL acetonitrile + 400 µL water.	Linearity range: 38.6 – 1650 ng.L^{-1} LOD: 38.6 ng.L^{-1} LOQ: 128.8 ng.L^{-1} Repeatability (RSD) at 670 ng.L^{-1}: 5.1% Mineral water with spike levels of 100 – 500 ng.L^{-1}: % Recovery – 98.9 – 102.1%; RSD – 5.9 – 7.8% River water with spike levels of 100 – 500 ng.L^{-1}: % Recovery – 98.2 – 107.7%; RSD – 4.3 – 7.9% Ground water with spike levels of 100 – 500 ng.L^{-1}: % Recovery – 93.6 – 101.9%; RSD – 3.9 – 6.1%	[41] (2008)
Surface and ground water	Fourteen pesticides	SPE-LC-ESI-MS/MS	Cartridge – Oasis HLB; Conditioning – 5 mL methanol-dichloromethane 1:1 (v/v), 10 mL water; Load – 250 mL with pH 6; Drying – vacuum 10 min; Elution – 10 mL methanol-dichloromethane 1:1 (v/v); Evaporation – N_2 stream; Dissolution – 1 mL methanol.	Linearity range: 10 – 250 ng.L^{-1} Ground water: LOD: 0.5 ng.L^{-1} LOQ: 1.6 ng.L^{-1} % Recovery (% RSD) at 40 ng.L^{-1}: 102% (1%) % Recovery (% RSD) at 200 ng.L^{-1}: 88% (1%) Surface water: LOD: 1.1 ng.L^{-1} LOQ: 3.6 ng.L^{-1} % Recovery (% RSD) at 40 ng.L^{-1}: 99% (5%) % Recovery (% RSD) at 200 ng.L^{-1}: 84% (9%)	[42] (2010)
Tap, pound and river water	Thirty-three pesticides (including carbofuran and chlorfenvinphos)	SPE-LC-ESI-MS/MS	Cartridge – Chromabond HR-X; Conditioning – 5 mL methanol, 5 mL water; Load – 50 mL with pH 6; Washing – 5 mL water with 5% methanol; Drying – N_2 15 min; Elution – 3 mL methanol and 3 mL methanol-ethyl acetate 75:25 (v/v); Evaporation – N_2 stream; Dissolution – 1 mL mobile phase (90% water-10% acetonitrile).	LOQ: 20 ng.L^{-1} Mineral water: % Recovery (% RSD) at 0.04 µg.L^{-1}: 58% (21%) % Recovery (% RSD) at 0.2 µg.L^{-1}: 61% (20%) Natural water: % Recovery (% RSD) at 0.04 µg.L^{-1}: 83% (22%) % Recovery (% RSD) at 0.2 µg.L^{-1}: 82% (11%)	[43] (2011)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Reservoir, river and pool water	Five carbamate pesticides	MSPE-LC-DAD	- Mix 200 mL water sample with 15 mg G-Fe_3O_4; - Shake 15 min; - Separate G-Fe_3O_4 from the suspension with a magnet and decant; - Transfer the residual solution and G-Fe_3O_4 to a centrifuge tube; - Aggregate with a magnet and separate the solution with a pipette; Elution – mix 0.5 mL acetone with the particles and vortex 10 min. Separate with magnet. Repeat 3 times; Evaporation – evaporate the combined solutions with N_2 stream; Dissolution – 200.0 µL methanol.	Linearity range: 0.1 – 50 ng.mL^{-1} LOD: 0.02 ng.mL^{-1} Repeatability (RSD): 3.0% Reservoir water: % Recovery: 94.0 – 97.3% River water: % Recovery: 91.6 – 92.0% Pool water: % Recovery: 90.0 – 93.8%	[44] (2011)
River, ground water	Twelve pesticides (including carbofuran and chlorfenvinphos)	SPE-LC-DAD-UV	Cartridge – mixed sodium dodecyl sulphate (SDS)-tetrabutylammonium (TBA) admicelle-based SPE; Alumina cartridges were conditioned with 10 mL of 0.01 M hydrochloric acid. Hemicelles were formed on the alumina by passing 25 mL solution containing 40 mg of SDS at pH 2 and 250 mL of water, containing 30 µg of TBA; Elution – acid pesticides were eluted with 2 mL of 0.3 M NaOH:methanol 90:10 (v/v); neutral and basic pesticides were eluted with 1 mL THF.	Linearity range: 10-3000 µg.L^{-1} LOD – 60 ng.L^{-1} LOQ – 180 ng.L^{-1} River water: % Recovery - 96-107% Underground water % Recovery – 93-104%	[45] (2012)
Waste and surface water	Forty-three pesticides and metabolites	SPE-LC-QqQ-MS/MS	Cartridge – Oasis HLB; Conditioning – 5 mL dichloromethane-methanol 50:50 (v/v), 10 mL water; Load – 200 mL; Drying – vacuum 10 min; Elution – 10 mL dichloromethane-methanol 50:50 (v/v); Evaporation – N_2 stream at 40 °C; Dissolution – 1 mL methanol.	Linearity range: 15 – 10000 ng.L^{-1} LOD: 0.2 ng.L^{-1} LOQ: 0.6 ng.L^{-1} % RSD: 2 – 8 ng.L^{-1}	[46] (2013)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface water	Seventy micropollutants (pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenols, pharmaceuticals)(including carbofuran and chlorfenvinphos)	SPE-GC-MS/MS	Cartridge – Oasis HLB; Conditioning – 5 mL dichloromethane-ethyl acetate 50:50 (v/v), 5 mL hexane-dichloromethane 50:50 (v/v), 1 mL methanol, 1 mL water; Load – 1000 mL; Washing – 2 mL water; Drying – vacuum 30 min; Elution –5 mL hexane-dichloromethane 50:50 (v/v), 3 mL dichloromethane-ethylacetate 50:50 (v/v); Evaporation – N_2 stream at 35 °C; Dissolution – 500 µL methanol.	Linearity range: 0-4000 $ng.mL^{-1}$ LOD – 19.1 $ng.L^{-1}$ LOQ – 63.7 $ng.L^{-1}$ % Recovery: 89.2 – 99.4% %CV: 7.98 – 21.8%	[18] (2015)
			Microextraction		
Ultrapure water	Carbofuran	SPME-LC-DAD	Fiber – Polydimethylsiloxane-divinylbenzene (PDMS-DVB) Sample volume – 40 mL Salt addition – 9 g NaCl Adsorption time – 30 min; room temperature Desorption – 150 µL 40% methanol-60% 0.1 M ammonium acetate; 15 min; 70 °C	Linearity range: 10 – 50 $\mu g.L^{-1}$ LOD – 8.9 $\mu g.L^{-1}$ LOQ – 10.0 $\mu g.L^{-1}$ % Recovery: 100% Repeatability (RSD): 7.7% Reproducibility (RSD): 5.1%	[30] (2002)
River water	Twenty-three pesticides	SPME-GC-FTD SPME-GC-MS	Fiber – Polydimethylsiloxane (PDMS) Sample volume – 5 mL, methanol < 0.1%, pH 7 Salt addition – 15% (v/v) NaCl Adsorption time – 45 min; room temperature Desorption –10 min GC injector.	Linearity range: 0.05 – 10 $\mu g.L^{-1}$ GC-FTD: LOD – 0.030 $\mu g.L^{-1}$ % Recovery: 87% Reproducibility (RSD): 9% GC-MS: LOD – 0.050 $\mu g.L^{-1}$ Reproducibility (RSD): 11%	[47] (2002)
Drinking and river water	Seven organophosphorus and one carbamate pesticide	HF-LPME-GC-FTD	Fiber – Accurel Q 3/2 polypropylene hollow fiber membrane Sample volume – 5 mL Organic solvent – 3.0 µL toluene Extraction time – 20 min Volume of enriched solvent – 1.5 µL.	Linearity range: 0.300 – 100 $\mu g.L^{-1}$ LOD – 72 $ng.L^{-1}$ Repeatability (RSD): 10.2% Reproducibility (RSD): 12.0% % Recovery (%RSD): Drinking water: 89% (10.6%) River water: 88% (10.9%)	[48] (2005)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			Microextraction		
Surface water	Seven carbamate pesticides	SPME-GC-MS	Fiber – Polyacrylate (PA) Sample volume – 10 mL Salt addition – 60% (v/v) NaCl Adsorption time – 120 min; 25 °C Desorption –6.5 min, in the GC injector at 300 °C.	LOD – 2 $\mu g.L^{-1}$ Repeatability (RSD): 13%	[49] (2005)
River water	Eight carbamate pesticides	SDME-GC-MS	Sample volume – 3 mL Organic/extraction solvent – benzonitrile Organic solvent volume – 3.5 µL Extraction – 15 min at 250 rpm	Linearity range: 0.2 – 20 $\mu g.L^{-1}$ LOD: 80 $ng.L^{-1}$ LOQ: 280 $ng.L^{-1}$ Precision (RSD): 7.4%	[50] (2008)
River, rain, well and tap water	Four carbamate pesticides	DLLME-LC-DAD	Sample volume – 5 mL Dispersive solvent – acetone Dispersive solvent volume – 1 mL Extraction solvent – trichloromethane Extraction solvent volume – 70 µL Vortex – 5 s Separation – centrifugation 3500 rpm, 5 min Sedimented phase was dried under N_2 stream and reconstituted in 15.0 µL methanol	Linearity range: 5 – 500 $ng.mL^{-1}$ LOD: 1 $ng.mL^{-1}$ LOQ: 3.3 $ng.mL^{-1}$ Repeatability (RSD): 4.7% Recovery (RSD): River water – 82.0-86.0% (6.2-6.5%) Rain water – 88.0-90.5% (5.1-5.5%) Tap water – 86.0-88.5% (6.3-7.2%) Well water – 90.0-93.5% (4.3-5.8%)	[51] (2009)
Surface water	Five carbamate pesticides	DLLME-LC-DAD	Sample volume – 5 mL Dispersive solvent – acetonitrile Dispersive solvent volume – 1 mL Extraction solvent – trichloromethane Extraction solvent volume – 40 µL Shake a few seconds Separation – centrifugation 4000 rpm, 5 min Sedimented phase was dried under N_2 stream and reconstituted in 15.0 µL methanol	Linearity range: 5 – 1000 $ng.mL^{-1}$ LOD: 0.4 $ng.mL^{-1}$ Recovery (RSD): Surface water – 90.5-91.0% (4.2-7.7%)	[52] (2009)
Tap, river and rain water	Three carbamate pesticides	DLLME-LC-UV	Sample volume – 5 mL Dispersive solvent – acetonitrile Dispersive solvent volume – 1 mL Extraction solvent – chlorobenzene Extraction solvent volume – 35 µL Shake a few seconds Separation – centrifugation 4000 rpm, 5 min 20 µL of the sedimented phase was analyzed	Linearity range: 1 – 1000 $\mu g.L^{-1}$ LOD: 0.9 $\mu g.L^{-1}$ Repeatability (RSD): 3.7% Recovery (RSD): Tap water – 84.5-97.5% (4.4-5.8%) River water – 89.5-102.3% (3.1-5.8%) Rain water – 86.3-104.4% (2.7-5.9%)	[53] (2009)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
Microextraction					
Tap water	Three pesticides	DLLME-LC-MS/MS	Sample volume – 5 mL Dispersive solvent – acetonitrile Dispersive solvent volume – 2 mL Extraction solvent – carbon tetrachloride Extraction solvent volume – 60 µL Shake a few seconds Separation – centrifugation 2000 rpm, 5 min Sedimented phase was dried under N_2 stream and reconstituted in 100 µL methanol	Linearity range: 1 – 1000 $\mu g.L^{-1}$ LOQ: 0.2 $\mu g.L^{-1}$ Recovery (RSD): 62.7-120.0% (1.9-9.1%)	[54] (2010)
Lake water	Four carbamate pesticides	ST-DLLME-GC-MS/MS	Sample volume – 5 mL Dispersive solvent – acetonitrile Dispersive solvent volume – 0.50 mL Extraction solvent – toluene Extraction solvent volume – 50 µL Separation – after a setting time, another 0.5 mL of dispersive solvent (serving as demulsifier) was injected in the mixture Upper phase was collected and analyzed	Linearity range: 0.02 – 20 $ng.mL^{-1}$ LOD: 0.008 $ng.mL^{-1}$ Recovery (RSD): 97.4-99.0% (2.7-4.5%)	[55] (2010)
River, reservoir and well water	Six carbamate pesticides	UASEME-LC-DAD	Sample volume – 5 mL Emulsifier – Tween 20, 1.0 $\times 10^{-2}$ $mol.L^{-1}$ Emulsifier volume – 30 µL Extraction solvent – trichloromethane-chlorobenzene 1:1 (v/v) Extraction solvent volume – 150 µL Mix – 30 min ultrasonic bath Separation – centrifugation 3500 rpm, 5 min Sedimented phase was dried under N_2 stream and reconstituted in 20 µL methanol	Linearity range: 0.6 – 200 $ng.mL^{-1}$ LOD: 0.2 $ng.mL^{-1}$ Recovery (RSD): River water – 83.0-88.1% (4.1-5.1%) Reservoir water – 82.0-87.3% (3.6-5.3%) Well water – 81.0-95.7% (4.6-5.8%)	[56] (2010)
River water	Sixteen pesticides	SPME-GC-MS	Fiber – Polyacrilate (PA) Sample volume – 10 mL Adsorption time – 30 min; 50 °C Desorption –5 min, in the GC injector at 280 °C	Linearity range: 0.10 – 100 $ng.mL^{-1}$ LOD: 0.03 $ng.mL^{-1}$ LOQ: 0.10 $ng.mL^{-1}$ Recovery (RSD): 89.4-108.5% (4.8-10.5%	[24] (2010)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
Microextraction					
Tap, mineral, sea and river water	Three *N*-methylcarbamate pesticides	DLLME-LC-UV	Sample volume – 5 mL with 4.7% (w/v) NaCl Dispersive solvent – acetonitrile Dispersive solvent volume – 1.5 mL Extraction solvent – trichloromethane Extraction solvent volume – 126.0 µL Shake a few seconds Separation – centrifugation 4000 rpm, 10 min Sedimented phase was dried under N_2 stream and reconstituted in 50 µL methanol	Linearity range: 0.005 – 10 $\mu g.mL^{-1}$ LOD: 0.0005 $\mu g.mL^{-1}$ Recovery (RSD): Tap water – 91.35-93.19% (3.07-4.82%) River water – 97.83-101.35% (2.91-5.06%) Sea water – 63.45-67.82% (3.81-3.90%) Mineral water – 92.64-93.15% (3.61-4.46%)	[57] (2011)
Sea, lake and tap water	Four carbamate pesticides	SPME-LC-DAD	Fiber – graphene-coated fiber Sample volume – 10 mL Adsorption time – 40 min; room temperature 800 rpm Desorption – 50 µL methanol, 2 min.	Linearity range: 2.4 – 400 $ng.mL^{-1}$ LOD: 0.8 $ng.mL^{-1}$ LOQ: 2.4 $ng.mL^{-1}$ Recovery (RSD): Tap water – 84.2-90.0% (3.4-6.2%) Sea water – 84.8-88.2% (2.8-6.6%) Lake water – 85.6-93.0% (2.8-5.1%)	[58] (2011)
Surface water	Fifty-eight pesticides, pharmaceuticals and personal care products	ST-DLLME-LC-MS/MS	Sample volume – 10 mL with 1% $MgSO_4$ (w/v), pH 8 Dispersive solvent – acetone Dispersive solvent volume – 0.75 mL Extraction solvent – octanol Extraction solvent volume – 120 µL Separation – after a setting time, another 0.75 mL of water (serving as demulsifier) was injected in the mixture Upper phase was collected, the volume was increased to 250 µL with methanol and analyzed	Linearity range: 0.125-25 $\mu g.L^{-1}$ LOQ: 0.125 $\mu g.L^{-1}$ Recovery (RSD): Intra-day: 86-121% (7-20%) Inter-day: 85-112% (8-25%)	[59] (2015)
LLE					
Tap and river water	Carbofuran and three derivative pesticides	LLE-GC-MS	Sample volume – 500 mL - 100 g sodium chloride and 25 mL dichloromethane were added to the sample - Shake 5 min. Let it rest for 5 min - Repeat the procedure for dichloromethane (25 mL) - Add 10 g anhydrous sodium sulphate to the extract and evaporate almost to dryness with N_2 - Adjust to 0.5 mL with hexane	Linearity range: 1-500 $\mu g.L^{-1}$ LOD: 1.7 $\mu g.L^{-1}$ LOQ: 5.7 $\mu g.L^{-1}$ Recovery (%CV): Tap water – 96% (2%) River water – 103% (1%)	[25] (2003)

Table 2.1 – Studies found in literature relative to the determination of carbofuran in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
LLE					
Mineral water	Three carbamate pesticides	LLE-LC-UV	Sample volume – 2.00 mL - 1.5% (w/v) sodium chloride and 4.00 mL acetonitrile were added to the sample - Ultrasonic bath for 10 min - Keep at – 20 °C for 3 h - Separate 1.0 mL of the organic phase and analyze	Linearity range: 0.033-10.0 $mg.L^{-1}$ LOD: 10 $\mu g.L^{-1}$ LOQ: 33 $\mu g.L^{-1}$ % Recovery (%CV): 94.7-96.3% (5.5-8.5%)	[60] (2010)
Surface and ground water	One organophosphorus and two carbamate pesticides	LLE-LC-DAD	Sample volume – 500 mL - 100 mL of solvent mixture (2% diethyl ether in hexane) was added to the sample - Collect the organic solvent. Repeat 2 times with 25 mL of solvent mixture - Add 20 g anhydrous sodium sulphate to the combined organic solvent - Evaporate in rotary vacuum to 5mL	Linearity range: 5-20 $\mu g.L^{-1}$ % Recovery: 90.13%	[17] (2012)

DAD: diode array detector; DLLME: dispersive liquid-liquid microextraction; ECD: electron capture detector; ESI: electron spray ionization; FLD: fluorescence detector; FTD: flame thermionic detector; GC: gas chromatography; HF: hollow fiber; LC: liquid chromatography; LLE: liquid-liquid extraction; LLME: liquid liquid microextraction; LOD: limit of detection; LOQ: limit of quantification; LPME: liquid-phase microextraction; MEKC: micellar electrokinetic chromatography; MS: mass spectrometry; MSPE: magnetic solid-phase extraction; NPD: nitrogen phosphorus detector; QqQ: triple quadrupole mass spectrometer; RSD: relative standard deviation; SBSE: stir bar sorptive extraction; SDME: single drop microextraction; SPE: solid-phase extraction; SPME: solid-phase microextraction; ST: solvent terminated; TOF: time of flight detector; TSD: thermionic specific detector; UASEME: ultrasound-assisted surfactant-enhanced emulsification microextraction.

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques.

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface water	Eleven pesticides	On-line SPE-LC-MS/MS	Cartridge – Oasis HLB; Conditioning – 850 µL water-methanol 90:10 (v/v); Load – 10 mL; Washing – 1.75 mL water-methanol 90:10 (v/v); Elution – 750 µL water-methanol 90:10 (v/v).	Linearity range: 5-500 $ng.L^{-1}$ LOD:0.7 $ng.L^{-1}$ % Recovery (100 $ng.L^{-1}$): 83% %RSD (50 $ng.L^{-1}$): 3.4%	[61] (2002)
River water	Over ninety compounds (phenols, carboxylic acids, aromatic sulfonates, aromatic amines, pharmaceuticals, surfactants, dyes and pesticides)	SPE-LC-ESI-MS	Cartridge – C_{18} and Oasis HLB; Conditioning – 5 mL methanol, 5 mL water; Load – 400 mL for fractions 1 + 2 (C_{18}), 300 mL for fraction 3 Oasis HLB; Washing – 2 mL water in each Cartridge; Drying – N_2 30 min; Elution – C_{18}: 2 × 3 mL hexane-dichloromethane 3:1 (v/v), 2 × 3 mL methanol-acetone-ethylacetate 2:2:1 containing 0.1% formic acid; Oasis HLB: 2 × 3 mL methanol-acetone 1:1	LOD: 2 $ng.L^{-1}$	[62] (2003)
Drinking and surface water	Twenty-eight pesticides	On-line SPE-LC-MS/MS	Cartridge – Cyclone and Hypercarb; Conditioning – methanol-water 10:90 (v/v); Load – 10 mL; Washing – 10 mL methanol-water 10:90 (v/v); Elution – eluent A: methanol-water 97:3 (v/v), 1mM ammonium acetate; eluent B: methanol-water 3:97 (v/v), 1mM ammonium acetate.	Linearity range: 3-300 $ng.L^{-1}$ Ultraclean water: %RSD (3 $ng.L^{-1}$) – 3.7% %RSD (50 $ng.L^{-1}$) – 6.9% LOD – 0.3 $ng.L^{-1}$ Drinking water: %RSD (3 $ng.L^{-1}$) – 1.4% %RSD (50 $ng.L^{-1}$) – 5.8% LOD – 0.3 $ng.L^{-1}$ River water: %RSD (3 $ng.L^{-1}$) – 5.1% %RSD (50 $ng.L^{-1}$) – 2.1% LOD – 0.6 $ng.L^{-1}$	[63] (2003)
River, ground and drinking water	Twenty-one pesticides, phenols and phtalates	On-line SPE-LC-MS	Cartridge – PLRP-s; Conditioning – 6 mL acetonitrile, 4 mL water; Load – 20 mL; Elution –acetonitrile-water 30:70 (v/v)	Linearity range: 50-10000 $ng.L^{-1}$ LOD – 20.4 $ng.L^{-1}$ % RSD (1 $\mu g.L^{-1}$) – 1.6% % Recovery – 100%	[64] (2004)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Water	Six organophosphate pesticides and two organonitrogen pesticides	SPE-GC-NPD	Cartridge – C_{18}; Conditioning – 10 mL ethyl acetate, 5 mL water; Load – 500 mL; Drying – N_2, 20 min; Elution – 10 mL ethyl acetate; Evaporate to dryness in rotary evaporator and dissolve in 0.5 mL isooctane.	Linearity range: 0.09-2.0 µg.L^{-1} LOD – 0.03 µg.L^{-1} LOQ – 0.09 µg.L^{-1} Repeatability (RSD, 0.1 µg.L^{-1}) – 4.6% % Recovery – 100%	[65] (2006)
Ground, surface and wastewater	Fifty-four compounds (organochlorine and organophosphorus insecticides, herbicides, polychlorinated biphenyls, polycylic aromatic hydrocarbons, brominated diphenyl ethers, phenols, pentachlorobenzene)	SPE-GC-MS/MS	Cartridge – C_{18}; Conditioning – 6 mL methanol, 6 mL ethyl acetate-dichloromethane 50:50 (v/v), 6 mL methanol, 6 mL water; Load – 100 mL; Washing – 3 mL water; Drying – air, 15 min; Elution – 5 mL ethyl acetate-dichloromethane 50:50 (v/v); Evaporate to dryness under N_2 stream and dissolve in 1 mL hexane.	Linearity range: 5-500 ng.L^{-1} LOD – 5 ng.mL^{-1} LOQ – 25 ng.mL^{-1} % Recovery (%RSD): Spike 25 ng.L^{-1} – 98% (10%) Spike 250 ng.L^{-1} – 68% (12%)	[66] (2007)
Waste water	Fourteen compounds (pharmaceuticals, pesticides, caffeine, triclosan, bisphenol A and three of its metabolites)	SPE-GC-MS	Cartridge – Oasis HLB; Conditioning –5 mL ethyl acetate, 5 mL methanol, 5 mL water; Load – 300 mL (100 mL influent + 200 mL effluent); Washing – 6 mL water; Drying – N_2 stream, 15 min; Elution –2 × 4 mL ethyl acetate; Evaporate under N_2 stream until 1 mL.	LOD – 21 ng.L^{-1} % Recovery (%RSD) – 85% (11%) Repeatability (%RSD) – 6% Reproducibility (%RSD) – 7%	[67] (2007)
Surface water	Eighty-eight pesticides	SPE-GC-MS/MS	Cartridge – C_{18}; Conditioning – 10 mL acetonitrile-dichloromethane 1:1 (v/v), 5 mL methanol, 3 mL water; Load – 250 mL with 2.5 mL methanol; Drying – vacuum, 2 h; Elution – 3 mL acetone, 3 mL hexane-acetone 1:1 (v/v), 3 mL hexane; Evaporate to dryness in N_2 stream and dissolve in 1 mL cyclohexane.	Linearity range: 0.03-0.5 µg.L^{-1} LOD – 0.004 µg.L^{-1} LOQ – 0.012 µg.L^{-1} % Recovery (%RSD): Spike 0.03 µg.L^{-1}– 80% (12%) Spike 0.1 µg.L^{-1} – 78% (11%) Spike 0.5 µg.L^{-1}– 94% (9%)	[68] (2008)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface water	Thirty-six compounds (pesticides, polycyclic aromatic hydrocarbons, endocrine disruptors, organochlorine compounds)	SPE-LC-FLD-MS/MS SPE-GC-MS	Cartridge – Strata-X; Conditioning – 4 mL acetonitrile, 4 mL methanol-isopropanol-acetonitrile 1:1:1 (v/v), 20 mL water; Load – 500 mL; Drying – air, 15 min; Elution – 4 mL methanol-isopropanol-acetonitrile 1:1:1 (v/v), 4 mL acetonitrile.	LC-FLD-MS/MS Linearity range: 4-170 $\mu g.L^{-1}$ LOD – 0.1 $ng.L^{-1}$ LOQ – 0.5 $ng.L^{-1}$ % Recovery (%RSD) – 83.5-85.5% (3.4-6.2%) GC-MS Linearity range: 50-1200 $\mu g.L^{-1}$ LOD – 32 $ng.L^{-1}$ LOQ – 56 $ng.L^{-1}$ % Recovery (%RSD) – 83-88% (0.9-7.8%)	[69] (2009)
River water	Ninety-seven compounds (pharmaceuticals, plasticizers, personal care products, acid herbicides, triazines, organophosphorus compounds, phenylureas, organochlorine biocides, polycyclic aromatic hydrocarbons, benzothizoles)	SPE-GC × GC-TOF-MS	Cartridge – Strata-X; Conditioning – 10 mL ethyl acetate, 10 mL methanol, 10 ml water (pH=2); Load – 6 mL; Drying – air, 30 min; Elution – 5 × 2 mL ethyl acetate; Evaporate until 100 µL under N_2 stream.	Linearity range: 2/10-1000/4000 $\mu g.L^{-1}$ LOD – 0.1 $ng.L^{-1}$ LOQ – 0.5 $ng.L^{-1}$ % Recovery – 98-101% Repeatability (%RSD) – 3-5%	[70] (2010)
Tap, pound and river water	Thirty-three pesticides (including carbofuran and chlorfenvinphos)	SPE-LC-ESI-MS/MS	Cartridge – Chromabond HR-X; Conditioning – 5 mL methanol, 5 mL water; Load – 50 mL with pH 6; Washing – 5 mL water with 5% methanol; Drying – N_2 15 min; Elution – 3 mL methanol and 3 mL methanol-ethyl acetate 75:25 (v/v); Evaporation – N_2 stream; Dissolution – 1 mL mobile phase (90% water-10% acetonitrile).	LOQ: 20 $ng.L^{-1}$ Mineral water: % Recovery (% RSD) at 0.04 $\mu g.L^{-1}$: 113% (36%) % Recovery (% RSD) at 0.2 $\mu g.L^{-1}$: 170% (60%) Natural water: % Recovery (% RSD) at 0.04 $\mu g.L^{-1}$: 129% (36%) % Recovery (% RSD) at 0.2 $\mu g.L^{-1}$: 135% (42%)	[43] (2011)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Surface, ground, effluent and waste water	Around one hundred and fifty compounds (polycyclic aromatic hydrocarbons, octy/nonyl phenols, polychlorinated biphenyls, polybrominated diphenyl ethers, pesticides and its metabolites)	SPE-GC-TOF-MS	Cartridge – C_{18}; Conditioning – 6 mL methanol, 6 mL ethyl acetate-dichloromethane 50:50 (v/v), 6 mL methanol, 6 mL water; Load – 250 mL; Washing – 3 mL water; Drying – air, 15 min; Elution – 5 mL ethyl acetate-dichloromethane 50:50 (v/v); Evaporate to dryness under N_2 stream at 40 °C and dissolve in 0.5 mL hexane.	Surface, ground, effluent and waste water: Limit of identification (LOI) – 0.1 $\mu g.L^{-1}$	[71] (2011)
River, ground water	Twelve pesticides (including carbofuran and chlorfenvinphos)	SPE-LC-DAD-UV	Cartridge – mixed sodium dodecyl sulphate (SDS)-tetrabutylammonium (TBA) admicelle-based SPE; Alumina cartridges were conditioned with 10 mL of 0.01 M hydrochloric acid. Hemicelles were formed on the alumina by passing 25 mL solution containing 40 mg of SDS at pH 2 and 250 mL of water, containing 30 µg of TBA; Elution – acid pesticides were eluted with 2 mL of 0.3 M NaOH:methanol 90:10 (v/v); neutral and basic pesticides were eluted with 1 mL THF.	Linearity range: 10-3000 $\mu g.L^{-1}$ LOD – 13 $ng.L^{-1}$ LOQ – 40 $ng.L^{-1}$ River water: % Recovery - 78-103% Underground water % Recovery – 80-100%	[45] (2012)
River water	Thirty-nine pesticides	SPE-GC-MS	Cartridge – Oasis HLB; Conditioning – 5 mL ethyl acetate, 5 mL methanol, 5 mL water; Load – 500 mL; Drying – vacuum, 1 h; Elution – 6 mL ethyl acetate. After the extraction, ~1 g of anhydrous sodium sulphate was added to the sample bottle to remove any residual water and the bottle was rinsed three times with ~4 mL of dichloromethane. This, volume reduced to 1 mL under N_2 stream, was added to the ethyl acetate fraction which was concentrated to 200 µL.	Linearity range: 100-800 $ng.L^{-1}$ LOD – 9.6 $ng.L^{-1}$ LOQ – 31.7 $ng.L^{-1}$ River water: % Recovery (%RSD) – 90.0-99.3% (4.5-9.6%)	[72] (2012)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SPE		
Mineral, surface and ground water	Sixty-six compounds (pesticides, octyl/nonyl phenols, polychlorinated biphenyls, polybrominated diphenyl ethers)	SPE-GC-MS	Cartridge – C_{18}; Conditioning – 6 mL methanol, 6 mL ethyl acetate-dichloromethane 50:50 (v/v), 6 mL methanol, 6 mL water; Load – 250 mL; Washing – 3 mL water; Drying – air, 30 min; Elution – 5 mL ethyl acetate-dichloromethane 50:50 (v/v); Evaporate to dryness under N_2 stream at 40 °C and dissolve in 0.5 mL hexane.	Linearity range: 10-250 µg.L^{-1} Mineral water: LOD – 10 ng.L^{-1} LOQ – 15 ng.L^{-1} Spike 100 ng.L^{-}1: Recovery (%RSD) – 110% (24%) Ground water: LOD – 10 ng.L^{-1} LOQ – 23 ng.L^{-1} Spike 100 ng.L^{-}1: Recovery (%RSD) – 100% (8%) Surface water: LOD – 10 ng.L^{-1} LOQ – 15 ng.L^{-1} Spike 100 ng.L^{-}1: Recovery (%RSD) – 99% (12%)	[73] (2012)
Surface water	Twenty-five pesticides and degradation products	On-line SPE-LC-MS/MS	Cartridge – PLRP-s; Conditioning – 1 mL acetonitrile, 1 mL water; Load – 5 mL; Washing – 1 mL water; Elution – mobile phase (acetonitrile-water 10:90 (v/v)).	LOD – 0.11 ng.L^{-1} LOQ – 0.36 ng.L^{-1} % RSD – 5.1	[74] (2014)
River water	Seventy micropollutants (pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenols, pharmaceuticals)(including carbofuran and chlorfenvinphos)	SPE-GC-MS/MS	Cartridge – Oasis HLB; Conditioning - 5 mL dichloromethane-ethyl acetate 50:50 (v/v), 5 mL hexane- dichloromethane 50:50 (v/v), 1 mL methanol, 1 mL water; Load – 1000 mL; Washing – 2 mL water; Drying – vacuum, 30 min; Elution – 5 mL hexane- dichloromethane 50:50 (v/v), 3 mL dichloromethane-ethyl acetate 50:50 (v/v); Evaporation - N_2 stream at 35 °C; Dissolution - 500 µL methanol.	Linearity range: 0-4000 ng.mL^{-1} LOD – 26.5 ng.L^{-1} LOQ – 88.4 ng.L^{-1} % Recovery: 98.2 – 102% %CV: 7.79 – 8.43%	[18] (2015)
			Microextraction		
Ground and drinking water	Thirty-five pesticides	SPME-GC-ECD/TSD	Fiber – PDMS-DVB Sample volume – 3 mL Adsorption time – 60 min; 60 °C; 600 rpm Desorption – 5 min, in the GC injector at 250 °C.	Linearity range: 0.01-1.0 µg.L^{-1} LOD – 0.005 µg.L^{-1} Precision (RSD): 13.9-16.4% Recovery: 97.3-104.5%	[75] (2002)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			Microextraction		
Ground water	Five pesticides	SPME-GC-MS	Fiber – PDMS-DVB Sample volume – 10 mL Adsorption time – 20 min Desorption – 5 min, in the GC injector at 250 °C	Linearity range: 0-25 ng.L^{-1} LOD – 3 ng.L^{-1} Recovery: >95%	[76] (2002)
Ground water	Forty-four pesticides	SPME-GC-MS/MS	Fiber – PDMS-DVB Sample volume – 3 mL Adsorption time – 60 min; 60 °C; 900 rpm Desorption –GC injector at 250 °C, 5 min	Linearity range: 0.001-1.0 ng.L^{-1} LOD – 0.002 µg.L^{-1} Repeatability (RSD) – 8.6%	[77] (2004)
Surface and drinking water	Eight carbamate and organophosphorus pesticides	SDME-GC-NPD	Sample volume – 1.8 mL Organic/extraction solvent – isooctane Organic solvent volume – 1 µL Extraction – 14 min at 350 rpm, room temperature	Linearity range: 0.5-30 µg.L^{-1} LOD – 0.2 µg.L^{-1} LOQ – 0.5 µg.L^{-1} Repeatability (RSD): 10%	[78] (2005)
Surface water	Nine organophosphorus pesticides	In-tube SPME-capillary LC-UV	GC capillary column – polysiloxane polymer with 95% methyl-/5% phenyl-substituted backbone (30 cm × 0.25 mm I.D., 0.25 µm coating thickness) Sample volume – 1 mL Washing – 14 µL water Desorption –10 µL methanol	Linearity range: 0.05-0.5 µg.mL^{-1} LOD – 1 ng.mL^{-1} % Recovery: 80-102%	[79] (2007)
Ground and waste water	Sixty compounds (pesticides, octyl/nonyl phenols, pentachlorobenzene and polycyclic aromatic hydrocarbons)	SPME-GC-TOF-MS	Fiber – Carbowax/divinylbenzene (CW/DVB) Sample volume – 4 mL, 10% NaCl Adsorption time – 45 min; room temperature Desorption – GC injector at 250 °C, 5 min	Linearity range:0.01-5 µg.mL^{-1} LOQ – 0.05 µg.L^{-1}	[80] (2007)
Drinking and surface water	Forty-six pesticides	SPME-GC-MS	Fiber – PDMS-DVB Sample volume – 18 mL Adsorption time – 45 min; 60 °C; 500 rpm Desorption – GC injector at 250 °C, 5 min	Linearity range: 25-250 ng.L^{-1} LOD – 24 ng.L^{-1} LOQ – 74 ng.L^{-1} Repeatability (RSD): 6.0%	[81] (2007)
Waste water	Nine compounds (triazines, organophosphorus, phenylureas, dinitroaniline and phthalate)	In-tube-SPME-capillary-LC-DAD	GC TRB-5 capillary column – 5% diphenyl-95% polydimethylsiloxane (PDMS) (40 cm × 0.32 mm I.D., 3 µm coating thickness) Sample volume – 4 mL Washing – 100 µL water Desorption – mobile phase (acetonitrile-water 60:40 (v/v))	Linearity range: 0.1-10 ng.mL^{-1} LOD – 20 ng.L^{-1} Reproducibility (RSD): 10-14% % Recovery: 103%	[82] (2011)

Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			Microextraction		
Tap and ground water	Twelve organophosphate pesticides	Vortex assisted LLME-GC-MS	Sample volume – 10 mL - 40 µL toluene was added to the sample - The mixture was vortexed for 3 min (3 × 60 s at 2000 rpm interrupted by 5 s manual shaking) - Separate by centrifugation 4000 rpm, 5 min - Recover ~20 µL of toluene layer	Linearity range: 75-500 $ng.L^{-1}$ LOD – 9 $ng.L^{-1}$ Repeatability (RSD): 2.6% % Recovery: 83% % Recovery (RSD): Tap water – 106% (8%) Groundwater – 105% (6%)	[83] (2012)
River, coastal and waste water	Nine compounds (eight pesticides and one phatalate)	In-tube SPME-UHPLC-ESI-MS/MS	GC TRB-5 capillary column – 5% diphenyl-95% polydimethylsiloxane (PDMS) (40 cm × 0.32 mm I.D., 3 µm coating thickness) Sample volume – 4 mL Desorption – 40 µL methanol	Linearity range: 0.25-25 $\mu g.L^{-1}$ LOD – 0.025 $\mu g.L^{-1}$ Repeatability (RSD): 5.06% % Recovery: Deionized water – 134% Effluent wastewater – 92% Influent wastewater – 45% Surface water – 94%	[84] (2013)
			LLE		
Ground and reclaimed water	One hundred and eighty-three compounds (pharmaceuticals, pesticides, polycyclic aromatic hydrocarbons, volatile organic compounds and flame retardants)	LLE-GC-MS	Sample volume – 200 mL, acidified up to pH 3 (with H_2SO_4 1 M) - Add 250 mg NaCl and 25 mL hexane were added to the sample - Shake 3 min. Let it rest for 5 min - Repeat the procedure three times - Combine the organic phases - Evaporate almost to dryness with a rotary evaporator - Dissolve in 2 mL of hexane	Linearity range: 0.1-1 $\mu g.L^{-1}$ LOD – 0.3 $ng.L^{-1}$ LOQ – 1.0 $ng.L^{-1}$ Repeatability (RSD) – 5.6% % Recovery – 100.1%	[5] (2012)

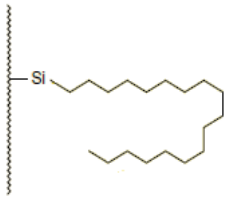
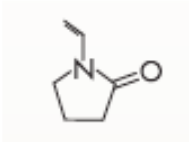
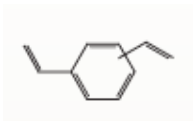
Table 2.2 - Studies found in literature relative to the determination of chlorfenvinphos in water, using different extraction and chromatographic techniques (cont.).

Sample matrix	Target analytes	Analytical method	Extraction procedure	Analytical parameters	Reference (Year)
			SBSE		
Estuarine and sea water	Twenty-four compounds (pesticides, polycyclic aromatic hydrocarbons, phenols)	SBSE-thermal desorption-GC-MS	Stir bar – commercial Twister™, 2.0 cm long, glass encapsulated magnetic stir bar, externally coated with PDMS Sample volume – 100 mL with 10 g NaCl Extraction – 12 h, room temperature, 800 rpm	Linearity range: 0-200 ng.L^{-1} LOD – 1.9 ng.L^{-1} LOQ – 6.0 ng.L^{-1} Estuarine water: Repeatability (RSD) – 5.2-5.5% % Recovery – 97.7-98.1% Sea water: Repeatability (RSD) – 5.2-5.7% % Recovery – 91.2-95.4%	[85] (2007)
River water	Seventy-seven compounds (pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls)	SBSE-thermal desorption-GC-MS/MS	Stir bar – commercial Twister™, 0.5 mm × 20 mm long, glass encapsulated magnetic stir bar, externally coated with PDMS Sample volume – 200 mL with 10 g NaCl Extraction – 24 h, room temperature, 800 rpm	Linearity range: 5.00-200 ng.L^{-1} LOQ – 5.00 ng.L^{-1} Repeatability (RSD) – 9-12% % Recovery – 101%	[86] (2012)

DAD: diode array detector; DLLME: dispersive liquid-liquid microextraction; ECD: electron capture detector; ESI: electron spray ionization; FLD: fluorescence detector; FTD: flame thermionic detector; GC: gas chromatography; HF: hollow fiber; LC: liquid chromatography; LLE: liquid-liquid extraction; LLME: liquid liquid microextraction; LOD: limit of detection; LOQ: limit of quantification; LPME: liquid-phase microextraction; MEKC: micellar electrokinetic chromatography; MS: mass spectrometry; MSPE: magnetic solid-phase extraction; NPD: nitrogen phosphorus detector; QqQ: triple quadrupole mass spectrometer; RSD: relative standard deviation; SBSE: stir bar sorptive extraction; SDME: single drop microextraction; SPE: solid-phase extraction; SPME: solid-phase microextraction; ST: solvent terminated; TOF: time of flight detector; TSD: thermionic specific detector; UASEME: ultrasound-assisted surfactant-enhanced emulsification microextraction.

The stationary phases that constitute C_{18} and Oasis HLB cartridges are depicted in Table 2.3.

Table 2.3 – Stationary phase of C_{18} and Oasis HLB SPE cartridges.

Cartridge	Stationary Phase	
C_{18} (Octadecyl bonded, endcapped silica)		
Oasis HLB (Hydrophilic-Lipophilic Balanced Copolymer)	 Hydrophilic monomer Retention of polars	 Lipophilic monomer Reversed-phase retention

Silica-based C_{18} cartridges are recommended for the reversed phase extraction of nonpolar to moderately polar compounds [87]. Oasis HLB cartridges, due to their chemical composition, have a retention capacity for a wide polarity spectrum of analytes [88]. While developing a routine method for the analysis of carbamates in water samples by SPE-LC-ESI-MS, Nogueira, et al. [31] tested several types of SPE cartridges, including Oasis HLB and two C_{18} from different brands. The authors verified that, for the extraction of the carbamates in study, C_{18} cartridges yielded the highest average recoveries. The same conclusion was reached in another study by the same authors [33], where a multiresidue method for the analysis of twelve pesticides, from different classes, in water is presented. Here, the authors compared three types of cartridges with different polarities including, once again, C_{18} and Oasis HLB cartridges. Acceptable recoveries were obtained for the pesticides studied in all three cartridges, for which octadecilsilica (C_{18}) showed better effective results (76 – 111%) than *N*-vinylpyrrolidane divinylbenzene (Oasis HLB) (30 – 123%). There are, however, other studies that chose Oasis HLB for the extraction of carbamates, and specifically of carbofuran [36, 40]. Dujaković, et al. [42] developed a method for the determination of pesticides by SPE-LC-ESI-MS/MS, and concluded that higher recoveries were achieved with Oasis HLB cartridges, when

comparing with C_{18} cartridges. Particularly for the case of linuron and carbofuran, the recoveries were significantly lower when using the C_{18} cartridge (41 – 75% for linuron and 50 – 87% for carbofuran) compared to Oasis HLB (72 – 108% and 83 – 95% for linuron and carbofuran, respectively). Considering the extraction of chlorfenvinphos from water matrices by SPE, a similar tendency as that of carbofuran is observed. Several authors report the extraction of pesticides, where chlorfenvinphos is included, by SPE with silica-based cartridges C_{18} [65, 66, 68], while several others chose Oasis HLB for the development of their studies [61, 67]. Furthermore, two studies were found, regarding the determination of pesticides and other pollutants, which include carbofuran and chlorfenvinphos. Masiá, et al. [46] developed an analytical method for the screening of pesticides and other pollutants in water samples by SPE-LC-QqQ-MS/MS. Terzopoulou, et al. [18] presented a multi-residue method for the determination of seventy organic micropollutants in surface waters by SPE-GC-MS/MS. Carbofuran and chlorfenvinphos were within the pesticides studied in both of these studies, and the validation of each method presented was performed with Oasis HLB cartridges. Different solvents are used in SPE with the function of conditioning, washing and elution [89]. As can be confirmed in Table 2.1 and Table 2.2, the most common elution solvents used for carbofuran and chlorfenvinphos are acetonitrile, methanol or less commonly ethyl acetate when the extractive phase is a silica-bonded phase, as C_{18}. In order to elute the compounds from the polymeric sorbent of Oasis HLB cartridges, ethyl acetate and mixtures of methanol or hexane with dichloromethane were the most frequently used.

Microextraction techniques have been gathering increasing importance due to their sensibility, simplicity of use, short sample pretreatment time and high enrichment factor. Moreover, these extraction methods require low solvent volumes, or sometimes none, are automatable, and can be applied to matrices in gaseous, aqueous or solid state [90]. Solid-phase microextraction was developed by Pawliszyn and co-workers in early 1990s [91], and since then, the technique has been successfully applied to the sample preparation and analysis of different types of matrices. The SPME combines sampling with preconcentration in a single step. In this procedure, a fiber of fused-silica coated with a stationary phase is exposed to the sample matrix for a given period of time to extract the organic compounds. The adsorption is based on equilibrium partioning

between the coated fiber and the sample. After the adsorption step, the fiber is retracted, and posteriorly the concentrated extracts are desorbed into the analytical instruments. Influencing factors on the extraction of analytes from the fiber include the fiber type, ionic strength, sample pH, extraction time, sample agitation and extraction temperature. There are numerous fibers commercially available, but the most used within pesticide residue analysis are polydimethylsiloxane (PDMS), PDMS/divinylbenzene (DVB) and polyacrylate (PA) [90]. The fibers can be classified by polarity or extraction type mechanism. Some characteristics of the fibers most commonly used for pesticide analysis are presented in Table 2.4.

Table 2.4 – Classification of the SPME fibers most used in pesticide analysis.

Fiber coating	Coating type	Polarity
PDMS	Absorbent	Nonpolar
PDMS/DVB	Adsorbent	Bipolar
PA	Absorbent	Polar

Absorbent-type fibers extract by the partitioning of analytes into a liquid-like coating. The coating ability to retain the analytes depends primarily on the thickness of the coating but also on the size of the analyte. Even though the polarity of the fiber coating may enhance the attraction of the analyte to that particular coating, it is the thickness of the fiber that retains the analytes. Adsorbent-type fibers contain porous particles suspended in a liquid phase. The particles retain the analytes in the pores or on the surface. The fibers can also be categorized by their polarity and, as can be seen, PA fibers are polar, PDME coated fibers are nonpolar and the bipolar fibers, such as PDMS/DVB, are primarily nonpolar, but can extract some polar analytes efficiently [92-94].

As can be observed in Table 2.1 and Table 2.2, these types of fiber coatings are also the most reported in the studies found for the analysis of carbofuran and chlorfenvinphos in water matrices. Even though SPME technique was initially developed to be used in GC, it was adapted to LC by specially designed interfaces or by desorption of the extracted analytes into a small amount of solvent placed in a LC autosampler vial

[95]. For both pesticides in study, the preferred analytical method following SPME is GC, coupled with mass spectrometry (MS) [24, 47, 49, 76, 77, 80, 81], but the use of a GC coupled with a flame thermionic detector (FTD) [47] for the detection of pesticides including carbofuran, and GC coupled with an electron-capture detector (ECD) tandem thermoionic specific detector (TSD) [75] for the detection of chlorfenvinphos among other pesticides, have also been reported. Comparing the limits of detection obtained with the different detectors, no large differences are observable. Gonçalves and Alpendurada [75] developed a SPME-GC-ECD/TSD method for the detection of pesticides in waters, and for chlorfenvinphos, the limit of detection obtained was of 0.005 $\mu g.L^{-1}$. A value which is within the range observed (0.002 – 0.024 $\mu g.L^{-1}$) in the reports on the extraction of chlorfenvinphos by SPME. Similarly, Lambropoulou, et al. [47], proposed an SPME-GC-FTD method for the analysis of pesticides in water samples, and the limit of detection obtained for carbofuran was 0.030 $\mu g.L^{-1}$, which is one of the lowest LODs obtained for the analysis of carbofuran by a method using SPME. Even though few literature exists on the hyphenation of SPME with liquid chromatography [96], two studies were found, which analyze carbofuran SPME extracts from water samples by liquid chromatography (LC) followed by diode array detection (DAD) [30, 58]. The limit of detection for carbofuran analysis by SPME-LC-DAD, reported by López-Blanco, et al. [30], is one of the highest among the studies found (8.9 $\mu g.L^{-1}$). Nonetheless, the method for the determination of carbamates by SPME-LC-DAD, proposed by Zhao, et al. [58], allowed to obtain a limit of detection of 0.8 $\mu g.L^{-1}$ for carbofuran. These results indicate that SPME is a simple but effective technique, which can be successfully coupled with various types of detectors, not compromising the yielded results.

A more recent microextraction technique, which has been increasingly used on the preparation of water samples is the dispersive liquid-liquid microextraction (DLLME). This extraction and preconcentration method was developed by Rezaee, et al. [97], and consists of a ternary component system, where the extraction solvent and the dispersive solvent are quickly injected, with a syringe, into the aqueous sample. The resulting mixture is shaken, and a cloudy solution (water/dispersive solvent/extraction solvent) is formed in the tube. Afterwards, centrifugation is used to separate the fine particles of

the extraction solvent, and two distinct phases are formed. The sedimented phase, containing the extraction solvent, is taken with a microsyringe and analyzed. The steps involved in the DLLME procedure are represented in Figure 2.3.

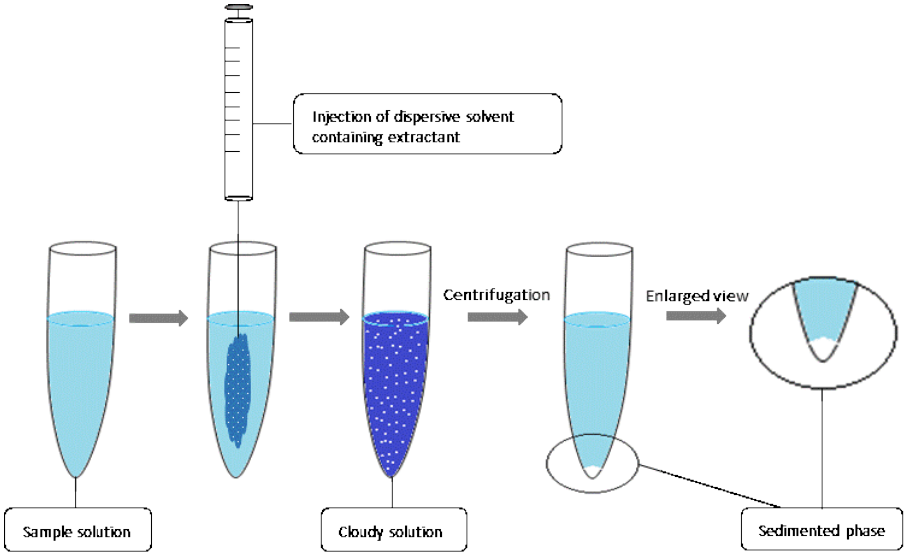


Figure 2.3 – Schematic illustration of DLLME.

DLLME has been used for the extraction of pesticides from several matrices such as fruit juice samples [98, 99], tea [100],watermelon and cucumber [101], bananas [102], table grapes and plums [103], honey [104], soils [105, 106]. Still, most of the available literature is based on the use of DLLME in water samples [53, 55, 57, 107-111]. As depicted in Table 2.1, there are several studies on the utilization of dispersive liquid-liquid microextraction for the preconcentration of carbofuran from water samples. The limits of detection achieved by the several methods proposed are in the range of 0.008 - 80 $\mu g.L^{-1}$. Most of the methodologies proposed coupled DLLME with the analysis by liquid chromatography with various detectors, such as LC-DAD [51, 52], LC-UV [53, 57] and LC-MS/MS [54]. Among these studies, the most common dispersive solvent is acetonitrile, the extraction solvent mostly used is trichloromethane, although some authors also mention chlorobenzene and carbon tetrachloride. In their study, Chen, et al. [55] adopted an alternative version of the DLLME. The authors proposed a low-density extraction solvent terminated dispersive liquid-liquid microextraction (ST-DLLME) followed by analysis by GC-MS/MS. The method is very similar to the traditional

DLLME. Two main characteristics differ: the extraction solvent has lower density than water and thus, after separation, the extraction phase is the upper one; the separation of the cloudy mixture is not performed by centrifugation, but instead by the injection of an aliquot of the dispersive solvent, which acts as a demulsifier. Afterwards, the extracted pesticides were analyzed by GC-MS/MS. The method proposed by Chen, et al. [55] allowed to reach detection levels, for the studied pesticides, in the range of 0.001 – 0.050 $\mu g.L^{-1}$ (0.008 $\mu g.L^{-1}$ for carbofuran), which are among the lowest values reported for the extraction of carbofuran from water matrices. Furthermore, it shows that the direct analysis of carbofuran is possible and, when in conjunction with DLLME, can be a powerful tool for the detection and quantification of this pesticide in water.

Despite the existence of a number of reports regarding the use of DLLME for organophosphorus pesticides [107, 108, 112, 113], including one of the first studies from the creators of the technique [114], the author could not find references to the extraction of chlorfenvinphos from water samples by DLLME. The conditions proposed in the several studies found, concerning the extraction of pesticides of the same class as chlorfenvinphos, (organophosphorus), are diverse. Berijani, et al. [114], in one of the first studies where DLLME technique was utilized, intended to analyze thirteen organophosphorus pesticides (OPPs) in water samples by DLLME followed by the analysis on GC and flame photometric detection (FPD). Chlorobenzene and acetone were chosen as the extraction and dispersive solvents, respectively. The limits of detection obtained by this method were between 0.003 and 0.02 $\mu g.L^{-1}$. Farajzadeh, et al. [107], proposed a DLLME-LC-UV method for the analysis of three OPPs in water samples. In their optimized method, chloroform and methanol were chosen as the extraction and the dispersive solvents, respectively. The limits of detection for the three pesticides were within the range of 2 – 3 $\mu g.L^{-1}$. Fu, et al. [108] analyzed a carbamate and an OPP in water and fruit juice samples by DLLME followed by LC separation and fluorescence detection (FLD). In their proposed method, the extraction solvent was tetrachloroethane and the dispersive solvent was acetonitrile. The detection limits obtained by this method were slightly better, and were in the range of 0.0123 – 0.0160 $\mu g.L^{-1}$. Two other studies were reported where DLLME is applied, but with an extraction solvent lighter than water. In their study, Wu, et al. [113], analyze five

organophosphorus pesticides in environmental samples by DLLME-LC-DAD. In the proposed method, the extraction solvent is 1-dodecanol and the dispersive solvent is methanol. After the extraction and separation of the two phases, the recovery of the extractant solvent is accomplished by the solidification of the floating organic droplet (dodecanol), through the immersion into an ice bath [113]. This method allowed to obtain detection limits in the range of 0.1 – 0.3 $\mu g.L^{-1}$. On another study, Farajzadeh, et al. [112] also use an extraction solvent lighter than water in their method. Three OPPs were extracted by DLLME and analyzed by GC-FID and GC-MS at lower concentrations. Cyclohexane and acetone were used as the extraction and dispersive solvent, respectively. The analysis by GC-FID yielded higher limits of detection (3 – 4 $\mu g.L^{-1}$), whereas the limits of detection for the analysis by GC-MS were of 0.003 $\mu g.L^{-1}$ for the three studied pesticides. The comparison between the different methods proposed for OPPs extraction by DLLME from water samples is difficult, given the disparity of experimental conditions amongst them. The extraction and dispersive solvents combinations proposed were different in all studies. Chlorobenzene, chloroform and tetrachloroethane were proposed as extraction solvents for the traditional DLLME methods, which was expected since those are some of the most used extraction solvents. Likewise, acetone, methanol and acetonitrile, which were used in some of the proposed methods, are amongst the most used dispersive solvents [115]. Regarding the method of analysis of the extract, it seems that the methods that used GC, either coupled with FPD [114] or with MS [112], yielded lowest values of limits of detection.

In sum, DLLME has become a powerful tool for pretreatment of water samples, due to its simplicity, reliability, low cost and reduced use of organic solvents. The application of this method will be discussed in greater detail in section 2.3.

As stated before, chromatography is still the most widely used method for the analysis of pesticides in several matrices, but particularly in water. Gas chromatography coupled with selective detectors (nitrogen-phosphorus (NPD), electron capture (ECD) and flame photometric (FPD)) or mass spectrometry (MS) is one of the most popular techniques for pesticide determination. GC-NPD allows for the determination of organophosphorus pesticides, through the analysis by the phosphorus mode. Chlorinated pesticides are commonly analyzed by electron capture detectors. GC-FPD

and GC-FID can also be used for the determination of organophosphorus pesticides. Nowadays, GC-MS is, probably, the most used method for the detection, and especially for the identification and confirmation of pesticides in environmental matrices. In order to overcome analysis interference of matrix components, and to achieve regulated limits for pesticides, quadrupole instruments must operate in selected ion monitoring (SIM) mode and ion trap instruments in MS/MS, which allows for the increase in selectivity and sensitivity [89]. Liquid chromatography is also widely used in the determination of pesticides, particularly thermally labile and polar pesticides [116]. LC methods differ within each other in the length of the column used, the stationary phase, the mobile phase, elution mode and detector. Acetonitrile, methanol and/or water are the solvents most frequently used as mobile phase, either in gradient or isocratic mode. Stationary phase usually consist of bonded silica, such as octyl-silica (C_8) and octadecyl-silica (C_{18}). Ultraviolet (UV) and diode array detectors (DAD) are among the most commonly available and thus, are frequently coupled with LC. However, UV detectors have lack of selectivity and sensitivity, thus, due to the possibility of wavelengths selection in the determination provided by DAD detectors, these are preferred. The coupling of MS with LC allows for the better identification and increased recoveries, due to the elimination of matrix interferences, which might occur in other type of detectors [27].

2.1.2.2 Biosensors

Chromatographic methods have been the most frequently used, in the detection of pesticides in the environment. Nonetheless, these techniques present some drawbacks, such as complexity, cost and are time consuming [117]. Efforts have been made in order to develop new methods for the detection and quantification of contaminants that are easy to use and more economic. For the development of biosensors, researchers considered the ability of many pesticides to inhibit some enzymes (for example acetylcholinesterase) from insects and other pests. Thus, these biosensors are based on the quantification of the inhibitor, measuring the enzymatic activity in absence and in the presence of the inhibitor [118]. When an inhibitor is present, some of the active sites of the enzyme are blocked, the electroactive product diminishes and the signal

decreases. The concentration of the pesticide is related with this decrease [119]. As can be observed in Figure 2.1, the development and use of biosensors for detection and quantification of pesticides, and particularly of carbofuran and chlorfenvinphos, is significant. Table 2.5 and Table 2.6 present a summary of the characteristics of biosensors reported in some studies, for the analysis of carbofuran and chlorfenvinphos, respectively.

As mentioned before, organophosphorus and carbamate pesticides are designed to inhibit acetylcholinesterase (AChE), thus, this enzyme has been used the most in the detection of these pesticides. As such, biosensors developed for the detection of carbofuran and chlorfenvinphos also have this characteristic, as can be observed in Table 2.5 and in Table 2.6. Inhibitory effects are measured by AChE biosensors. When the analyte is not present in solution, acetylthiocoline substrate is converted into thiocoline and acetic acid, by AChE. Thiocoline is then oxidized by the applied voltage. When a pesticide is present, acetylcholine conversion is diminished or even null [120]. The anodic oxidation current is inversely proportional to the pesticide concentration in the samples. When developing a biosensor, the attachment of the enzyme onto the surface of the working electrode is extremely important. Sensitivity, stability, response time and reproducibility of the sensor are largely dependent on the adequate immobilization of the enzyme onto the electrode. Enzyme immobilization can be achieved through several methods such as, covalent binding, entrapment, crosslinking or physical adsorption. Covalent binding consists on the attachment of the enzyme to the transducer surface using a chemical reaction, for instance the linkage to activated surface groups. Entrapment is characterized by the physical trapping of the enzyme into a film or coating. Crosslinking combines features of both covalent bonding and entrapment. In this process a polymerization agent is used to provide additional chemical linkages between the entrapped enzyme and the film or coating. Immobilization by adsorption involves the association of the enzyme with a film or coating through hydrophobic, hydrophilic and/or ionic interactions [121]. The interaction of the analyte with the enzyme produces a physicochemical change, which is converted into an electrical signal by a transducer.

Table 2.5 - Studies found in literature relative to the determination of carbofuran in water, using biosensors.

Target analytes	Electrode material/ Immobilization matrix	Enzyme	Type of transducer/ technique	Immobilization method	Analytical parameters	Reference (Year)
Two pesticides	Photolithographic conducting copper track, graphite-epoxy composite applied by screen-printing	AChE or BChE	Amperometric	Crosslinking	For BChE: LOD – 0.047 μg.L^{-1} Recovery in tap water: 101.9-104.2%	[119] (2001)
Five pesticides	Activated controlled pore glass	AChE or BChE	Spectrophotometric detection	Crosslinking	LOD: AChE electric eel – 3.8 μg.L^{-1} AChE bovine – 5.1 μg.L^{-1} AChE human – 5.5 μg.L^{-1} BChE horse – 60 μg.L^{-1}	[122] (2001)
Four *N*-methylacarbamate pesticides	Screen-printed working electrode - 7,7,8,8-tetracyanoquinodimethane modified graphite working electrode	AChE	Amperometric	Photopolymerization with poly(vinyl alcohol)bearing styrylpyridinium groups	Linear range – 0.2-166 μg.L^{-1} LOD – 0.2 μg.L^{-1}	[123] (2004)
Four pesticides	Sensitive fluorescence probe used as pH indicator	AChE	Florescence detector	------	Linear range – 3.5-30 μg.L^{-1} LOD – 0.2 μg.L^{-1}	[124] (2004)
Two carbamate pesticides	Silica gel	AChE	Potentiometric Conductimetric	Covalent binding	Potentiometric: Linear range – 0.02-8.0 mg.L^{-1} LOD – 0.02 mg.L^{-1} %RSD – 2.4% Conductimetric: Linear range – 0.02-8.0 mg.L^{-1} LOD – 0.02 mg.L^{-1} %RSD – 4.0%	[125] (2005)
Three carbamate pesticides	Sol-gel matrix on 7,7,8,8-tetracyanoquinodimethane-modified screen-printed electrode	AChE	Amperometric	Entrapment	LOD – 0.8-10 ×10^{-9} M	[126] (2006)
Carbofuran	Enzyme-linked immunosorbent assay (ELISA)	Monoclonal antibodies (McAb)	------	------	%Recovery: 94.2-130.0% %RSD: 1.8-8.8%	[127] (2008)
Two pesticides	Gold immunochromatographic assay (GICA)	Bispecific monoclonal antibody (BsMcAb), McAb	------	------	BsMcAb: Visual LOD – 64 μg.L^{-1} McAb: Visual LOD – 32 μg.L^{-1}	[128] (2009)

Table 2.5 - Studies found in literature relative to the determination of carbofuran in water, using biosensors (cont.).

Target analytes	Electrode material/ Immobilization matrix	Enzyme	Type of transducer/ technique	Immobilization method	Analytical parameters	Reference (Year)
Three pesticides	Gold nanoparticles and silk fibroin modified platinum electrode	AChE	Amperometric	Covalent binding	Linear range – 0.2-100 $\times 10^{-9}$ M LOD – 0.1 $\times 10^{-9}$ M	[129] (2009)
Carbofuran	Magnetic beads	AChE	Amperometric	Magnetic field	Linear range – 0.1-20 $\mu g.L^{-1}$ LOD – 0..34-0.96 $\mu g.L^{-1}$	[130] (2009)
Carbofuran	Glassy carbon electrode	McAb	Amperometric	Silica sol-gel technology	Linear range: 1 $\times 10^{-3}$-100 $\mu g.mL^{-1}$ 50-200 $\mu g.mL^{-1}$ LOD – 0..33 $ng.mL^{-1}$ %RSD: 2.7-5.3%	[131] (2011)
Carbofuran	Halloysite nanotubes	Estereases from *eupenicillium shearii FREI-29* endophytic fungus	Amperometric	Adsorption	Linear range – 5.0-100.0 $\mu g.L^{-1}$ LOD – 1.69 $\mu g.L^{-1}$ LOQ – 5.13 $\mu g.L^{-1}$ %Recovery: 103.8-106.7%	[132] (2015)

AChE: acetylcholinesterase; BChE: butyrylcholinesterase; BsMcAb: bispecific monoclonal antibody; LOD: limit of detection; LOQ: limit of quantification; McAb: monoclonal antibody; RSD: relative standard deviation.

Table 2.6 - Studies found in literature relative to the determination of chlorfenvinphos in water, using biosensors.

Target analytes	Electrode material/ Immobilization matrix	Enzyme	Type of transducer/ technique	Immobilization method	Analytical parameters	Reference (Year)
Four organophosphate pesticides	Monolayer of 11-mercaptomonoundecanoic acid (MUA)assembled on the gold surface of sensor	AChE	Piezoelectric	Self-assembled monolayer	LOD – 48.5 $\mu g.L^{-1}$ %RSD – 8%	[133] (2005)
Two organophosphorus pesticides	Activated magnetic microbeads	Genetically engineered AChE (B394)	Amperometric	Magnetic controlled affinity immobilization	LOD – 1.3×10^{-11} M	[134] (2007)
Three pesticides	Graphite and 7,7,8,8-tetracyanoquinodimethane screen printed electrode	AChE	Amperometric	Entrapment	------	[135] (2008)
Three organophosphorus pesticides	Photocrosslinkable polymer polyvinyl alcohol/Screen printed electrode	AChE	Amperometric	Entrapment	LOD – 17 $\mu g.L^{-1}$ % Recovery: 94.50-108.41%	[136] (2012)
Three organophosphorus pesticides	Multiwalled carbon nanotube modified electrode	AChE	Amperometric	Covalent linkage	Linear range: 0.005-0.1 $\mu g.mL^{-1}$ 0.1-12.5 $\mu g.mL^{-1}$ LOD – 4.90 $ng.L^{-1}$	[137] (2014)
Two organophosphorus pesticides	Lipase based liquid phase sensor	Lypase	Voltammetric	------	Linear range – 100-900.0 µM LOD – 84.45 µM LOQ – 253.03 µM	[138] (2014)

AChE: acetylcholinesterase; LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation.

Several types of transducers can be used, however the most commonly used are the electrochemical, and specifically the amperometric ones. This type of transducers is based on the oxidation and reduction of the electroactive substances at the working electrode. This electrode has a specific potential related to a reference electrode. The produced current is proportional to the concentration of the electroactive product [139]. A transducer with electrochemical detection has the advantages of being low cost, easy to use, portable, and simple to construct [140]. As portrayed in Table 2.5 and Table 2.6, there are numerous materials which can be used for the electrode and supporting substrate. These materials usually are conductive materials exhibiting low currents in an electrolyte solution, free of electroactive species. Metals (platinum [129], gold [133], silver and stainless steel), carbon-based materials (glassy carbon [131], graphite [119, 123, 135], carbon black and carbon fiber) and new hybrid materials are among the most frequently used. The immobilization matrix may be just the support or may also be involved in the mechanism of signal transduction mediation [141].

Comparing the limits of detection achieved by chromatographic methods (Table 2.1 and Table 2.2) and by biosensors analysis (Table 2.5 and Table 2.6) it is clear that, in general, biosensors are not able to reach LODs as low as the chromatographic analytical methods. The limits of detection reported by the considered studies with biosensors are, for the most part, of the order of $\mu g.L^{-1}$. A possible approach for the improvement of detection limits might be the development of different immobilization approaches and the use of new materials. For example, Kesik, et al. [137] developed a biosensor based on a conducting polymer using multi walled carbon nanotubes. Acetylcholinesterase was successfully immobilized by covalent linkage on the modified graphite electrode. The biosensor was used to detect three organophosphorus pesticides, including chlorfenvinphos, and under optimal conditions, the limit of detection was 0.0049 $\mu g.L^{-1}$ for this pesticide, for five minutes of incubation time. The biosensor was also tested in real water samples, and it was able to detect the presence of those pesticides in very low concentrations (detection limits: 0.0005 – 0.005 $\mu g.L^{-1}$). Hence, the presence of covalent binding between enzyme and immobilization matrix resulted in a stable and long life biosensor with high selectivity. However, the biosensor is not able to distinguish between the different pesticides [137]. Biosensors, based on

AChE inhibition show high sensitivity, are low cost, portable, and provide rapid results [142]. Yet, analytical applications of this type of sensors are still limited due to their usual inability to differentiate between various contaminants in a single sample.

Enzymatic methods should not be regarded as substitutes of the traditional chromatographic methods, but as a complement; namely as, a screening method for the rapid detection of contaminants in environmental matrices. In an event of deliberate contamination of a water distribution system, biosensors could provide the first warning of the presence of contaminants. Afterwards, chromatographic methods could be used to identify and quantify the threat, helping in the decision of the necessary action to be taken.

In sections 2.2 and 2.3 two methods, which allow the detection and quantification of carbofuran and chlorfenvinphos in waters, are presented. The first method, consisting on the rapid analysis by direct injection onto LC-DAD, intended to determine both pesticides at high levels of concentration. The second method, which involves the preconcentration by DLLME followed by GC-MS analysis, aimed to detect, quantify and confirm the presence of carbofuran and chlorfenvinphos in water samples, up to the levels required by European legislation [19].

2.2 Method for fast detection and quantification of carbofuran and chlorfenvinphos in waters by DI-LC-DAD

2.2.1 Introduction

In the extreme case of a deliberate contamination of a water distribution system, there is the need of an immediate response. Presumably, the levels of contaminant(s) (namely pesticide(s)) in the water should be high, at least in the concentration front of contamination and in places with stagnant water. These concentration levels of pesticide should diminish as the contamination progresses along the pipes. In order to take proper measures, the detection, identification and quantification of the pesticide is of main importance. Thus, analytical methods that can provide indicative and quantitative results are extremely necessary. These methodologies should also be able to deliver said results quickly, but reliably. In a distribution system the water flowing in the pipes is in direct contact with the deposits there formed. Hence, said methodologies should also be accurate in the presence of leached compounds from the pipe deposits. The method now presented aims to quickly detect and quantify the presence of carbofuran and chlorfenvinphos, at high concentration levels, in waters. Basically, it consists on the analysis by the direct injection of water samples onto a liquid chromatograph with diode array detector (DI-LC-DAD). Even though the proposed method is not able to quantify the pesticides presence at very low levels of contamination, it is a useful way of immediately understand the extent of a deliberate contamination event. This methodology should be able to give an almost immediate answer to the existence of these pesticides in water which, subsequently, enables the adoption of appropriate measures in order to contain the contamination, and protect the population. Then, the DI-LC-DAD method for carbofuran and chlorfenvinphos detection and quantification in waters is presented and validated.

2.2.2 Experimental section

2.2.2.1 Chemicals and reagents

Carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl-methylcarbamate) with a purity of 99.9%, and chlorfenvinphos ([EZ]-2-chloro-1-(2,4-dichlorophenyl)ethenyl diethyl phosphate) standards were purchased from Fluka, Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was from VWR BDH Prolabo (Fontenay-sous-Bois, France).

2.2.2.2 Standards preparation

The individual stock solutions (100 $mg.L^{-1}$) of carbofuran and chlorfenvinphos were prepared in distilled water. Mixture and individual standards of carbofuran and chlorfenvinphos were prepared by diluting an appropriate amount of each stock solutions in distilled water. All solutions were stored at 4 °C.

2.2.2.3 Instrumentation

Carbofuran and chlorfenvinphos samples were analysed in a Hitachi Elite LaChrom apparatus with a L-2130 pump, a L-2200 autosampler, a L-2300 column oven and a L-2455 diode array detector. The quantification of both pesticides was made by direct injection of 99 µL in a Purospher STAR LiChroCART RP-18 endcapped (240×4 mm, 5 µm) reversed phase column from Merck (Darmstadt, Germany), at 30 °C. The mobile phase consisted of 60% (v/v) of acetonitrile and 40% (v/v) of water, with a flow rate of 1 $mL.min^{-1}$. Carbofuran quantification was made at 220 nm and chlorfenvinphos at 240 nm.

2.2.2.4 Deposits

This analytical method is intended to detect and quantify carbofuran and chlorfenvinphos in water, specifically water from distribution systems. Considering that the water flowing in the pipes is in direct contact with the deposits there formed, there was the need to confirm if unwanted compounds leached from the deposits were able to interfere with the analysis. These deposits may have different characteristics, depending also on the type of pipe and water composition of the specific region. Therefore, recovery assays were performed with three different types of deposits.

The deposits used in the recovery tests were collected from real drinking water distribution systems, specifically, from cast iron pipes that were required to be replaced. The deposits were kindly supplied by Dr. Gabriela Schaule (IWW Water Centre, Germany).

Prior to their use, the deposits samples (herein called S3 and S4) were dried in an oven (until no weight variation was observed). Afterwards, the dried deposits were sieved and kept in dry conditions until their utilization. Previous work developed within LEPABE/FEUP led to a comprehensive characterization of these deposits [143], thus, for a better understanding, the nomenclature herein was kept consistent. With the results obtained, the authors of the study classified the S3 and S4 samples as tubercle and white deposits, respectively.

Clay is the main component of the mineral fraction of soils [144, 145] and, unlike deposits, is a material that was available in large quantities. Therefore, clay was chosen as model deposit, and used in the validation of the extraction method. This material has been used before in adsorptions studies within our group, thus, a characterization of clay was presented in a previous work [146].

The principal properties that characterize the adsorbents are presented in Table 2.7.

Table 2.7 - Physical-chemical properties of the real deposits and clay (from [[143, 146])**.**

	S3	**S4**	**Clay**
Deposit classification	Tubercle	White	n.d.
ICP-OES analysis (wt.% of the main elements at dry basis)	Fe: 97 P:1 Mn:1	Ca: 97 Fe: 1 Mg: 1	Al_2O_3: 34 SiO_2: 49
S_{BET} ($m^2.g^{-1}$)	36	1	n.d.
pH_{pzc}, 20 °C	6.1	9.9	
pH in water, 20 °C	7.2	9.0	
Main components identified by XRD	Goethite	Calcite ($CaCO_3$)	n.d.
Organic matter content (wt.%)	1.0	0.2	12

ICP-OES: inductively coupled plasma-optical emission spectrometry; n.d.: not determined; XRD: X-ray diffraction.

2.2.3 Results and discussion

The purpose of this work was to develop a rapid and straightforward method for the direct determination of carbofuran and chlorfenvinphos in water, at high concentration levels. As such, it is worth mentioning, at this point, that the requisite of rapidity of the method was fulfilled, since the time required for the analysis by LC-DAD was of 15 minutes (run time of the method for the detection of both pesticides).

The obtained results are shown along the following sections.

2.2.3.1 Method validation

In order to evaluate the DI-LC-DAD method, the limits of detection and quantification, the linearity, precision and accuracy, were calculated.

2.2.3.1.1 DI-LC-DAD linearity range, limits of detection and quantification and precision

Under the selected conditions, the proposed method was evaluated in terms of linearity range, correlation coefficient, limits of detection (LOD) and quantification (LOQ) and precision, for carbofuran and chlorfenvinphos analysis in water.

Twelve analytical standards of each pesticide were directly injected, at least twice, in order to perform the calibration. A linearity range of 0.05 – 100 mg.L^{-1} was obtained for carbofuran and chlorfenvinphos. The obtained calibration curves are presented in Figure 2.4 , along with the respective 95% confidence intervals.

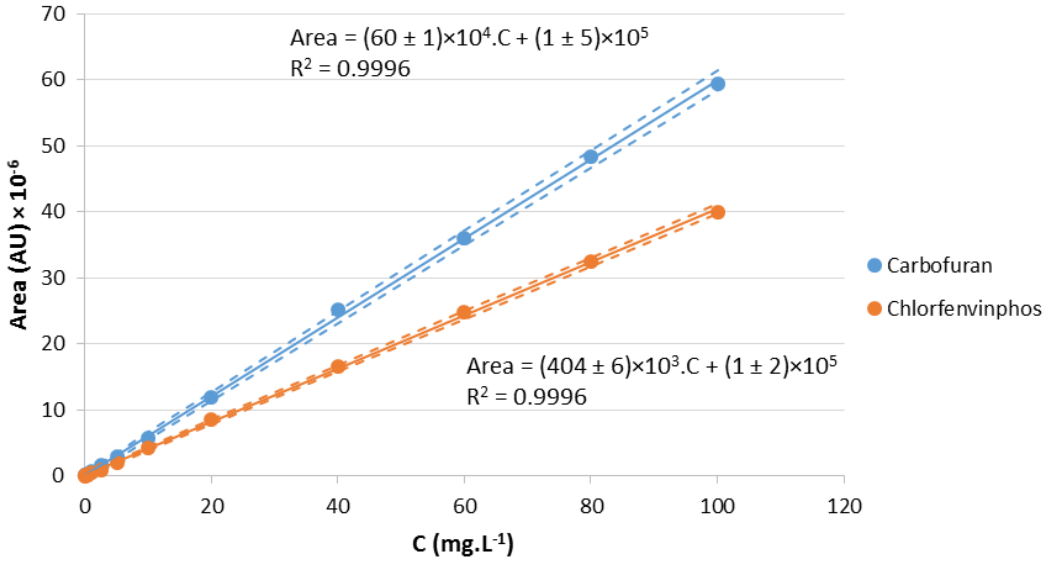


Figure 2.4 – Calibration curves for carbofuran and chlorfenvinphos in water, by DI-LC-DAD.

The limits of detection and quantification were calculated based on the standard deviation (SD) of the response at the lowest concentration (0.05 mg.L^{-1}) and the slope (S) of the calibration curve, according to the following equations [147]:

$$LOD = 3.3\frac{SD}{S} \quad (2.1)$$

$$LOQ = 10\frac{SD}{S} \quad (2.2)$$

Precision was evaluated by a repeatability study, and was expressed as the relative standard deviation (%RSD) of six different replicate measurements of aqueous standard samples at four different concentration levels (0.1, 1, 10 and 60 $mg.L^{-1}$), for each pesticide. The results are summarized in Table 2.8.

Table 2.8 - Linearity results, detection and quantification limits and precision (% RSD) for each pesticide studied.

	Carbofuran	**Chlorfenvinphos**
Calibration Range ($mg.L^{-1}$)	0.05-100	0.05-100
R^2	0.9996 (N=12)	0.9996 (N=12)
LOD ($mg.L^{-1}$)	0.004	0.011
LOQ ($mg.L^{-1}$)	0.013	0.251
% RSD *(n = 6)*		
0.1 ($mg.L^{-1}$)	1.8	3.5
1 ($mg.L^{-1}$)	0.4	0.6
10 ($mg.L^{-1}$)	6.2	0.1
60 ($mg.L^{-1}$)	0.5	0.1

The limits of detection were of 0.004 and 0.011 $mg.L^{-1}$, for carbofuran and chlorfenvinphos respectively.

The criteria that allow the verification of the adequacy of a method, were also determined [148]. The relative standard deviations of the slope were of 0.8% for carbofuran and 0.6% for chlorfenvinphos. Correlation coefficients of 0.9996 were obtained for each pesticide. Furthermore, it was verified, for both pesticide calibration curves, that the confidence limits for the interception contain the origin. The calibration curves obtained for carbofuran and chlorfenvinphos can, therefore, be considered adequate for the purpose of analysis.

The method showed to be precise for the analysis of both pesticides, as can be confirmed by the %RSD lower than 10%, even for the analysis at lower concentrations.

2.2.3.1.2 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value found and the reference (or true) value. In this work, accuracy was evaluated through recovery assays, using different spiked samples at three levels (0.1, 1 and 10 $mg.L^{-1}$). These test were performed for carbofuran and chlorfenvinphos, in four distinct water matrices.

Since this method was intended to be used for the detection and quantification of both pesticides in water, in the case of a contamination event, several recovery tests were considered. Usually, the water flowing in the pipes is in direct contact with the deposits there formed. These deposits may have different characteristics, depending also on the type of pipe and water composition of the specific region. According to Echeverría, et al. [149], these deposits can be classified into three categories, namely: brown, tubercle and white deposits. Thus, besides the recovery assays performed with tap water, others were performed with water that has been in contact with different deposits, which had been recovered from water distribution pipes. Additionally, recovery tests were also made with water in contact with clay, due to its later utilization in several experiments included in Chapter 3. Thus, it was important to verify if leached compounds from the deposits and clay could interfere with the analysis of carbofuran and chlorfenvinphos, by the DI-LC-DAD method.

The results obtained in these experiments are condensed in Table 2.9.

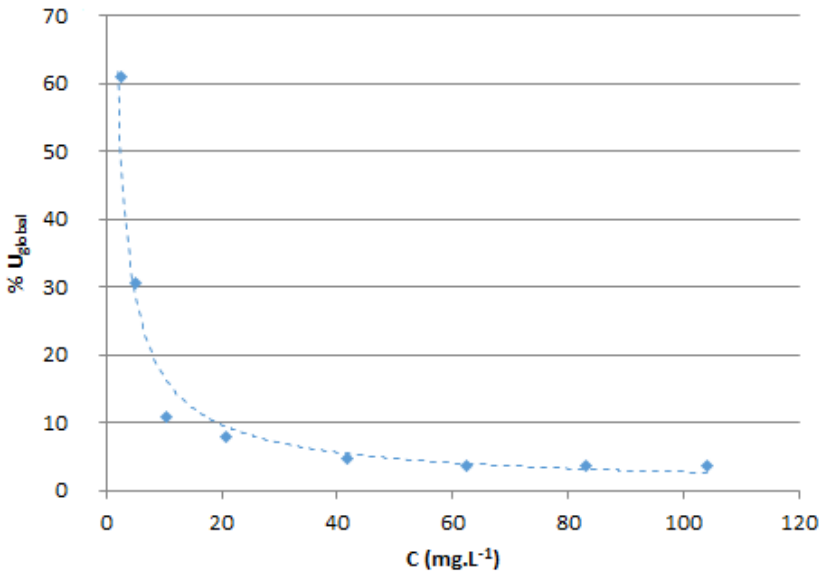
Table 2.9 – Recovery values obtained with the DI-LC-DAD method.

	Recovery (%) (n=3)			
	Tap water	**S3**	**S4**	**Clay**
Carbofuran				
0.1 ($mg.L^{-1}$)	92±4	109±14	99±6	97±4
1 ($mg.L^{-1}$)	97.5±0.3	95±1	94±1	88±1
10 ($mg.L^{-1}$)	109.1±0.1	108.1±0.2	103±4	95±1
Chlorfenvinphos				
0.1 ($mg.L^{-1}$)	102±8	95±21	88±19	112±9
1 ($mg.L^{-1}$)	88±16	102±1	101±4	105±1
10 ($mg.L^{-1}$)	103.8±0.2	86.1±0.3	77±4	74±1

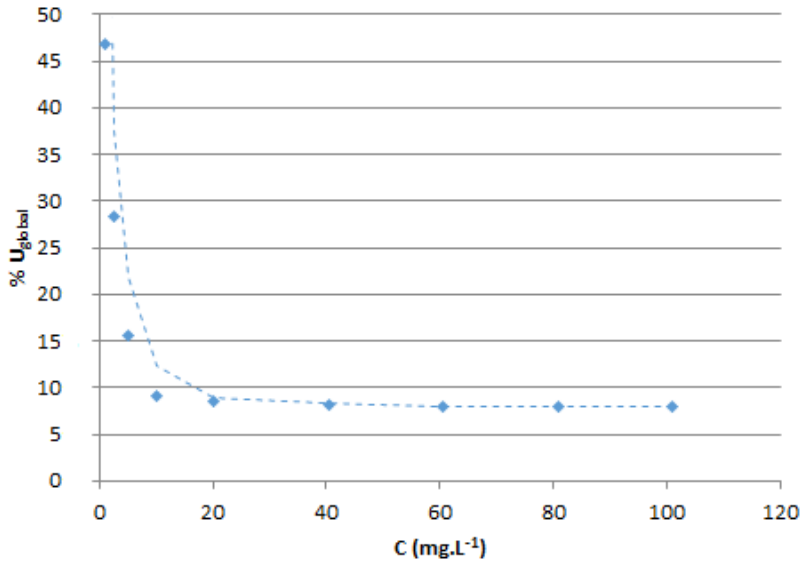
As a whole, the recovery percentage values were satisfactory. An average recovery of 99, 104, 99 and 93% was obtained for carbofuran in tap water, S3 deposit, S4 deposit and clay, respectively. Similarly, the average recoveries of 98, 94, 89 and 97% were obtained for chlorfenvinphos in tap water, S3 deposit, S4 deposit, and clay, respectively. The results are acceptable and demonstrate that this method can be used in the analysis of water from drinking water distribution systems.

2.2.3.1.3 Global uncertainty

The global uncertainty (U) associated to this study was evaluated through the *bottom-up* approach/EURACHEM procedure, described by Ratola, et al. [150]. Global uncertainty for the different levels of concentration of each pesticide are represented in Figure 2.5.



(a)

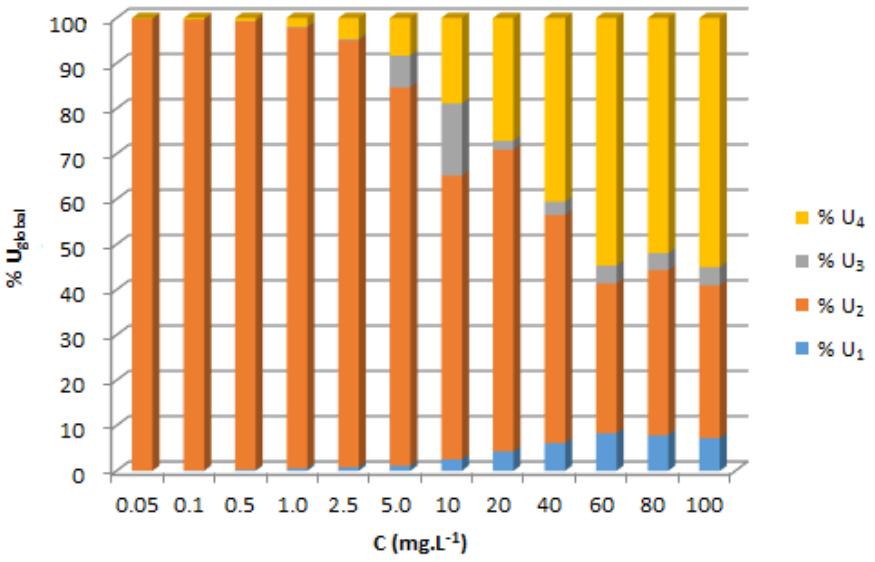


(b)

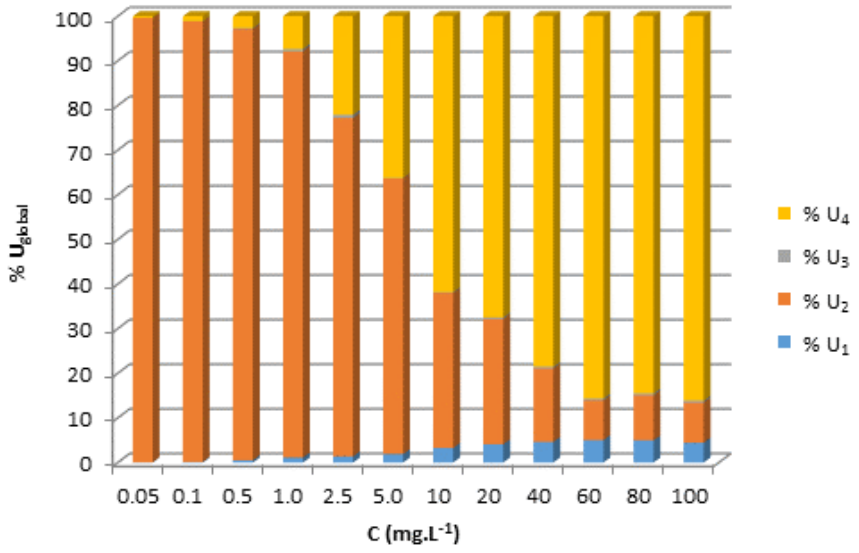
Figure 2.5 – Global uncertainty of the DI-LC-DAD method for (a) carbofuran and (b) chlorfenvinphos quantification in waters.

As can be observed, an almost constant global uncertainty is reached for intermediate and higher concentration levels. However, for the lower levels of the calibration range the global uncertainty rises, even reaching values higher than 100%. Thus, in Figure 2.5 only the global uncertainty for concentrations higher than 2.5 $mg.L^{-1}$

for carbofuran and 1 mg.L^{-1} for chlorfenvinphos are represented. This approach also enables the evaluation of the weight of the individual sources of uncertainty, clarifying which are more relevant, as can be seen in Figure 2.6.



(a)



(b)

Figure 2.6 – Relative weights of each individual source of uncertainty of the DI-LC-DAD method for (a) carbofuran and (b) chlorfenvinphos analysis in waters.

As observed in Figure 2.6, it was considered that the uncertainty could come from four sources [150]: the uncertainty associated with standard preparation (U_1); the uncertainty associated with the calibration curve (U_2); the uncertainty associated with precision (U_3); and the uncertainty associated with accuracy (U_4). Observing the distribution of uncertainty for carbofuran, it appears that the uncertainty associated with the calibration curve (U_2) has high importance throughout the whole range, but is especially important at the lowest levels of concentration. The uncertainty associated with accuracy (U_4) has some importance at the higher levels of the calibration range, but its relative importance reduces as the lower concentrations are reached. For carbofuran as for chlorfenvinphos, the uncertainty associated with the standards preparation (U_1) is more relevant for the higher levels of concentration, and progressively diminishes with the concentration. The global uncertainty distribution for chlorfenvinphos also shows that the relative importance of U_4 is much higher, particularly for high concentrations, than that observed in carbofuran distribution. This indicates a higher difficulty for chlorfenvinphos analysis in matrices with some interferents. As for carbofuran, the main source of uncertainty at low levels of concentration is the calibration curve (U_2). This fact was expected, since at lower concentration levels the contribution of precision (U_3) for the global uncertainty is inconstant and not very relevant for carbofuran; for chlorfenvinphos, the relative contribution is almost inexistent, which indicates a high precision of the method for the analysis of this compound. Hence, it was verified that the relative contribution of these four sources is definitively dependent on the calibration levels, being the uncertainty associated to the calibration curve (U_2) the main responsible for such variation, for the two pesticides.

2.3 Method for detection and quantification of carbofuran and chlorfenvinphos in waters by DLLME-GC-MS *

2.3.1 Introduction

Pesticides, including carbamate and organophosphorus pesticides, have been widely used in agriculture pest control with the objective of increasing agricultural productivity [100]. Due to their widespread distribution and persistent properties, pesticides have become an important class of emerging pollutants, presenting serious risks to human and animal health [24]. As stated before, due to the pesticides toxicity, the European Union establishes maximum concentrations of 0.1 $\mu g.L^{-1}$ for individual pesticides and 0.5 $\mu g.L^{-1}$ for the sum of all pesticides in water destined to human consumption [152].

Carbofuran is a systemic pesticide widely used in several agricultural crops as insecticide, nematicide and acaricide [153], while chlorfenvinphos is applied as insecticide and acaricide [154]. They are powerful cholinesterase inhibitors and, therefore, they are highly toxic for both wildlife and humans. The extensive use of these compounds and their accumulation in the environment demands the development of highly sensitive, fast and simple analytical methods for the determination of their trace levels to ensure water and food quality and to protect consumers against possible health risks [52].

Due to the low concentrations ($\mu g.L^{-1}$ or less) and complex matrices in which they are found, these compounds cannot be directly analysed with conventional methods such as liquid chromatography (LC), liquid chromatography coupled with mass spectrometry (LC-MS) or gas chromatography coupled to mass spectrometry (GC-MS). Thus, there is the need of an extraction/preconcentration step before their final determination. Despite being one of the oldest preconcentration and matrix isolation techniques in analytical chemistry [155], conventional liquid-liquid extraction (LLE) is still

* Adapted from (except section 2.3.4.5): [151] Sousa, R., V. Homem, J.L. Moreira, L.M. Madeira, and A. Alves, *Optimisation and application of dispersive liquid-liquid microextraction for simultaneous determination of carbamates and organophosphorus pesticides in waters.* Analytical Methods, 2013. 5(11): p. 2736-2745.

used in sample preparation for the determination of pesticides [156-158]. However, recent analytical methods have focused on minimizing sample reagent consumption (and thus cost of the analysis), maintaining high selectivity and recoveries, and speeding up the sample treatment process. Several reports of other preconcentration and quantification techniques for the analysis of pesticides in water have thus emerged, such as solid-phase extraction (SPE) [33, 36, 67, 159-161], solid-phase microextraction (SPME) [24, 30, 96, 162] and liquid-phase microextraction (LPME) [155, 163].

In 2006, a new microextraction technique was introduced by Assadi and co-workers [97]. This method was named dispersive liquid-liquid microextraction (DLLME) and is a very simple, rapid and cost-efficient process for the extraction of organic compounds from water samples. The basic principle of DLLME is the dispersion of an appropriate mixture of the extraction solvent (immiscible with water) and dispersive solvent (miscible both in water and in the extraction solvent) within an aqueous solution. The rapid injection of the solvents in the sample generates a cloudy solution, consisted of tiny droplets dispersed among the aqueous solution, generating a very high contact area between the extraction solvent and the aqueous sample [54, 99, 108]. Since its development, DLLME has been successfully applied in the extraction and determination of several analytes, such as polycyclic aromatic hydrocarbons (PAHs) [97, 164], polybrominated diphenyl ethers (PBDEs) [165], phthalate esters (PEs) [166], polychlorinated biphenyls (PCBs) [167, 168], metals [169], benzene, toluene, ethylbenzene and xylenes (BTEX) [170], pyrethroids [171], organosulfur pesticides (OSPs) [172], organophosphorus pesticides (OPPs) [100, 112] and carbamate pesticides [51-53, 55].

Carbofuran and chlorfenvinphos have already been extracted by DLLME in several matrices, though it wasn't found any study in which they were analyzed simultaneously. Table 2.10 displays the main studies found, involving the determination of carbofuran by DLLME in aqueous matrices (some studies already mentioned in Table 2.1). To the author best knowledge, there are no published studies of chlorfenvinphos extraction by DLLME in water matrices. Effectively, because these compounds belong to two different classes of pesticides, it is expected that they have different chemical behavior. Therefore, chlorfenvinphos and carbofuran could act as model molecules of each class

of pesticides, namely organophosphorus and carbamate. So, this study will allow the extension of this type of analysis to other compounds of the same chemical family.

Table 2.10 – Studies found regarding carbofuran extraction from water samples by DLLME.

Analyte	Separation Technique	Extraction Conditions			LOD ($\mu g.L^{-1}$)	Reference (Year)
		Sample Volume (mL)	Extraction Solvent	Dispersive Solvent		
4 carbamate pesticides	LC-DAD	5	Trichloromethane	Acetone	1	[51] (2009)
5 carbamate pesticides	LC-DAD	5	Trichloromethane	Acetonitrile	0.4	[52] (2009)
3 carbamate pesticides	LC-UV	5	Chlorobenzene	Acetonitrile	0.9	[53] (2009)
3 multiclass pesticides	LC-MS/MS	5	Carbon tetrachloride	Acetonitrile	0.02 (LOQ)	[54] (2010)
Carbamate pesticides	GC-MS/MS	5	Toluene	Acetonitrile	0.008	[55] (2010)
3 carbamate pesticides	LC-UV	5	Trichloromethane	Acetonitrile	0.5	[57] (2011)
Multiresidue	LC-MS/MS	10	Octanol	Acetone	0.125 (LOQ)	[59] (2015)

LOD – limit of detection; LOQ – limit of quantification

On the other hand, due to the distinctive chemical behavior, the optimal extraction conditions for each compound may be different. Therefore, it is important to define a compromise in order to enable both carbofuran and chlorfenvinphos determination in a single procedure. Usually, the optimal extraction conditions are defined based on a single-factor-at-a-time approach, analysing the effects of each variable independently and keeping all other conditions constant. However, this methodology does not take into account the interaction between variables and, consequently, their effect on the process response. The application of an experimental design methodology (DoE – Design of Experiments) can overcome these disadvantages (multivariate analysis).

An analytical method for the simultaneous evaluation of two pesticides, carbofuran and chlorfenvinphos, in aqueous samples by DLLME followed by GC-MS analysis was developed and validated. The optimisation of some important DLLME parameters (type and volume of extraction and dispersive solvents, sample volume, extraction time, and use of NaCl) was performed using a DoE approach. This methodology was validated and

employed to the determination of the target compounds in spiked real water samples. Lastly, a comparison was made between the application of DLLME and SPE procedures for the identification and quantification of carbofuran and chlorfenvinphos in aqueous samples. The main objective was to obtain a rapid and reliable method which, in case of a deliberate contamination, is capable of unmistakably identify and quantify both pesticides in water, up to the levels required by European legislation.

2.3.2 Experimental design and process optimization

As mentioned above, the definition of the optimal process conditions has been traditionally done using a single-factor-at-a-time approach, which does not consider the interaction between variables. The application of statistical experimental design and response surface methodology (RSM), a multivariate statistical approach, provides a new perspective for the optimization of a process response. These methodologies are useful to determine the effects of different factors and of their interactions in the process response within a certain range and with a minimum number of experiments. However, when the number of factors that may influence the response is too high, it is usual to previously develop a screening design. This kind of approach is an efficient way to identify the most significant effects and separate them from the less important ones, reducing the number of factors that should be studied in the RSM.

The optimization procedure involves studying the response of the statistically significant factors, estimating the coefficients by fitting the experimental data to the response functions, predicting the response of the fitted model and checking the adequacy of the model [173-175]. When the response surface methodology is applied, a mathematical relationship between dependent and independent variables is established. Usually, a second-order polynomial equation is applied, i.e.:

$$\boldsymbol{Y = b_0 + \sum_{i}^{n} b_i x_i + \sum_{i}^{n} b_{ii} x_i^2 + \sum_{j>i}^{n} \sum_{i=1}^{n} b_{ij} x_i x_j} \qquad 2.3$$

where *Y* refers to the process response, x_i to the codified independent variable, b_0 to the interception term, b_i is the influence of the variable *i* in the response, b_{ii} is a

parameter that determines the shape of the curve and b_{ij} corresponds to the effect of the interaction among variables *i* and *j*.

The natural variables (X_i) must be converted into dimensionless codified values (x_i):

$$x_i = \frac{(X_i - X_0)}{\Delta X} \qquad 2.4$$

where X_0 denotes the value of variable *i* in the center of the domain ($x_i=0$) and ΔX refers to the difference of that variable between $x_i=+1$ and $x_i=0$.

The statistical analysis continues with an ANOVA test, which evaluates the model fitting adequacy. If the F-ratio obtained is higher than the Fisher's F-value (similarly if F-probability is less than 0.05 for a 95% confidence level), then the response variation can be attributed to the model, not to random errors. In order to determine the parameters and/or interactions with statistical meaning it is usual to use the Student's t-test. So, if t-probability is smaller than 0.05, the parameter or interaction is considered to be significant.

In this study, a L_{27} (3^{13}) Taguchi fraction factorial design was employed as screening design to test the factors that would influence the process response. Then, the significant variables were optimized using a central composite factorial design (CCD), considering the peak areas in the GC-MS as response. Experimental data analysis was developed using the JMP 5.0.1 software.

2.3.3 Experimental section

2.3.3.1 Chemicals and reagents

Carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate), with a purity of 99.9%, and chlorfenvinphos standard ([EZ] -2-chloro-1-(2,4-dichlorophenyl)ethenyl] diethyl phosphate) were purchased from Fluka, Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), acetone (AC), methanol (MeOH), chlorobenzene (CB), carbon tetrachloride (TCC) and tetrachloroethylene (TCE) were HPLC grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3.3.2 Standards preparation

The individual stock solutions of carbofuran and chlorfenvinphos were prepared in methanol at a concentration of 50 $mg.L^{-1}$. Mixture standards of carbofuran and chlorfenvinphos were prepared by diluting an appropriate amount of each of the stock solutions in distilled water. All solutions were stored at 4 °C.

To evaluate the accuracy and applicability of the proposed method, the extraction of carbofuran and chlorfenvinphos in different water samples (tap, mineral and river waters) was performed. River waters were collected from different areas in the northern region of Portugal. Tap water and mineral water (plain and sparkling) samples were taken from our laboratory (LEPABE/FEUP) and local supermarket in Porto, respectively. The river and tap water samples were stored in amber glass bottles, kept at -20 °C and protected from light until they were processed. Before the extraction, water samples were filtered using VWR quantitative filter papers with particle retention between 5-10 µm (West Chester, USA). It was verified that these filters do not exhibit retention towards the compounds studied. Besides that, mineral sparkling water samples were degassed on ultrasound before being processed.

2.3.3.3 DLLME procedure

For the optimized DLLME, 10 mL of sample or standard was placed in a 15.0 mL plastic tube with conical bottom. 500 µL of methanol (dispersive solvent) were blended with 80 µL of chlorobenzene (extraction solvent); the mixture was rapidly injected into the sample solution using a syringe, at room temperature, and then vortexed for 4 min. The resultant cloudy solution was centrifuged for 5 min at 3000 rpm. After the centrifugation process, the sedimented phase was removed and 1 µL was injected in the GC-MS for analysis.

2.3.3.4 SPE procedure

With the purpose of comparing with DLLME, three types of SPE cartridges (with different sorbents) were tested: LC–18 (non–polar silica based – Supelco; 6 mL, 1 g), HR–P (polymeric polystyrene-divinylbenzene resin based – Chromabond; 3 mL, 200 mg) and Oasis HLB (copolymeric reversed–phase – Waters; 6 mL, 200 mg). The extraction conditions used for each SPE cartridge were the recommended by the suppliers.

One extraction methodology for LC–18 and HR–P cartridges and another for Oasis HLB cartridges were used. Each LC–18 and HR–P cartridge was previously conditioned with 5 mL of methanol followed by 5 mL of water. After the conditioning step, the aqueous sample (15 mL) was passed through the cartridge at a flow rate of 1 mL.min^{-1}. Then, the retained compounds were eluted with 5 mL of methanol at 1 mL.min^{-1}. The eluent was passed through a drying cartridge (Chromafix; Dry (L)) and evaporated to dryness under a gentle stream of nitrogen. The dry residues were re–dissolved in 1000 µL of ethyl acetate.

Oasis HLB cartridges were conditioned with 5 mL of ethyl acetate followed by 5 mL of methanol and 5 mL of water. Then, the aqueous sample was passed at a flow rate of 1 mL.min^{-1}, followed by a washing step (6 mL of water). Elution was performed with 2x4 mL of ethyl acetate (1 mL.min^{-1}). The eluent was passed through a drying cartridge (Chromafix; Dry (L)) and evaporated to dryness under a gentle stream of nitrogen. The dry residues were re–dissolved in 1000 µL of ethyl acetate. Afterwards 1 µL was injected into GC–MS (the same volume was used for the other cartridges).

2.3.3.5 Instrumentation

A Varian 4000 GC-MS apparatus (Walnut Creek, CA, USA), equipped with an ion trap mass detector, was used. The injection port was kept at 290 °C. For the separation of the analytes, the gas chromatograph was equipped with a DB-5MS 30 m x 0.25 mm i.d. x 0.25 µm film thickness column (Walnut Creek, CA, USA). Helium (purity 99.9999%) was employed as carrier gas with a constant flow of 1 mL.min^{-1}. The column temperature

was held at 130 °C for 2 minutes, then programmed to increase at 20 °C.min^{-1} to 275 °C, and held for 1 min. The MS transfer line temperature was kept at 250 °C. The mass spectrometer was operated in the electronic ionization (EI) mode. Monitoring ions in the selected ion-storage (SIS) mode are listed in Table 2.11.

Table 2.11 - Quantification and qualifier ions of each individual compound studied in the GC-MS and respective retention times.

Compound	Retention time (min)	Quantification ion (*m/z*)	Qualifier ions (*m/z*)
Carbofuran	6.549	164	149, 131
Chlorfenvinphos	8.274	323	267, 269

2.3.4 Results and discussion

In this study, DLLME combined with GC-MS was applied to determine simultaneously carbofuran and chlorfenvinphos in water samples. In DLLME several variables affect the extraction recovery. In order to obtain the optimum experimental extraction conditions a two-step design (Taguchi screening design and CCD for optimization) was used.

2.3.4.1 Screening design

As mentioned above, several factors can affect the DLLME extraction procedure such as type of extraction and dispersive solvents, solvents volumes, ionic strength, etc. A screening study was thus implemented to determine which are the most important factors involved in this extraction. Seven factors were selected: volume of the extraction solvent (X_1), volume of the dispersion solvent (X_2), extraction solvent (X_3), dispersion solvent (X_4), sample volume (X_5), ionic strength (X_6) and extraction time (X_7). The extractions were tested using a concentration of each pesticide of 50 µg.L^{-1}, which is an intermediate level of concentration in the range of linearity.

In DLLME, the extraction solvent should have: (a) higher density than water; (b) good chromatographic behavior; (c) low solubility in water; (d) high extraction capability for the analytes of interest and (e) should form a stable two-phase system in the presence of a dispersive solvent when injected into an aqueous solution [53, 108]. Based on these considerations, chlorobenzene, carbon tetrachloride and tetrachloroethylene were chosen for this study. For the selection of the dispersive solvents, the main point is its miscibility in the organic phase (extraction solvent), and in the aqueous phase (sample solution) [108]. Acetone, acetonitrile and methanol, which have these characteristics, were selected for this purpose.

It is expected that extraction and dispersion volumes may affect the results. Lower volumes of extraction solvent should enhance the extraction efficiency by reducing the sedimented phase. However, it is necessary to reach a compromise between the minimum quantity required for injection into the GC-MS and the enrichment factor. The volume of dispersive solvent directly affects the formation of the cloudy solution (water/dispersive solvent/extraction solvent) and the degree of dispersion of the extraction solvent into the aqueous phase and, consequently, the efficiency of extraction. It is expected that the cloudy formation may not be stable and may cause incomplete extraction if low volumes of dispersive solvent are used [176].

Sample volume is another factor that affects the extraction efficiencies in microextraction techniques. The effects of sample volume are not only a function of the amount of analytes in solution, but also of the analytes solubility in the extraction phase. However, the solubility of the target analytes and of the organic extraction solvent in the aqueous phase are usually decreased with the increase of ionic strength, which is favorable for reaching high recovery [26]. Therefore, the ionic strength effect was also tested.

Finally, the extraction time was also investigated. In DLLME, extraction time is defined as the interval between injecting the mixture of dispersive and extraction solvents and centrifugation. Usually, a short extraction time is enough to reach the equilibrium due to the high contact area originated by the homogeneous dispersion of the extraction solvent after the formation of the cloudy solution. Thus, the transition of the analyte from the aqueous to the extraction phase can be very fast [177].

A L_{27} Taguchi fraction factorial design was selected to determine the influence of these factors on the extraction, using the peak areas as the response. The main effects were determined by the probability (F-probability) calculated for each factor. The investigated range and the p-values for each factor are shown in Table 2.12.

Table 2.12 – Experimental factors (X), levels involved and F-probability obtained with Taguchi fraction design.

Factors	Coded levels (x_i)			F-probability	
	-1	**0**	**1**	**Carbofuran**	**Chlorfenvinphos**
X_1. Volume of the extraction solvent	60	80	100	**0.02**	**0.03**
X_2. Volume of the dispersion solvent	50	750	1000	0.47	0.15
X_3. Extraction solvent	CB	TCC	TCE	**<0.0001**	0.12
X_4. Dispersion solvent	AC	ACN	MeOH	0.11	**0.01**
X_5. Sample volume (mL)	8	10	12	**0.03**	**<0.0001**
X_6. Ionic strength (% w/w NaCl)	0	2.5	5	0.11	0.70
X_7. Extraction time (min)	0	5	10	0.11	**0.02**

CB – chlorobenzene; TCC – carbon tetrachloride; TCE – tetrachloroethylene; AC – acetone; ACN – acetonitrile; MeOH – methanol

A F-probability ≤ 0.05 represents a significant effect on the extraction efficiency, whereas 0.05 < F-probability ≤ 0.10 indicates relative effect on the extraction. As can be seen, for carbofuran three factors have a strong effect on its extraction (type and volume of extraction solvent and sample volume – shown in bold). With regard to chlorfenvinphos, four factors influence significantly the response (volume of extraction solvent, type of dispersive solvent, sample volume and extraction time).

Among the factors identified, two are discrete variables (nature of extraction and dispersion solvent) and must be defined prior to the optimization using CCD. Therefore, after this exploratory runs, only three factors were assessed applying response surface methodology: volume of the extraction solvent, sample volume and extraction time. The other factors were fixed accordingly to the Student's t-test, i.e., a compromise has been established between the results obtained for both pesticides, choosing the lowest t-probability (data not shown). Hence, the subsequent experiments were performed using

chlorobenzene and methanol as extraction and dispersion solvents, respectively, 500 µL of dispersion solvent and 0% w/w of NaCl.

2.3.4.2 Central composite design

After the screening design, a central composite design was applied (Table 2.13). The experiments performed are summarized in Table 2.14, as well as the responses based on the experimental runs and the predicted values. Four assays were performed in the center of the cubic domain (runs 6, 8, 12 and 18), providing a relative standard deviation of 9% for carbofuran and 5% for chlorfenvinphos, which correspond to acceptable values in this kind of experiments.

Table 2.13 – Experimental range and levels of process variables for the CCD.

Parameter	**Coded levels (x_i)**				
	-1.682	**-1**	**0**	**+1**	**+1.682**
$V_{\text{extraction solvent}}$ (µL)	60	68	80	92	100
V_{sample} (µL)	2	4	7	10	12
$t_{\text{extraction}}$ (min)	0	2	5	8	10

Table 2.14 – Experimental design and response (peak area) based on experimental data and predicted values (±standard deviation) proposed by the CCD (X_1 – volume of extraction solvent (μL), X_2 – sample volume (μL), X_3 – extraction time (min)).

Run	Pattern	X_1	X_2	X_3	Carbofuran		Chlorfenvinphos	
					Exp (x10^6)	Pred (x10^6)	Exp (x10^6)	Pred (x10^6)
1	+++	92	10	8	10	9±1	31	28±4
2	---	68	4	2	7	7±1	17	18±4
3	+-+	92	4	8	6	4±1	17	11±4
4	-++	68	10	8	12	10±1	36	32±4
5	00a	80	7	0	7	7±1	23	20±4
6	000	80	7	5	10	10±1	25	25±3
7	+--	92	4	2	8	9±1	14	17±4
8	000	80	7	5	11	10±1	25	25±4
9	-+-	68	10	2	11	11±1	33	37±4
10	--+	68	4	8	3	4±1	2	4±4
11	0a0	80	2	5	4	4±1	8	7±4
12	000	80	7	5	9	10±1	23	25±3
13	00A	80	7	10	1	3±1	4	10±4
14	a00	60	7	5	11	11±1	33	30±4
15	A00	100	7	5	10	12±1	21	26±4
16	0A0	80	12	5	10	12±1	33	37±4
17	++-	92	10	2	13	11±1	28	25±4
18	000	80	7	5	11	10±1	26	25±3

As mentioned above, using the response surface methodology a mathematical relationship between dependent and independent variables was determined. The experimental data were fitted to a second-order polynomial equation and the coefficients of the quadratic model were calculated by a least-square regression analysis. The comparison between the model prediction and the experimental response is given in the parity plot of Figure 2.7. As can be seen, the values predicted by the second-order model agree very reasonably with the experimental data. Table 2.15 shows the second-order equations and the model suitability using the ANOVA test. The mean squares were determined dividing the sum of squares for each variation source by their degrees of freedom (DF). The model F-ratio was obtained by dividing the model mean square by the residual mean square.

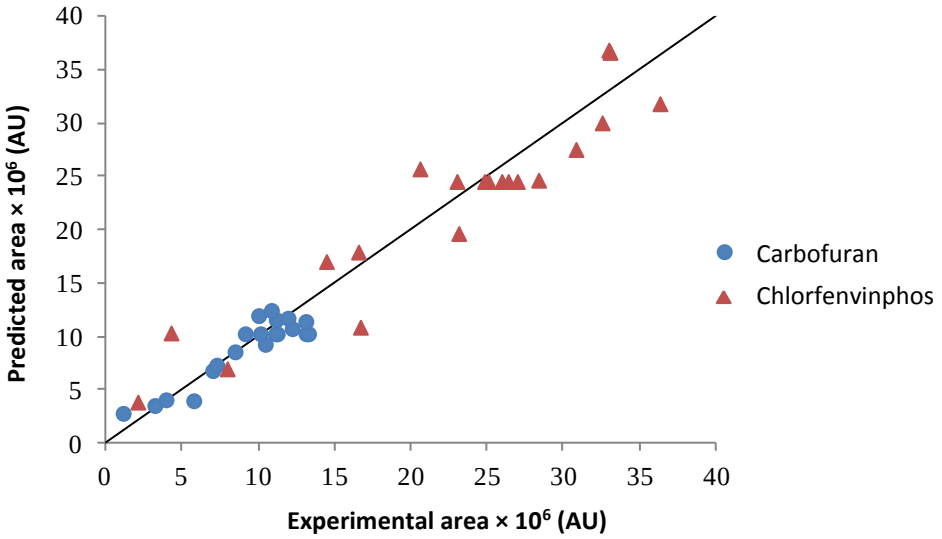


Figure 2.7 – Parity plot comparing the process response with the model predictions.

Table 2.15 – Second-order polynomial equation and ANOVA test for the response functions.

Compound	Source	DF	Sum of squares	Mean square	F-ratio	Prob>F
Carbofuran						
$Y=1.0x10^{7}+7.2x10^{4}x_1+2.5x10^{6}x_2-1.4x10^{6}x_3-4.8x10^{5}x_1x_2-3.3x10^{5}x_1x_3+6.1x10^{5}x_2x_3+5.7x10^{5}x_1^2-7.2x10^{5}x_2^2-1.9x10^{6}x_3^2$						
(R^2 = 0.89)						
	Model	9	$1.74x10^{14}$	$1.93x10^{13}$	7.01	0.0058
	Error	8	$2.21x10^{13}$	$2.76x10^{12}$		
	Lack of fit	5	$1.92x10^{13}$	$3.84x10^{12}$		
	Pure error	3	$2.90x10^{12}$	$9.65x10^{11}$		
Chlorfenvinphos						
$Y=2.4x10^{7}-1.3x10^{6}x_1+8.9x10^{6}x_2-2.8x10^{6}x_3-2.8x10^{6}x_1x_2+2.0x10^{6}x_1x_3+2.3x10^{6}x_2x_3+1.2x10^{6}x_1^2-9.8x10^{5}x_2^2-3.4x10^{6}x_3^2$						
(R^2 = 0.88)						
	Model	9	$1.53x10^{15}$	$1.70x10^{14}$	6.64	0.0069
	Error	8	$2.05x10^{14}$	$2.57x10^{13}$		
	Lack of fit	5	$4.56x10^{12}$	$4.01x10^{13}$		
	Pure error	3	$2.05x10^{14}$	$1.52x10^{12}$		

Comparing the F-ratio obtained with the Fisher's F-value (F-ratio>$F_{9,8}$=3.39), it can be concluded that variations that occur in the responses for both pesticides should be

associated to the model, rather than to experimental error. A similar conclusion can be drawn through the F-Probability values (Prob-F<0.05).

Subsequently, the significant variables and interactions were identified by the Student's t-test (bold values in Table 2.16). It was considered that a variable is very significant if the Prob>|t| was less than 0.05 and relatively significant if Prob>|t| was greater than 0.05 and less than 0.10 (95% confidence level). Therefore, sample volume (x_2) and extraction time (x_3, x_3^2) were the most significant variables for both pesticides.

Table 2.16 – Results from the Student's t-test to determine the significant variables.

	Term	**t-ratio**	**Prob>|t|**		**Term**	**t-ratio**	**Prob>|t|**
Carbofuran				**Chlorfenvinphos**			
Main effects	Intercept	12.42	<0.0001	Main effects	Intercept	9.72	<0.0001
	b_1	0.16	0.8771		b_1	-0.94	0.3747
	b_2	**5.56**	**0.0005**		**b_2**	**6.46**	**0.0002**
	b_3	**-3.00**	**0.0101**		**b_3**	**-2.03**	**0.0767**
Interactions				Interactions			
	b_{12}	-0.82	0.4373		b_{12}	-1.58	0.1533
	b_{13}	-0.56	0.5927		b_{13}	1.10	0.3021
	b_{23}	1.04	0.3282		b_{23}	1.26	0.2432
Quadratic effects				Quadratic effects			
	b_{11}	1.20	0.2627		b_{11}	0.83	0.4290
	b_{22}	-1.52	0.1665		b_{22}	-0.68	0.5151
	b_{33}	**-3.95**	**0.0042**		**b_{33}**	**-2.38**	**0.0446**

Three-dimension response surface and two-dimension contour plots of the predicted responses were also obtained using the JMP software (considering only the significant parameters). These plots are represented in Figure 2.8 and show the response surface obtained by plotting peak area vs. sample volume and extraction time, with the extraction volume fixed at 80 µL. As can be seen, the maximum response for carbofuran was obtained for a sample volume higher than 10 mL and an extraction time

between 4 and 6 min. On the other hand, the maximum response for chlorfenvinphos was achieved for a volume greater than 10 mL and an extraction time between 2 and 7 min. These results are in accordance with what was referred previously. Therefore, the following were established as best conditions for the simultaneous carbofuran and chlorfenvinphos extraction: 80 µL of chlorobenzene, 500 µL of methanol, 10 mL of aqueous sample and 4 min of extraction.

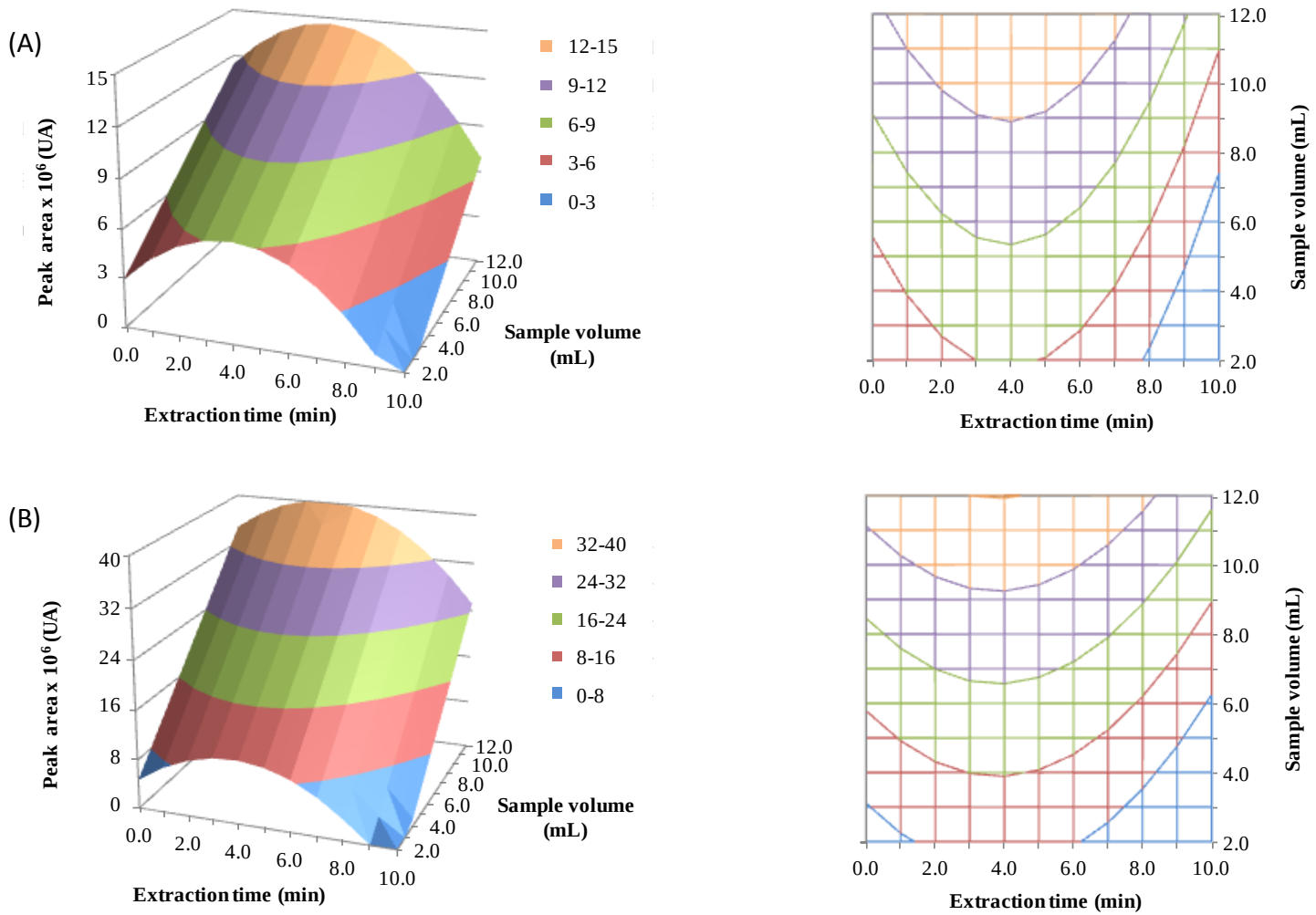


Figure 2.8 – Response surface and contour plots for (A) carbofuran and (B) chlorfenvinphos (50 µg.L^{-1} carbofuran and chlorfenvinphos, 80 µL chlorobenzene, 500 µL methanol).

2.3.4.3 Method validation

In order to evaluate the developed DLLME-GC-MS methodology, the limits of detection and quantification, the linearity, precision and accuracy, as well as the global uncertainty, were evaluated.

2.3.4.3.1 DLLME-GC-MS linearity, limits of detection and quantification and precision

Under the selected conditions, the proposed method was evaluated in terms of linearity range, correlation coefficient, limit of detection (LOD) and quantification (LOQ) and precision. The limits of detection and quantification were calculated based on the signal to noise ratio (S/N) of individual peaks, assuming a ratio of 3:1 to LODs and 10:1 for the LOQs [178]. Precision was evaluated by a repeatability study of extracted aqueous standard samples at three different concentration levels (1, 50 and 250 $\mu g.L^{-1}$ for carbofuran and 1, 50 and 200 $\mu g.L^{-1}$ for chlorfenvinphos). The results are presented in Table 2.17.

Table 2.17 – Linearity results, detection and quantification limits and precision (% RSD) for each compound studied.

	Carbofuran	**Chlorfenvinphos**
Calibration Range ($\mu g.L^{-1}$)	0.1-250	0.1-200
R^2	0.9987 (N=9)	0.9987 (N=8)
LOD ($\mu g.L^{-1}$)	0.04	0.02
LOQ ($\mu g.L^{-1}$)	0.14	0.07
% RSD *(n = 12)*		
1 ($\mu g.L^{-1}$)	18.8	19.3
50 ($\mu g.L^{-1}$)	9.5	7.0
250 ($\mu g.L^{-1}$)	8.0	12.2*

*For chlorfenvinphos the maximum concentration was 200 $\mu g.L^{-1}$
R^2: determination coefficient; N: number of calibration standards; n: number of replicates; LOD: limit of detection; LOQ: limit of quantification; %RSD: relative standard deviation percentage.

It can be seen that the linear range of calibration curves of carbofuran and chlorfenvinphos using DLLME ranged from 0.1 to 250 $\mu g.L^{-1}$ and 0.1 to 200 $\mu g.L^{-1}$, respectively. The LOD and LOQ were 0.04 $\mu g.L^{-1}$ and 0.14 $\mu g.L^{-1}$for carbofuran, and for chlorfenvinphos 0.02 $\mu g.L^{-1}$ and 0.07 $\mu g.L^{-1}$, respectively. Overall, the detection limits using this methodology were lower than those found in the literature (Table 2.10). Moreover, these results show that the developed method is capable of identify and quantify both pesticides in water, up to the levels required by European legislation (0.1 $\mu g.L^{-1}$) [152]. The relative standard deviation (RSD) values ranged from 8.0 to 18.8% for carbofuran and from 7.0 to 19.3% for chlorfenvinphos. These results show a satisfactory precision of the extraction methodology, for both pesticides.

Therefore, these results demonstrate that DLLME procedure can be applied prior to the chromatographic analysis, allowing pesticide determination at low levels of concentration.

2.3.4.3.2 Real samples analysis

In order to evaluate the accuracy and applicability of the proposed method, the extraction and determination of carbofuran and chlorfenvinphos was performed in several aqueous matrices. For this study it was used tap, river and mineral (plain and sparkling) water. To check the presence of interferences due to the matrix, each water sample was spiked at two concentration levels (50 and 200 $\mu g.L^{-1}$) and two replicate experiments were performed for each level. Accuracy results, expressed through analytical recovery tests (the observed value divided by the expected value) are presented in Table 2.18. The lowest recoveries were observed in the sparkling water 1 (ranging from 58-85%). These results might be explained by the high concentration of carbon dioxide in that water, though a degasification (by ultrasound) of each sparkling water was made. The recoveries for the extraction in the other matrices were in the range of 75-120% (average value of 98.2% was reached). Given the results obtained, it can be assumed that there is no relevant matrix effect.

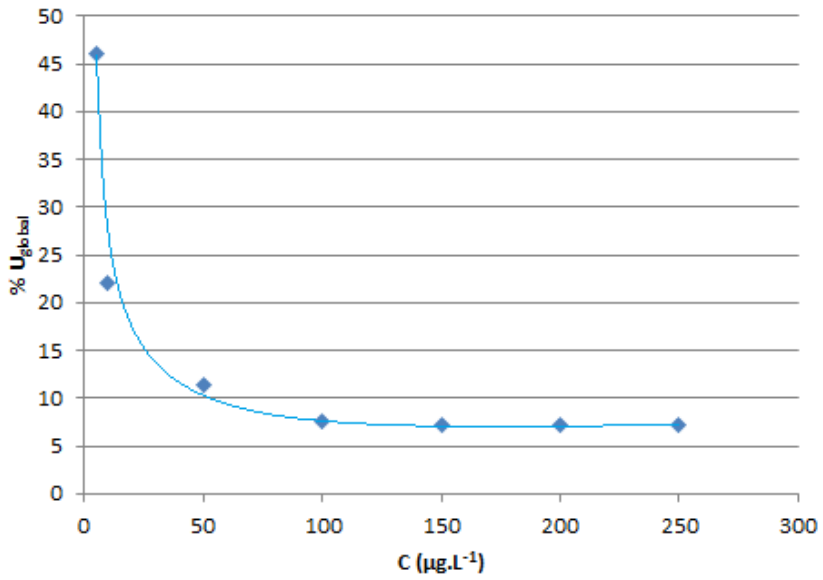
Table 2.18 – Average recoveries of carbofuran and chlorfenvinphos in water samples at different spiked levels.

	Carbofuran		**Chlorfenvinphos**	
%Rec (average, n=4)	50 µg.L^{-1}	200 µg.L^{-1}	50 µg.L^{-1}	200 µg.L^{-1}
Douro River	102	86	104	85
Cávado River	117	102	114	97
Ave River	75	88	103	90
Plain Water 1	115	104	108	96
Plain Water 2	120	100	120	84
Plain Water 3	86	115	93	95
Sparkling Water 1	58	74	85	69
Sparkling Water 2	106	80	106	82
Sparkling Water 3	114	93	101	82
Tap Water	95	104	84	88

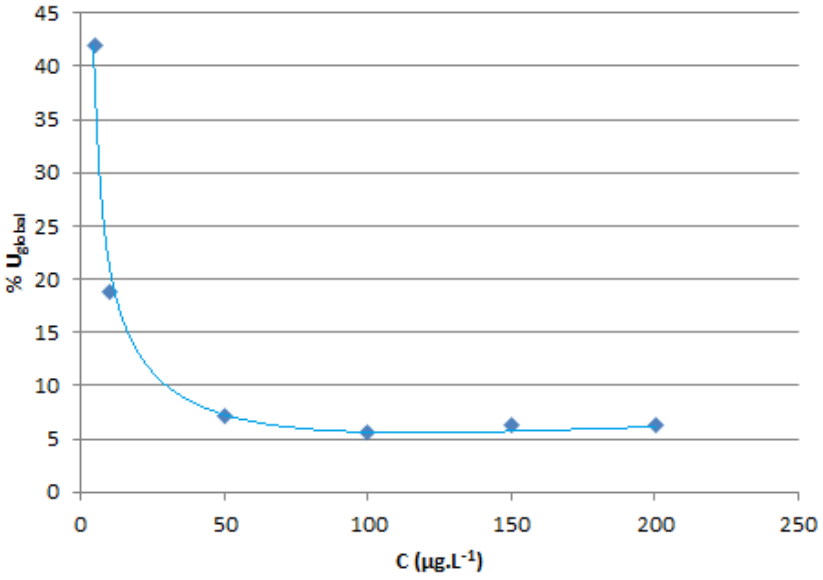
n: number of replicates.

2.3.4.3.3 Global uncertainty

The global uncertainty (U) associated to this study was evaluated through the *bottom-up* approach/EURACHEM procedure, described by Ratola, et al. [150]. Global uncertainty for the different levels of concentration of each pesticide are represented in Figure 2.9.



(a)



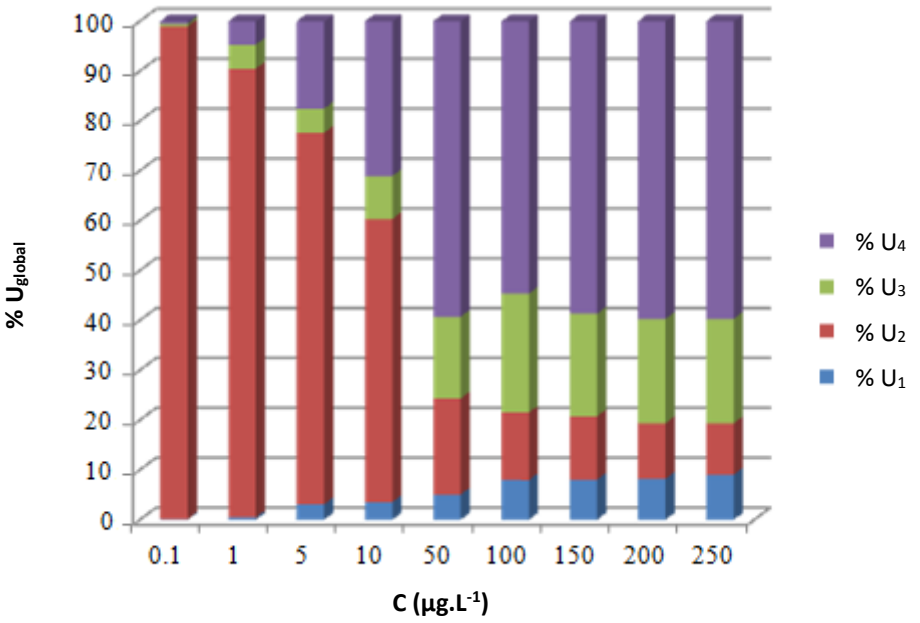
(b)

Figure 2.9 - Global uncertainty of the DLLME-GC-MS method for (a) carbofuran and (b) chlorfenvinphos quantification in waters.

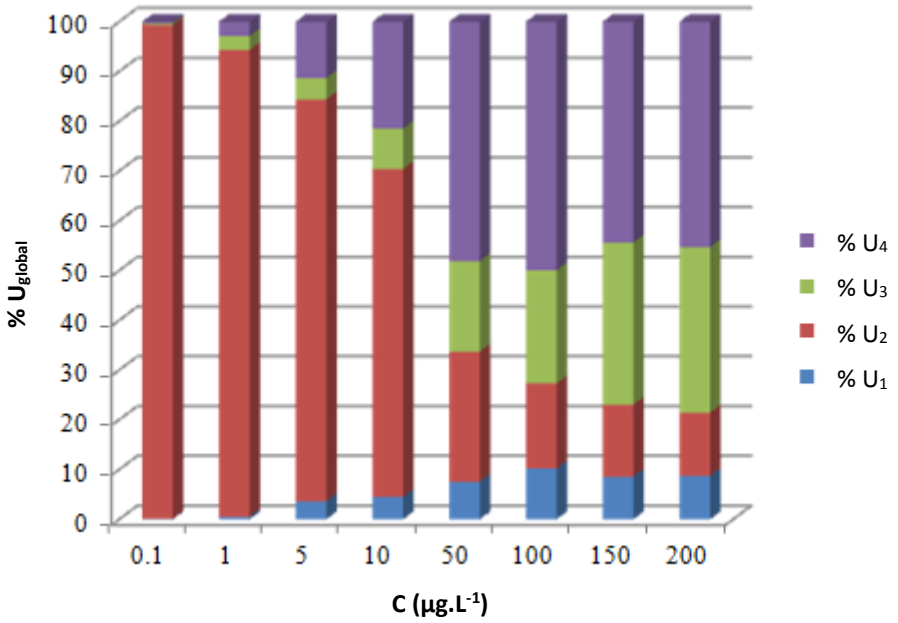
A constant uncertainty was achieved for the upper and intermediate levels of the calibration ranges (7% for carbofuran and 6% for chlorfenvinphos). However, when the standard concentrations are lowered, approaching the detection limits, the global uncertainty rises exponentially.

This approach also weights the individual sources of uncertainty and makes possible the identification of those that are more relevant. In this study, four main contributions were considered, namely: the uncertainty associated to standard preparation (U_1); the uncertainty associated to the calibration curve (U_2); the uncertainty associated to precision (U_3) and the uncertainty associated to accuracy (U_4).

As can be observed in Figure 2.10, both for carbofuran and for chlorfenvinphos, the relative contribution of the uncertainty associated to the standard preparation (U_1) decreases when the concentration diminishes. However, its relative weight to the global uncertainty is always below 3%, for both compounds. The contribution of the uncertainty associated to the calibration curve (U_2) showed an increasing importance as it reaches the lower concentrations, being almost the only source of uncertainty for the lower concentrated standards. The contribution of precision (U_3) diminishes as the concentration decreases, while the contribution of the uncertainty associated to accuracy (U_4) has an important role at the highest concentrations.



(a)



(b)

Figure 2.10 – Relative weight of each individual source of uncertainty for (a) carbofuran, (b) chlorfenvinphos analysis by DLLME-GC-MS for all standard concentrations.

2.3.4.4 Suitability of the extraction methodology to other pesticides

Although the optimisation of the extraction process was performed for carbofuran and chlorfenvinfos, this methodology was also applied to the extraction of other 13 pesticides, which are presented in Table 2.19. The choice of these compounds was made in order to have pesticides from different chemical families and, consequently, with different chemical properties. The optimized DLLME process was used for the extraction of the mix of 13 pesticides in distilled water and in Cávado River water. With these experiments it was possible to obtain the average recovery (%Rec) for each compound. The results obtained were in the range of 64-134%, as shown in Table 2.20. These values of %Rec could not be explained solely by the octanol-water partition coefficient (K_{ow}) and the solubility in water (S_w) of each compound, given that there wasn't any clear trend. More information would be necessary to explain these results, since the equilibrium occurring in the extraction depends on the solubility of each compound in the matrix, but also on the solubilities on the extraction solvent (chlorobenzene) and in the dispersive solvent (methanol).

Table 2.19 – List of the 13 pesticides tested (plus carbofuran and chlorfenvinphos) and some of their properties [179].

Compound	Type of chemical	Chemical Family	log K_{OW}	S_W (mg L^{-1})
Carbofuran	Insecticide	Carbamate	2.30	320
Chlorfenvinphos	Insecticide	Organophosphorus	4.15	124
EPTC	Herbicide	Thiocarbamate	3.02	375
Desethylatrazine	Herbicide	Triazine	1.78	3200
Desethyl terbuthylazine	Herbicide	Chlorotriazine	2.23	532
Dimethoate	Insecticide	Organophosphorus	0.28	23300
Atrazine	Herbicide	Triazine	2.82	34.7
Terbuthylazine	Herbicide	Chlorotriazine	3.27	8.5
Alachlor	Herbicide	Chloroacetanalide	3.37	240
Metalaxyl	Fungicide	Xylylalanine	1.70	26000
S-Metolachlor	Herbicide	Chloroacetanalide	3.24	530
Linuron	Herbicide	Urea	2.91	75
Pendimethalin	Herbicide	Dinitroaniline	4.82	0.3
Captan	Fungicide	Thiophthalimide	2.74	5.1
Methidathion	Insecticide	Organophosphorus	1.58	187

K_{ow}: octanol/water partition coefficient; S_w: water solubility.

Table 2.20 – Average recoveries obtained in the extraction of Cávado River water, with a concentration of 100 µg.L^{-1}.

Compound	%Rec (average, n=4)
EPTC	107
Desethylatrazine	77
Desethyl terbuthylazine	103
Dimethoate	64
Atrazine	103
Terbuthylazine	134
Alachlor	104
Metalaxyl	93
S-Metolachlor	106
Linuron	66
Pendimethalin	83
Captan	100
Methidathion	107

These results show that there is no significant matrix effect, and that the optimized DLLME process is a simple and robust method that can be extended to the extraction of different chemical families of pesticides.

2.3.4.5 Comparison between DLLME-GC-MS and SPE-GC-MS methods

The importance of the SPE methodology has been highlighted before (cf. section 2.1.2.1). Moreover, as is patent in Table 2.1 and Table 2.2, solid-phase extraction is still one of the most popular methods for the analysis of carbofuran and chlorfenvinphos in water samples. Thus, intending to compare different extraction procedures, an additional method for the analysis of both carbofuran and chlorfenvinphos in water samples, was developed. In this methodology the preconcentration of the samples was

achieved by solid phase microextraction, and the subsequent analysis was performed, as for DLLME, by GC-MS.

Some important differences between DLLME and SPE are shown in Table 2.21.

Table 2.21 – Comparison of DLLME with SPE method for the determination of the two studied pesticides.

	Carbofuran				**Chlorfenvinphos**			
Method	**SPE**			**DLLME**	**SPE**			**DLLME**
	LC-18	**HR-P**	**Oasis**		**LC-18**	**HR-P**	**Oasis**	
Linearity range ($\mu g.L^{-1}$)	2–100	2–100	2–100	0.1–250	2–100	2–100	2–100	0.1–200
LOD ($\mu g.L^{-1}$)	0.14	0.07	0.17	0.04	0.26	0.14	0.08	0.02
Sample volume (mL)	15			10	15			10
Extraction time (min)	60			A few seconds	60			A few seconds

Analysing Table 2.21, it can be seen that HR-P cartridges provided the best (lowest) limit of detection for carbofuran analysis. Chlorfenvinphos, on the other hand, was better extracted by Oasis HLB cartridges. Nevertheless, both cartridges performed well for the extraction of both compounds. The adequacy of Oasis HLB to the extraction of both of these pesticides comes as no surprise, when considering the various studies reporting the successful use of those cartridges for the extraction of carbofuran [18, 32, 36, 39-42] and chlorfenvinphos [18, 61, 62, 67, 72] from water samples (cf. section 2.1.2.1.). Comparing both extraction methodologies, it can be observed that DLLME has much shorter extraction times, as well as lower solvent (cf. section 2.3.3) and sample volume consumption. Furthermore, the limits of detection achieved with DLLME methodology were below the limits established by European Union legislation [19]. Although SPE yielded higher limits of detection than DLLME, lower LODs can be achieved if the sample volume is increased. This is the case in most of the studies concerning SPE methodology summarized in Table 2.1 and Table 2.2, in which several authors report low LODs (at $ng.L^{-1}$ level), but whose methodologies require water samples in the order

of the hundreds of millilitres. Thus, the great advantage of DLLME, when compared with SPE, is the possibility of achieving results with a simple, rapid, easy and low cost method of extraction; which does not require the use of large amounts of sample nor solvents.

2.4 Conclusions

Two analytical methods for the simultaneous analysis of carbofuran and chlorfenvinphos in water were developed and validated. The first method, consisting on the direct injection and analysis by LC-DAD, intended to be a rapid way of detecting a possible contamination. LODs of 4 and 11 $\mu g.L^{-1}$ were obtained for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed, in four different matrices (tap water and distilled water which had been in contact with S3 and S4 deposits and clay), at three levels of concentration, and average recoveries of 99% and 95% were obtained for carbofuran and chlorfenvinphos, respectively. These results show that there were no relevant interferences in the matrices.

A method involving the preconcentration of water samples by DLLME and analysis by GC-MS was also developed. LODs of 0.04 and 0.02 $\mu g.L^{-1}$ were achieved for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed in different matrices, such as river, tap, plain and sparkling water. Except for the results obtained for the sparkling water (recoveries ranging from 58-85%), the recoveries for the extraction in the other matrices were in the range of 75-120% (average value of 98.2% was reached). Given the results obtained, it can be assumed that no relevant matrix effect was observed when applying this method.

Both methods showed wide linearity (DI-LC-DAD: 0.05-100 $mg.L^{-1}$; DLLME-GC-MS: 0.1-250 $\mu g.L^{-1}$ for carbofuran and 0.1-200 $\mu g.L^{-1}$ for chlorfenvinphos), good repeatability and high recoveries in several matrices, within a short analysis period.

Therefore, both methods present the advantages of speed, simplicity, ease of operation and low consumption of sample volume and solvents; these methodologies are, thus, well fitted for detection and quantification of carbofuran and chlorfenvinphos in water, at lower and higher levels of concentration.

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Chapter 3

3 Quantification of carbofuran and chlorfenvinphos in deposits from drinking water networks by ultrasound extraction-LC-DAD

3.1 Introduction

Water distribution systems are vulnerable to contamination, either accidental or deliberate. As mentioned before, in the case of a contamination event, it is important to consider not only the presence of the contaminant in the water, but also the possibility of interaction of the contaminant with the deposits and/or biofilms formed at the inner surface of the pipes, as well as suspended particles. In order to account for the possibility of contamination of the deposits, there was the need to develop methods for the quantification of carbofuran and chlorfenvinphos in these materials (mostly of inorganic constitution).

The development of an extraction methodology of the two target compounds from deposits has to take into consideration the characteristics of such materials, which can be diverse, as already mentioned in section 1.1.2. The rates at which deposits are formed, their chemical composition, structure, morphology and solubilities of predominant mineralogical phases constituting them, are affected by some factors like the pipe material, the water chemistry parameters (pH, temperature, dissolved solids, and organic contents), disinfectants used, and hydraulic patterns of the system [1, 2]. Hence, one of the difficulties that may arise during the development of an analytical method for the detection and quantification of carbofuran and chlorfenvinphos in deposits is the diversity of characteristics of the matrices to be analyzed. Preferably, the analytical method to be developed should be able to detect and quantify the two pesticides in the various types of deposits. Also, using them as model compounds representative of the families of carbamates and organophosphorus, it may allow, in the future, the extension of the method to all compounds of both families.

The hypothesis of a contamination event, deliberate or accidental, and the possibility of interaction of the contaminant with the deposits in water distribution systems, is a matter that lacks further study. A method for the quantification of paraquat

in deposits was already presented by Santos, et al. [3]. However, no other studies concerning the quantification of pesticides in deposits were found in the literature. For this reason, it was decided that the methods of analysis of carbofuran and chlorfenvinphos in soils would be the starting point in the development of an analytical method for the detection and quantification of the two target compounds in the deposits.

Among the various matrices, for which there are reports of analytical methods for the detection of pesticides, it was considered that soils should be the most comparable to deposits. Thus, the studies found in the open scientific literature, regarding the analysis of carbofuran and chlorfenvinphos in soils, are presented in Table 3.1 and Table 3.2, respectively.

Analyzing both tables, it can be seen that the most frequent methodology for the extraction of these two pesticides from soil involves liquid-solid extraction (LSE). Soxhlet extraction, discontinuous LSE, microwave-assisted extraction, microwave-assisted Soxhlet extraction, ultrasound-assisted extraction and pressurized liquid extraction, for example, are all considered liquid-solid extraction methods. Even though Soxhlet extraction is a popular methodology for the extraction of pesticides from soils, due to its adoption in many standardized analytical methodologies [4], it is not the most utilized methodology for the pesticides in study. Soxhlet extraction uses drastic conditions (high temperatures for long periods of time), which may degrade thermolabile compounds such as N-methylcarbamates [5] and specifically carbofuran. Only one study [6] reports the use of this methodology for the extraction of seven pesticides (carbofuran included), from different classes, but no remarks are made by the authors concerning possible degradation of the pesticides during the extraction. Aiming to overcome the problem of degradation of N-methylcarbamates during Soxhlet extraction, Prados-Rosales, et al. [7] studied the feasibility of the focused microwave-assisted Soxhlet for the extraction of this class of pesticides from soil, and posterior analysis by LC-post column fluorescence derivatization detection. In their study, the authors compared the efficacy of extraction of the proposed method with a conventional liquid-solid extraction methodology, with analysis by LC-post column fluorescence derivatization detection, and concluded that the recoveries obtained by both methods were similar.

Table 3.1 – Studies found in literature relative to the quantification of carbofuran in soil samples (from 1979 to 2016).

Target analytes	Extraction of the soil	Detection method	Analytical parameters	Reference (Year)
Seven pesticides	Sample amount – 5 g Extraction – sonication for 15 min with 5 mL acetone Pre-concentration with SPE disks with C_8 and C_{18} adsorbent	GC-NPD	C_8: %Recovery - 76±14 C_{18}: %Recovery - 48±11	[8] (1993)
Seven pesticides	Sample amount – 50g Extraction – Soxhlet extraction with 200 mL methanol for 4 hours	LC-UV	----------	[6] (2001)
Carbofuran	Sample amount – 50 g Extraction – 1 hour shaking with a 100 mL mixture of acetone and methanol (1:1); filter the soil and extract again with 100 mL of a mixture of acetone, methanol and acetonitrile (1:1:1) by shaking for 1 hour	Thin layer chromatography	----------	[9] (2002)
***N*-methylcarbamates**	Sample amount – 1.5 g Extraction – microwave-assisted Soxhlet extraction with 100 mL of acetonitrile	LC-post column fluorescence derivatisation-detection	% Recovery – 45.0±2.2 Precision (%RSD): 2.34 – 7.53	[7] (2002)
***N*-methylcarbamates**	Sample amount – 5 g Extraction – continuous ultrasound-assisted extraction for 2 minutes with 5 mL of water at pH 10	LC-post column fluorescence derivatisation-detection	% Recovery: Organic soil – 87% Clayey soil – 91% Slimy soil – 92% Limy soil – 93% Sandy soil – 90%	[10] (2003)
Six carbamate pesticides	Sample amount – 5 g Extraction – 5 mL of methanol, 15 minutes in an ultrasonic bath at room temperature, followed by a second extraction with 4 mL methanol, 15 min ultrasounds and washing with 1 mL methanol	LC-post column fluorescence derivatisation-detection	Linearity range: 0.1 – 1 $\mu g.mL^{-1}$ LOD: 3.2 $\mu g.kg^{-1}$ % Recovery: 87.6 – 97.5	[11] (2003)
Seventeen pesticides	Sample amount – 300 mg Extraction – continuous subcritical water extraction; 2 $mL.min^{-1}$, for 20 or 90 minutes, at 270 °C	GC-MS	LOD – 30.9 $\mu g.kg^{-1}$ % Recovery: 25 min extraction – 97.3% 90 min extraction – 106.7% Repeatability (%RSD) – 2.3%	[12] (2003)
Carbofuran	Sample amount – 5 g Extraction – Shake for 2 hours with 12 mL of acetonitrile, at 250 rpm on a reciprocating shaker. The extraction was repeated twice with 10 mL acetonitrile followed by shaking for 1 hour. Separation was achieved by centrifuging at 2700 rpm for 10 min	LC-UV	----------	[13] (2007)

Table 3.1 – Studies found in literature relative to the quantification of carbofuran in soil samples (from 1979 to 2016) (cont.).

Target analytes	Extraction of the soil	Detection method	Analytical parameters	Reference (Year)
Carbamate and urea pesticides	Sample amount – 5 g Extraction – microwave-assisted extraction with 20 mL of acetonitrile at 70 °C for 10 minutes	LC-DAD	% Recovery (RSD): 71.5±2.3 – 105.0±1.4	[14] (2008)
Three pesticides	Sample amount – 5 g Extraction – shake 100 mL of a mixture 75/15 (v/v) water-acetonitrile (for carbofuran) at 150 rpm for 24 hours at room temperature (28 °C)	LC-DAD	% Extraction: Water as extraction solvent: 30 – 60% Acetonitrile (for carbofuran) as extraction solvent: 50-80%	[15] (2008)
Carbofuran	Sample amount – 10 g Extraction – shake 10 mL water for up to 24 hours at 1500 rpm	LC-UV	% Extraction – 55%	[16] (2010)
Carbofuran	Sample amount – 1 g Extraction – shake 20 mL water or a 0.01 mol.L^{-1} β-cyclodextrin solution, for 3 hours (after 24 hours of equilibration time)	Spectrophotometry	% Extraction: Soil I: 15 – 24% (water) 34 – 65% (β-cyclodextrin) Soil II: 20 – 30% (water) 46 – 72% (β-cyclodextrin) Soil III: 22 – 31% (water) 50 – 74% (β-cyclodextrin) Soil IV: 76 – 85% (water) 100% (β-cyclodextrin)	[17] (2011)
Carbofuran	Sample amount – 1 g Extraction – shake 10 mL of a 0.005 mol.L^{-1} $CaCl_2$ solution, for 24 hours	LC-UV	% Extraction: n.d. – 69%	[18] (2011)
Carbofuran	Sample amount – 1 g Extraction – shake 10 mL of a 0.005 mol.L^{-1} $CaCl_2$ solution, for 24 hours	LC-UV	% Extraction: Soil 1: 14.5 – 29.0% Soil 2: n.d. – 23.1%	[19] (2012)

Table 3.2 – Studies found in literature relative to the quantification of chlorfenvinphos in soil samples (from 1979 to 2016).

Target analytes	Extraction of the soil	Detection method	Analytical parameters	Reference (Year)
Eight organophosphorus pesticides	Sample amount – 70 g Extraction – add 100 mL of acetone and place in an ultrasonic bath for 15 minutes. Add 100 mL of a benzene-hexane solution (1:1) and tumble end-over-end, for 30 minutes.	GC-FID	% Extraction – 95%	[20] (1979)
Chlorfenvinphos and its metabolites	Sample amount – 100 g Extraction – Soxhlet extraction with 200 mL of acetone, with stirring and heat, for 2 hours. Repeat the extraction with 200 mL of acetone-water (1:1). Gather the extracts and concentrate to 130 mL. After adding NaCl, extract two times with methylene chloride (2×200 mL)	Thin layer chromatography	----------	[21] (1988)
Chlorfenvinphos and its metabolites	Sample amount – 100 g Extraction – Soxhlet extraction with 200 mL of acetone, with stirring and heat, for 2 hours. Repeat the extraction with 200 mL of acetone-water (1:1). Gather the extracts and concentrate to 130 mL. After adding NaCl, extract two times with methylene chloride (2×200 mL)	Thin layer chromatography	----------	[22] (1989)
Twenty pesticides	Sample amount – 2 g Extraction - supercritical fluid extraction with CO_2 and methanol as modifier, at 2.5 $mL.min^{-1}$, for 10 minutes, 40 °C, 400 atm	HPTLC-AMD	----------	[23] (1996)
Forty-four pesticides	Sample amount – 5 g Extraction – ultrasonic extraction with 5 mL of ethyl acetate for 15 min. The extraction was repeated 3 times.	GC-MS	Linearity range: 0.05 – 7.0 $\mu g.kg^{-1}$ LOD: 0.20 $\mu g.kg^{-1}$ % Recovery: 91 – 92 Repeatability (%RSD): 4.8 – 7.5	[24] (2005)
Twenty pesticides	Sample amount – 2.3 g Extraction – supercritical fluid extraction with 15 mL of ethyl acetate, at 120 °C, 42 MPa, 8% of methanol as modifier, 1.2 – 1.3 $mL.min^{-1}$	GC-MS/MS	Linearity range: 1.0 – 100 $\mu g.kg^{-1}$ LOD: 0.6 – 1.5 $\mu g.kg^{-1}$ LOQ: 1.9 – 5.2 $\mu g.kg^{-1}$ % Recovery: 93.9 – 101.3 Repeatability (%RSD): 7.2 – 8.5	[25] (2006)
Thirty pesticides	Sample amount – 1 g Extraction – pressurized liquid extraction with a mixture of acetone-dichloromethane (1:1), at 130 °C, 1500 psi	GC-MS	Linearity range: 10 – 750 $\mu g.L^{-1}$ LOD: 5.6×10^{-6} µg % Recovery (%RSD): 48% (8.0%)	[26] (2007)
Chlorfenvinphos	Sample amount – 5 g Extraction – microwave-assisted extraction with 20 mL of hexane-acetone (1:1), at 100 °C, for 10 minutes	Mercury film ultramicroelectrode	Linearity range: 10 – 750 $\mu g.L^{-1}$ LOD: 0.042 $\mu g.g^{-1}$ % Recovery (%RSD): 90.2 – 92.1 (2.8 – 3.4)	[27] (2007)

Table 3.2 – Studies found in literature relative to the quantification of chlorfenvinphos in soil samples (from 1979 to 2016) (cont.).

Target analytes	Extraction of the soil	Detection method	Analytical parameters	Reference (Year)
Twenty-four pesticides	Ultrasonic extraction: Sample amount – 20 g Extraction – 60 mL water-acetonitrile (1:2), for 2 minutes Pressurized liquid extraction: Sample amount – 5 g Extraction – 1 g silica gel was mixed with the soil. The extraction was made with water-acetonitrile (1:2), at 110 bar, for 20 minutes, with three cycles Extraction according to European Norm DIN 12393: Sample amount – 25 g Extraction – 50 mL of water and 100 mL of acetone (1:2), followed by partioning with 100 mL ethylacetate-cyclohexane (1:1) QuEChERS method: Sample amount – 10 g Extraction – 20 mL of acetonitrile, followed by a salting-out step with 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate dehydrate and 0.5 g di-sodium hydrogen citrate sesquihydrate	GC-MS LC-MS/MS	Linearity range: 0.010 – 2 $\mu g.mL^{-1}$ Ultrasonic extraction: Linearity range: 7 – 1400 $ng.g^{-1}$ LOD: 12 $ng.g^{-1}$ LOQ: 39 $ng.g^{-1}$ Pressurized liquid extraction: Linearity range: 20 – 4000 $ng.g^{-1}$ LOD: 6 $ng.g^{-1}$ LOQ: 19 $ng.g^{-1}$ Extraction according to European Norm DIN 12393: Linearity range: 4 – 800 $ng.g^{-1}$ LOD: 8.7 $ng.g^{-1}$ LOQ: 29 $ng.g^{-1}$ QuEChERS method: Linearity range: 20 – 4000 $ng.g^{-1}$ LOD: 22 $ng.g^{-1}$ LOQ: 73 $ng.g^{-1}$	[28] (2008)

Table 3.2 – Studies found in literature relative to the quantification of chlorfenvinphos in soil samples (from 1979 to 2016) (cont.).

Target analytes	Extraction of the soil	Detection method	Analytical parameters	Reference (Year)
One hundred and fifty pesticides	Matrix solid-phase dispersion: Sample amount – 10 g Extraction – 60 mL of dichloromethane-acetone-petroleum ether (1:1:1) was added to the soil and shaken for 1 hour. After separation of the extract, 20 mL of the extraction mixture was added and shaken for 10 minutes. Extracts were combined and 50 mL of petroleum ether was added. The extraction was carried out in two stages by the addition of water (150 mL and 10 mL) Liquid-liquid extraction: Sample amount – 2 g Extraction – the soil sample and 4 g of solid support (Florisil) were put in a mortar and blended. The homogeneous mixture was packed into a macro column with anhydrous sodium sulphate (5 g) and silica gel (2.5 g). The analytes were eluted with 15 mL hexane-acetone (8:2) and 15 mL hexane-acetone-diethyl ether (1:2:2)	GC-ECD GC-NPD	Linearity range: 0.010 – 0.500 $mg.kg^{-1}$ Matrix solid-phase dispersion: LOD: 0.001 – 0.020 $mg.kg^{-1}$ % Recovery – 107.1% % RSD – 0.4% Liquid-liquid extraction: LOD: 0.005 – 0.040 $mg.kg^{-1}$ % Recovery – 87.4% % RSD – 4.8%	[29] (2012)
Four pesticides	Sample amount – several amounts of adsorbent Extraction – After adsorption, the solid was separated and put in contact with 50 mL of water at a stirring speed of 150 rpm for 10 minutes	GC-MS	% Recovery – 3.02%	[30] (2015)

Additionally, and since this methodology requires less time of extraction, and thus less residence time of the analytes in the distillation flask, no degradation was observed [7]. The use of microwave radiation in the extraction of carbofuran [14] and chlorfenvinphos [27] from soils has also been tested, and high values of recovery were reported. Still, as can be observed in Table 3.1 and Table 3.2, liquid-solid extraction, with mechanical shaking, and ultrasound-assisted extraction are the two most used methodologies for the extraction of either pesticides. A relevant feature of these methods is that they can be performed at room temperature, which is recommended for the analysis of thermolabile compounds without altering them [5]. Commonly, these techniques use different organic solvents or mixtures, mixtures of water and solvents or aqueous alkaline media to achieve the extraction. Acetonitrile and methanol are the two solvents mostly reported for the extraction of carbofuran from soil samples [6, 7, 11, 13, 14]; for the extraction of chlorfenvinphos from soils, the most used solvent is acetone [20-22] or mixtures of acetone with other solvents [26, 27, 29]. Lesueur, et al. [28] compared four extraction methods for the analysis of twenty four pesticides in soil samples. All extracts were analyzed by GC-MS and LC-MS/MS, in order to detect and quantify each pesticide by the most adequate method. The authors presented a new ultrasonic solvent extraction method and compared the results with those obtained with the European Norm DIN 12393 for foodstuff (liquid-liquid extraction (LLE)), the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method and a pressurized liquid extraction (PLE) method. The lowest limits of detection (LOD) for the analysis of the twenty four pesticides from soil samples were achieved with the European Norm DIN 12393 (LLE) and the highest with the QuEChERS and PLE methods. For chlorfenvinphos, in particular, the lowest LOD was achieved with PLE-GC-MS method and the highest with the QuEChERS-GC-MS method (Table 3.2). Chlorfenvinphos was successfully extracted with all the four methodologies, however, the European Norm DIN 12393 (LLE) and the PLE methods were not able to extract the totality of pesticides. Furthermore, the QuEChERS method showed to be the most efficient methodology with recoveries in the range of 27.3 to 120.9%. The recoveries obtained with the ultrasound extraction (USE) method were in the range of 10.9 – 96.3%, for the European Norm DIN 12393 (LLE) were between 6.8 – 108.1% and for PLE between 12.2 – 153.2%. The authors finally suggest

the investigation of the use of acetone (instead of acetonitrile), as extraction solvent in the USE method, to increase the recoveries [28].

Considering the state of the art on the extraction of carbofuran and chlorfenvinphos from soil samples, it was decided to test similar methodologies, to those used in soils, for the extraction of the two pesticides from deposits.

3.2 Experimental section

3.2.1 Chemicals and reagents

Carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl-methylcarbamate) with a purity of 99.9%, and chlorfenvinphos ([EZ]-2-chloro-1-(2,4-dichlorophenyl)ethenyl diethyl phosphate) standards were purchased from Fluka, Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade), methanol (HPLC grade), n-hexane (for pesticide residue analysis) and acetone (for pesticide residue analysis) were from VWR BDH Prolabo (Fontenay-sous-Bois, France). Ethyl acetate (for analysis) was from Riedel-de-Haën (Seelze, Germany) and calcium chloride dehydrated was from Sigma-Aldrich (St. Louis, MO, USA).

3.2.2 Standards preparation

The individual stock solutions (100 $mg.L^{-1}$) of carbofuran and chlorfenvinphos were prepared in distilled water. Mixture and individual standards of carbofuran and chlorfenvinphos were prepared by diluting an appropriate amount of each stock solutions in distilled water. All solutions were stored at 4 °C.

3.2.3 Instrumentation and operational conditions

Carbofuran and chlorfenvinphos samples were analysed in a Hitachi Elite LaChrom apparatus with a L-2130 pump, a L-2200 autosampler, a L-2300 column oven and a L-

2455 diode array detector. The separation of both pesticides was made by injection of 99 µL in a Purospher STAR LiChroCART RP-18 endcapped (240×4 mm, 5 µm) reversed phase column from Merck (Darmstadt, Germany), at 30 °C. The mobile phase consisted of 60% (v/v) of acetonitrile and 40% (v/v) of water, with a flow rate of 1 $mL.min^{-1}$. Carbofuran quantification was made at 220 nm and chlorfenvinphos at 240 nm. The extraction was performed in an Ultrasonic Cleaner USC300D (45 kHz, 80 W) instrument, from VWR International (Leuven, Belgium).

3.2.4 Deposits preparation

The deposits used in the method development and validation were recovered from drinking water distribution systems, specifically, from cast iron pipes that were required to be replaced. The deposits were kindly supplied by Dr. Gabriela Schaule (IWW Water Centre, Germany).

Prior to their use, the deposits samples (S3 and S4) were dried in an oven (until no weight variation was observed). Afterwards, the dried deposits were sieved and kept in dry conditions up until its utilization. Previous work developed at LEPABE/FEUP led to a comprehensive characterization of these deposits [31], thus, for a better understanding, the nomenclature herein was kept consistent. With the results obtained, the authors of the study classified the S3 and S4 samples as tubercle and white deposits, respectively.

Clay is the main component of the mineral fraction of soils [32, 33] and, unlike deposits, is a material that was available in large quantities. Therefore, clay was chosen as model deposit, and used in the validation of the extraction method. This material has been used before in adsorptions studies by our group, thus, a characterization of clay was presented in a previous work [34].

The principal properties that characterize the deposits and the clay are presented in Table 3.3. Besides the different composition, it can be observed that the main differences between the deposits are the surface area and the organic matter content. Since these properties are relevant on the adsorption processes, it can be assumed that the existing differences indicate that the interaction between the pesticides and each deposit may be distinct.

Table 3.3 - Physical-chemical properties of the real deposits and clay (from [31, 34]).

	S3	**S4**	**Clay**
Deposit classification	Tubercle	White	Not determined
ICP-OES analysis (wt.% of the main elements at dry basis)	Fe: 97 P:1 Mn:1	Ca: 97 Fe: 1 Mg: 1	Al_2O_3: 34 SiO_2: 49
S_{BET} ($m^2.g^{-1}$)	36	1	Not determined
pH_{pzc}, 20 °C	6.1	9.9	4.8
pH in water, 20 °C	7.2	9.0	5.3
Main component identified by XRD	Goethite	Calcite ($CaCO_3$)	Not determined
Organic matter content (wt.%)	1.0	0.2	12

3.2.5 Spiking of the deposits with the pesticides

In order to obtain deposits with a known concentration of contaminant, there was the need to contaminate the deposit before the extraction step. Thus, 50 mg of deposit sample were put in contact with 10 mL of a carbofuran or chlorfenvinphos aqueous solution, of known concentration, at 20 °C for 24 hours. Separation was performed by centrifugation for 10 minutes, at 4000 rpm. The upper layer was analyzed by LC-DAD, and the difference between the amounts of pesticide at the beginning and at the end of the contamination, in the liquid phase, allowed for the determination of the amount of pesticide adsorbed in the solid phase.

3.2.6 Ultrasonic extraction procedure

The developed extraction procedure was based on several reported methods for the extraction of carbofuran and chlorfenvinphos by ultrasonic extraction [8, 11, 24], and is described below.

Following the contamination and separation steps, the deposit was freeze-dried overnight (12 hours). Then, 10 mL of a suitable organic solvent was added and the mixture was placed in an ultrasonic bath for 15 minutes, at room temperature. After the extraction, the mixture was separated by centrifugation (4000 rpm for 10 minutes) and the upper layer was analyzed by LC-DAD, in order to determine the amount of pesticide extracted. The comparison between the analytical responses of the extract of a previously contaminated deposit and an extract, from a "clean" deposit, which was spiked at the end of extraction (at the same level of contamination), allows the determination of the extraction percentage.

3.3 Results and discussion

The development of the extraction method for detection and quantification in deposits was focused on the analysis of chlorfenvinphos. Also, clay was chosen as model deposit because of its high adsorption capacity of chlorfenvinphos, but especially due to its large availability. Firstly, the selection of the most suitable extraction solvent was made and, afterwards, several extraction methods were tested, and the most adequate was chosen. Then, the developed method was validated for the extraction of chlorfenvinphos from clay, and its applicability to the extraction of other deposits was tested. Additionally, the extraction of carbofuran, with the developed method, from clay, S3 and S4 deposits, was also assessed.

3.3.1 Selection of the extraction solvent

One of the most important parameters that can influence extraction efficiency and selectivity is the solvent nature. Several solvents were tested for the extraction of chlorfenvinphos from clay, in order to minimize the effect of clay co-extractives and to improve recoveries, namely: acetone, acetonitrile, calcium chloride 0.1 M solution, ethyl acetate, hexane, methanol, water and a mixture of acetonitrile-water (80%/20% (v/v)). The choice was made based on the most reported solvents for the extraction of chlorfenvinphos from soils. Since the extraction methodology was yet to be optimized,

in these initial experiments, the extraction was performed by batch mode. The recoveries obtained with each extraction solvent are compared in Figure 3.1.

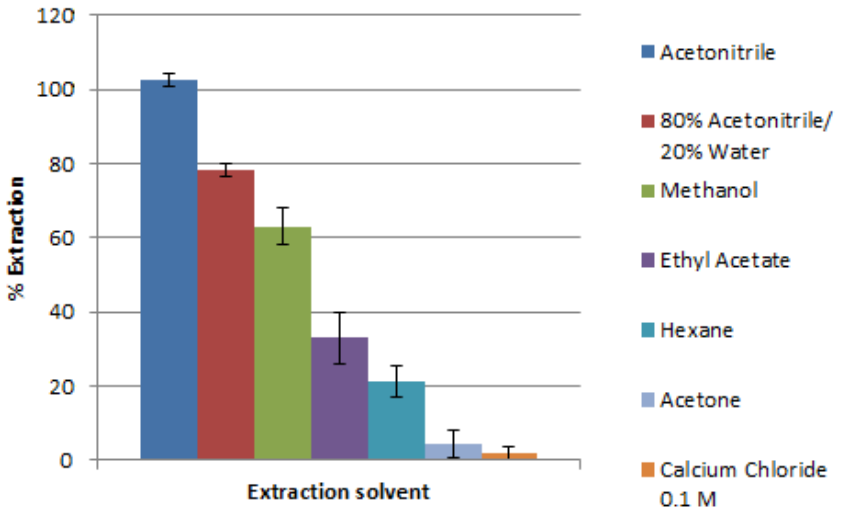


Figure 3.1 – Selection of the solvent for the extraction of chlorfenvinphos from clay. Deposit contamination: 50 mg of clay was put under magnetic agitation with 10 mL of a 10 $mg.L^{-1}$ chlorfenvinphos solution, for 24 hours, 20 °C. Batch extraction: 50 mg of contaminated clay was put under magnetic agitation with 10 mL of extraction solvent, 24 hours, 20 °C.

Water was tested as extraction solvent and discarded, since no chlorfenvinphos was detected after extraction. Therefore, in a contamination event, if some quantity of chlorfenvinphos adsorbs to the deposit, it should be improbable that the pesticide would desorb solely by the passage of water. The extraction percentage yielded by each solvent is depicted in Figure 3.1. Calcium chloride practically was not able to extract the pesticide from the solid matrix. Acetone was one of the organic solvents most reported (Table 3.2) for the extraction of chlorfenvinphos from soil samples. The extraction of chlorfenvinphos from clay with acetone yielded low recoveries (about 4.2%) and the analysis showed some interferences, probably due to the co-extraction of other compounds from the solid. Methanol, ethyl acetate and hexane were able to recover some amount of chlorfenvinphos (21.3 – 63.2%), as was the mixture acetonitrile-water (80%/20% (v/v)) with an extraction percentage of 78.2%. However, acetonitrile was the solvent that gave the best recovery for the extraction of chlorfenvinphos from clay samples, and thus it was the chosen solvent.

3.3.2 Selection of the extraction method

With the extraction solvent defined, several methods of extraction (adapted from reported methods [6, 8, 11, 15, 18, 21]) were tested. These methodologies are some of the most frequently used for the extraction of pesticides from soils (cf. Table 3.1 and Table 3.2). Considering the possibility of extension of the method applicability to the extraction carbofuran from deposits, and given its thermolability [35], only methods that did not involve the destruction of the matrix, or severe conditions, were considered. Also, since it is intended to be a fast methodology of detection and quantification of pesticides in deposits, it is important to avoid any extra steps, such as clean-up or pre-concentration steps. The percentages of extraction obtained with each methodology are presented in Figure 3.2.

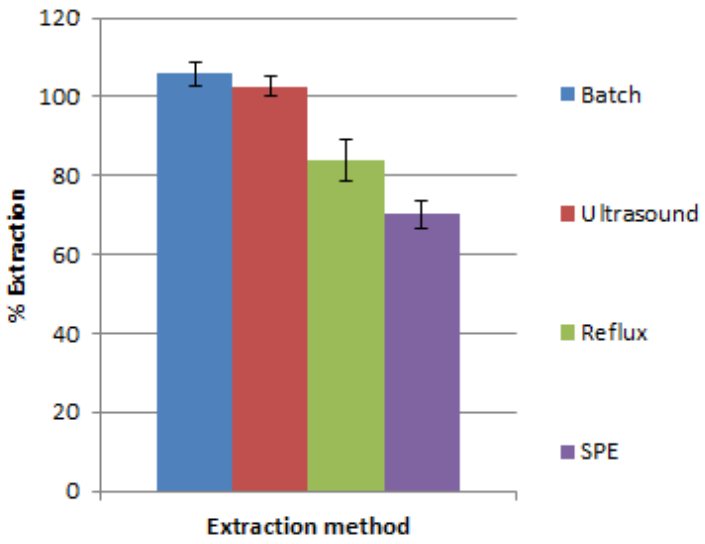


Figure 3.2 – Selection of the extraction methodology. Batch extraction: 50 mg of contaminated clay, with 10 mL of acetonitrile, 24 hours, 20 °C; Ultrasonic extraction: 50 mg of clay, with 10 mL of acetonitrile, 15 minutes, room temperature; Reflux extraction: 50 mg of clay, with 10 mL of acetonitrile, 5 hours; SPE: 10 mL of acetonitrile was passed through a SPE cartridge filled with 50 mg of clay, at room temperature.

It should be mentioned that Soxhlet extraction, for 6 hours with 40 mL of acetonitrile, was also tested, but no chlorfenvinphos was detected in the extract. The use of a higher volume of acetonitrile in the extraction procedure was a necessity of the

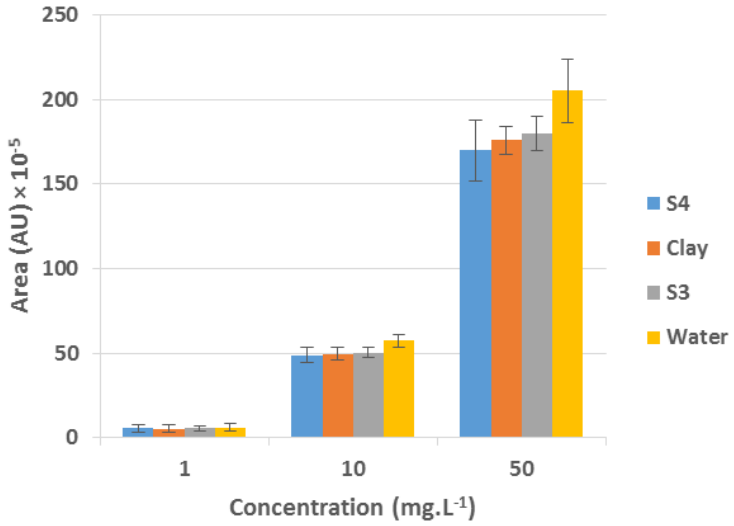
method, but this may have led to the rise of the detection limit. Possibly, an extra pre-concentration step was needed. Due to the complexity of the process, high solvent consumption and the possible necessity of a concentration step, the Soxhlet methodology was discarded. The comparison between the several methods (Figure 3.2), show that batch and ultrasonic extractions yielded the best percentages of extraction of chlorfenvinphos. Even though batch extraction presented a slightly higher recovery, the ultrasound-assisted extraction method was chosen, because it requires a shorter time of extraction.

From the evaluation of method performance using 10 mL acetonitrile as extractant in ultrasonic extraction during 15 min, it was concluded that these conditions exhibited excellent extraction capabilities; therefore, no further optimization was performed.

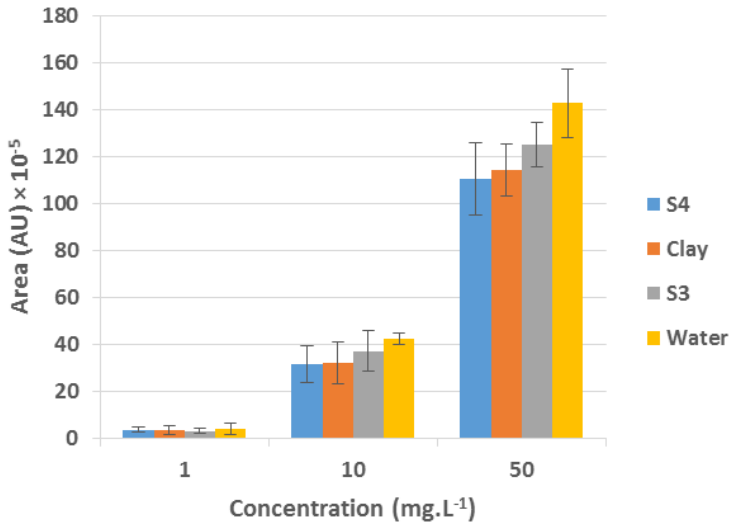
3.3.3 Study of matrix interferences

During the extraction procedure, the solvent should extract the compound or compounds in study from the deposits, however it can also extract other unwanted compounds, which may interfere with the analysis. The influence of matrix co-extractives on the response of analytes is a well-known phenomenon in the pesticide residue analysis, which can result in either decreased detection response [36] or increased analytical signal [37]. Thus, matrix interference studies were performed, in order to guarantee that the analytical response is not affected by the presence of possible interferents. The developed method was intended to be applied to the extraction of chlorfenvinphos and carbofuran, from various deposits. So, the analytical responses of both pesticides in distilled water were compared to the responses at the end of an extraction, for each deposit. Even though the extract is in acetonitrile, previous tests allowed to conclude that the analytical response of carbofuran and chlorfenvinphos in acetonitrile is comparable to that in water. Thus, the comparison of the analytical response of the spiked acetonitrile extracts was made with the water standards. Each deposit was put in contact with water, without any pesticide, and the extraction procedure was performed, as described in section 3.2.6. At the end, the acetonitrile extract was spiked with a known concentration of pesticide, and the

analytical response was compared to that obtained for the same concentration in a distilled water sample. The "blank" acetonitrile extract is expected to have the compounds that may be extracted simultaneously with the pesticides. The matrix interferences study was performed at three concentration levels of each pesticide, in each deposit, as shown in Figure 3.3. Also, each "blank" acetonitrile extract, without pesticide spike, was analyzed by LC-DAD, and no traces of chlorfenvinphos or carbofuran were detected.



(a)



(b)

Figure 3.3 – Study of matrix interferences on the analysis of (a) carbofuran and (b) chlorfenvinphos.

As can be observed, the analytical responses obtained for the spiked "blanks" are slightly lower than those obtained with the water solution. This is verified for both carbofuran and chlorfenvinphos. The fact that the extract is in acetonitrile and the possible presence of other compounds in solution, that may influence the analytical response, may justify the suppressed response. Despite the apparent differences, the analytical responses for both pesticides can be considered statistically similar. Thus, it can be concluded that the analytical responses of carbofuran and chlorfenvinphos are not significantly affected by the possible presence of co-extractives in solution.

3.3.4 Linearity, limits of detection and quantification, precision and accuracy

The calibration curve for the extraction of chlorfenvinphos from clay was obtained at five concentration levels of contamination, from 0.30 to 2.93 $mg.g^{-1}$. The narrow range of linearity (a factor of 10 between the extreme values is not verified) can be justified by the low contamination levels achieved on the deposit. In order to obtain greater contamination levels, aqueous solutions of higher concentrations of chlorfenvinphos should be used in the contamination step. Since the concentration of pesticide in the aqueous solution used in the experiments was already close to its solubility limit in water, a co-solvent would have to be used to prepare such solutions. On the other hand, a lower linearity range would probably be achievable, however, the associated uncertainty to those values would have to be considered.

In Figure 3.4 is presented the relation between the contamination level in the deposit and the area obtained after the analysis by LC-DAD, of the extract. In practice, this relation allows to determine the contamination level of the deposit, after the ultrasonic extraction with acetonitrile (in the aforementioned conditions) and subsequent analysis of the extract by LC-DAD (where the area value is obtained).

The high correlation coefficient confirms the linear relationship between the analytical signal and the degree of contamination of the clay with chlorfenvinphos, as presented in Figure 3.4.

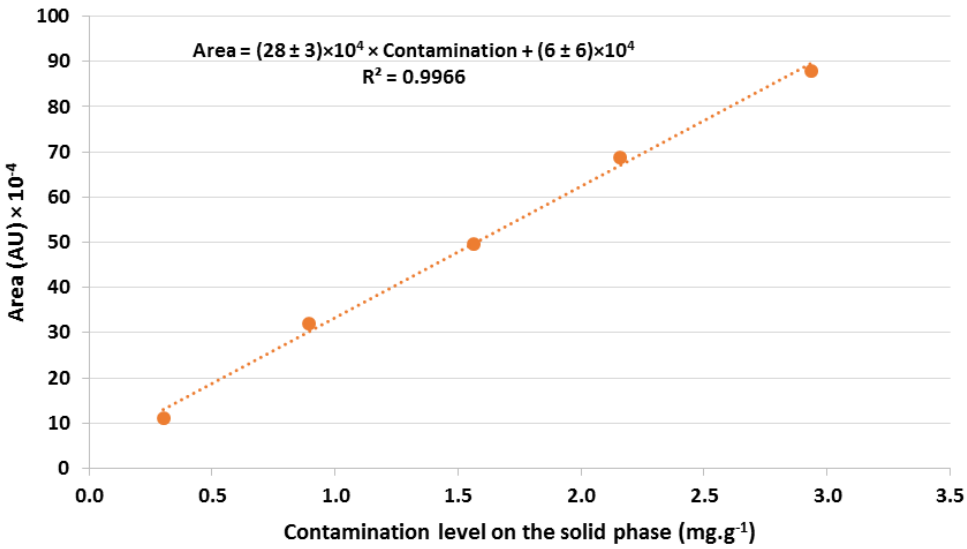


Figure 3.4 – Calibration curve for the extraction of chlorfenvinphos from clay, and analysis by LC-DAD.

The limits of detection and quantification of 0.35 and 1.05 $mg.g^{-1}$, respectively, were calculated based on the standard deviation of the response and the slope of the calibration curve at the lowest concentration (0.30 $mg.g^{-1}$), according to the equations 2.1 and 2.2, presented before.

The precision of the technique was evaluated in terms of repeatability (within-day relative standard deviation, %RSD) by the extraction and analysis of six replicate spiked clay samples at three contamination levels: 0.30, 1.57 and 2.93 $mg.g^{-1}$. The comparison between the contamination level obtained by the calibration curve and the real amount of chlorfenvinphos adsorbed onto the clay, allowed for the evaluation of the accuracy of the method. The results are summarized in Table 3.4.

Table 3.4 – Precision and accuracy of the method for the extraction of chlorfenvinphos from clay.

Contamination level ($mg.g^{-1}$)	Precision (%RSD)	Recovery (%)
0.30	14	106±1
1.57	10	101±8
2.93	8	101±6

The precision of the method is lower for the contamination levels close to the detection limit, however, for a non-automated procedure, these values of repeatability are acceptable. High average recoveries were obtained for the three contamination levels, with values between 101 and 106%.

The obtained recovery results are comparable to those reported in literature for the extraction of carbofuran (87-93%) [10] and chlorfenvinphos (91-92%) [24] from soils, with similar ultrasonic extraction methods. The limits of detection and quantification obtained with the developed USE-LC-DAD method are, in general, higher than those reported in the studies presented in Table 3.1 and Table 3.2. Nevertheless, if lower detection or quantification limits are desired, a preconcentration step, such as DLLME, could be coupled.

3.3.5 Global uncertainty

The *bottom-up* approach/EURACHEM procedure, described by Ratola, et al. [38], was used to estimate the global uncertainty (U) associated to this study. Global uncertainty for the different levels of contamination of chlorfenvinphos in clay is represented in Figure 3.5.

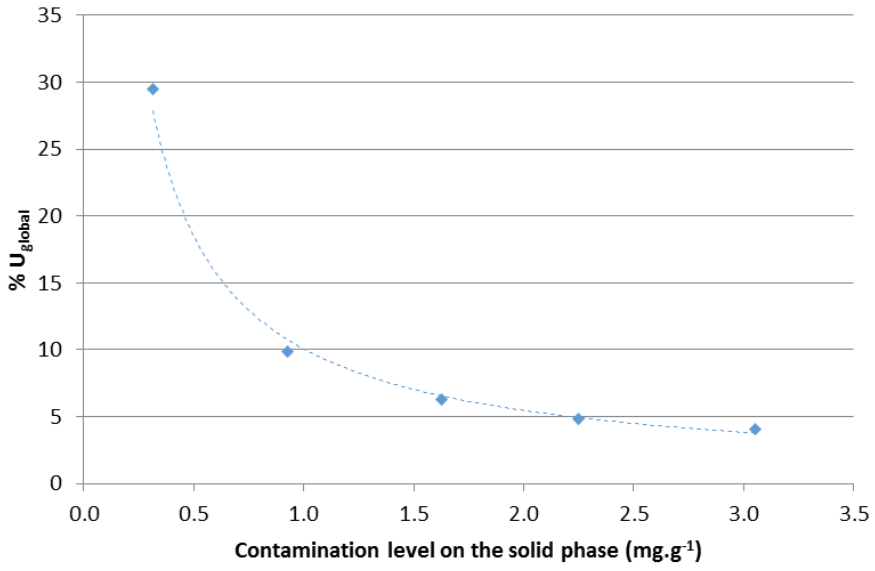


Figure 3.5 - Global uncertainty of the ultrasonic extraction of chlorfenvinphos from clay.

As can be seen, the global uncertainty is in the range of 5 to 10% for the higher contamination levels. However, for the lowest level of contamination evaluated, the global uncertainty rises to almost 30%. The weight of each individual source of uncertainty can also be evaluated by this approach, enabling the clarification of the more relevant. Four main contributions were considered in this study, namely: the uncertainty associated to standard preparation (U_1); the uncertainty associated to the calibration curve (U_2); the uncertainty associated to precision (U_3) and the uncertainty associated to accuracy (U_4). The importance of each type of uncertainty is presented in Figure 3.6.

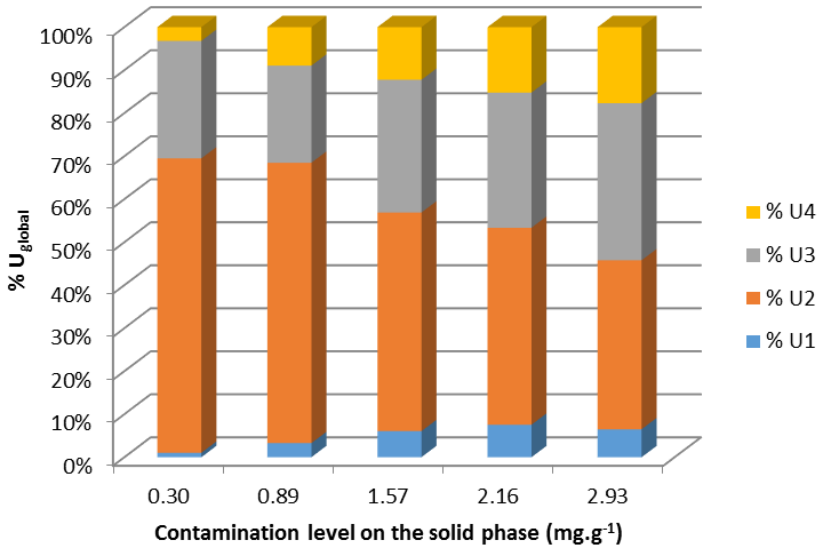


Figure 3.6 - Relative weight of each individual source of uncertainty for chlorfenvinphos analysis in clay.

The contribution of the uncertainty associated to standard preparation (U_1) is minor for the higher contamination levels, and diminishes further as the contamination levels lowers. On the other hand, the uncertainty associated to the calibration curve (U_2) appears to be the main source of uncertainty, representing almost 70% of the global uncertainty at the lowest level of contamination. Its contribution reduces as the contamination levels rise, and an increasing importance of the uncertainties associated to precision (U_3) and to accuracy (U_4) is noticed.

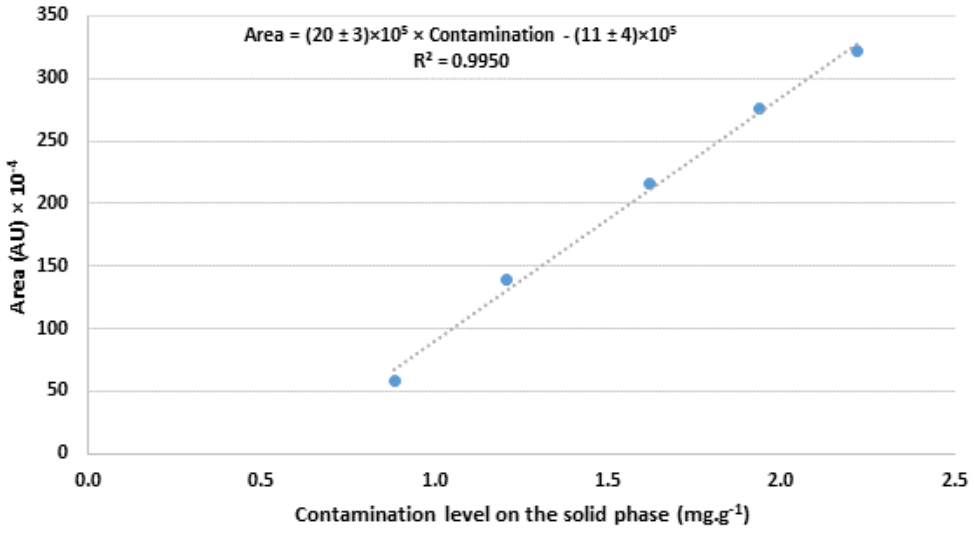
In sum, the developed extraction methodology showed to be adequate for the detection and quantification of chlorfenvinphos in clay samples, with satisfactory performance even at lower levels of contamination.

3.3.6 Suitability of the extraction method to different deposits and to the extraction of carbofuran

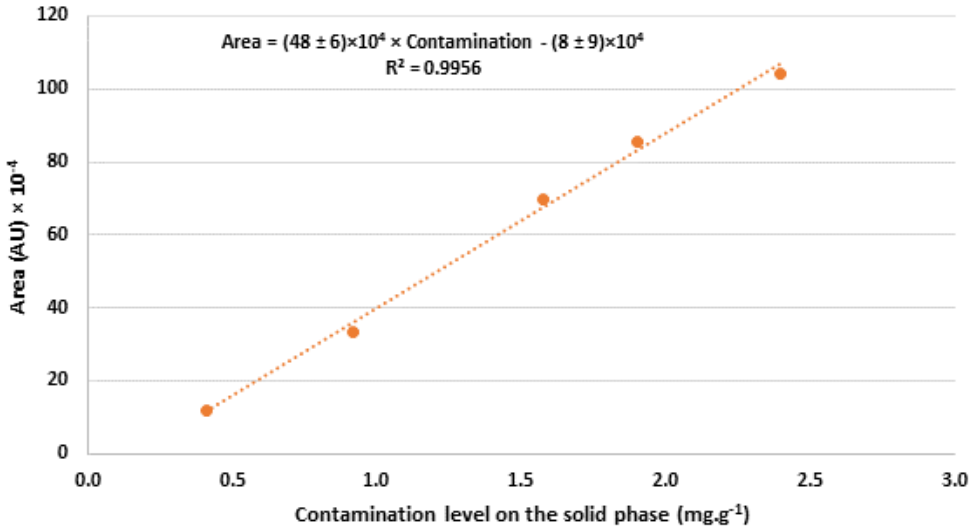
The applicability of the ultrasonic extraction method was also tested on the recovery of chlorfenvinphos from contaminated S3 and S4 deposits. The aim was to understand if the developed method was suitable for use with deposits with different characteristics. The method was tested as is, however, it should be mentioned that a matrix-matched calibration, or an optimization of the extraction is recommended if lower detection and quantification limits, or higher extraction percentages, are intended.

Just like with clay experiments, first the deposits were contaminated (section 3.2.5), and then extracted by the procedure described in section 3.2.6. The methodology was tested at five levels of contamination of chlorfenvinphos in each deposit, as shown in Figure 3.7.

The differences are clear, when comparing the two calibration curves presented in Figure 3.7, and also the calibration curve for clay (Figure 3.4). Even though the experimental conditions used in the contamination of the different deposits were the same, different contamination levels were achieved. As happens in soils with different characteristics, the interaction between one contaminant with different types of deposits appears to be different. As such, the developed method may be appropriate to detect and quantify chlorfenvinphos in the deposits tested, but not suitable for use with other unknown deposits. Also, considering these results, it is obvious that a calibration curve would have to be traced, for each deposit which is intended to be analyzed.



(a)



(b)

Figure 3.7 - Calibration curve for the extraction of chlorfenvinphos from S3 (a) and S4 (b) deposits, with analysis by LC-DAD.

As can be observed in Figure 3.7, the analytical response is linear in the range of contamination considered, for both deposits. However, and similarly to what was obtained for the quantification of chlorfenvinphos in clay, the calibrations curves for the quantification in S3 and S4 deposits have narrow ranges (factor of 10 between extremes is not verified). As previously referred, a wider linearity range of the calibrations curves is preferred, however, the use of solutions with higher concentrations, in the

contamination step, is limited to the solubility in water of the pesticide. Lower contamination levels should be achievable at the expense of higher associated uncertainty of the results.

The limits of detection and quantification were calculated according to equations 2.1 and 2.2, presented before. Detection limits of 0.23 and 0.11 $mg.g^{-1}$ were obtained for the extraction from S3 and S4 deposits, respectively. The limits of quantification obtained were of 0.71 $mg.g^{-1}$ for S3 and 0.32 $mg.g^{-1}$ for S4. The precision of the method, when applied to S3 and S4 deposits, was also tested at three levels of contamination, as presented in Table 3.5.

Table 3.5 - Precision and extraction percentages of the method for the extraction of chlorfenvinphos from S3 and S4 deposits.

Deposit	Contamination level ($mg.g^{-1}$)	Precision (%RSD)	Extraction (%)
S3	0.88	27	33±24
	1.62	10	65±9
	2.22	12	70±10
S4	0.41	14	16±13
	1.57	10	22±7
	2.39	8	21±7

Although the method shows a lack of precision on the quantification at lower contamination levels (especially on the analysis of S3), for the higher levels the method appears to be precise. The percentages of extraction obtained for the extraction from S3 were lower than those obtained for clay. Furthermore, it can be observed (Figure 3.7 (a)) that the trend line obtained for the extraction of this deposit crosses the x axis at 0.54 $mg.g^{-1}$. Thus, this extraction procedure is not appropriate for the analysis of chlorfenvinphos on S3 deposit at levels below 0.54 $mg.g^{-1}$. For the extraction of chlorfenvinphos from S4 deposit, the method appears to be suitable, however, low percentages of extraction were achieved.

This simple and rapid extraction methodology enabled the detection and quantification of chlorfenvinphos in S3 and S4 deposits, if the extraction percentages are taken into account. However, for appropriate quantification of chlorfenvinphos in S3 and S4 deposits, a matrix-matched calibration is recommended. When lower detection and quantification limits are intended, a pre-concentration step, such as DLLME, can be coupled.

This method was also tested for the extraction of carbofuran from clay, S3 and S4 deposits. The results are summarized in Table 3.6.

Table 3.6 – Extraction percentages obtained for the extraction of carbofuran from clay and S4 deposit, using the developed method.

Deposit	Contamination level ($mg.g^{-1}$)	Extraction (%)
Clay	0.34	102±8
S4	0.09	13±22
	0.12	13±18
	0.15	11±6

Carbofuran adsorbed poorly to clay, at low contamination levels. Yet, when some level of contamination of the clay was achieved, the obtained extraction percentage was acceptable. Further experiments, at higher levels of contamination, should be made, in order to confirm the applicability of the method.

In the conditions considered, the adsorption of carbofuran on S3 deposit was negligible (below the limit of detection). Thus, no extraction results are presented. Again, higher levels of contamination should be tested as well as longer times of contamination.

The method appears to be applicable to the extraction of carbofuran from S4 deposit. However, as happened to chlorfenvinphos, the extraction percentages achieved for this deposit were low.

The developed extraction method appears to be applicable to the rapid detection and quantification of carbofuran in clay and S4 deposit, if the extraction percentages are

considered. The results obtained were inconclusive in what regards to the extraction of carbofuran from S3 deposit.

These results indicate that carbofuran is not easily adsorbed by clay and S3 deposit, in the conditions tested. However, in a contamination event, greater concentration levels of contaminant would be present, as well as higher quantities of deposit. Thus, it cannot be presumed that clay or S3 deposit would not be contaminated by carbofuran.

3.4 Conclusions

An ultrasonic extraction method for the detection and quantification of chlorfenvinphos in clay samples was developed and validated. During the development of the method, the optimization of the extraction solvent, as well as the selection of the most suitable extraction methodology were performed. The method, consisting of ultrasonic extraction with acetonitrile of chlorfenvinphos from clay, was intended to allow the detection and quantification of a possible contamination, in a fast, simple and reliable way. The analytical response was linear, although in a narrow range. The limits of detection and quantification were of 0.35 and 1.05 $mg.g^{-1}$, respectively. Also, high average recoveries were obtained for the three contamination levels tested, with values between 101 and 106%.

The methodology was also tested in the extraction of chlorfenvinphos from two other types of deposits: S3, a tubercle deposit, and S4, a white deposit. A linear analytical response was observed, in the narrow range of contamination considered, for both deposits. Detection limits of 0.23 and 0.11 $mg.g^{-1}$ were obtained for the extraction of S3 and S4 deposits, respectively. However, since the trend line obtained for the extraction of S3 deposit crosses the x axis at 0.54 $mg.g^{-1}$, this extraction procedure is not appropriate for the analysis at levels below this value.

Additionally, the method was tested for the extraction of carbofuran from clay, S3 and S4 deposits. The obtained results indicate that the developed extraction method appears to be applicable to the rapid detection and quantification of carbofuran in clay and S4 deposit, whenever the extraction percentages are considered. Since no contamination was achieved for the S3 deposit, no conclusions about the applicability of the extraction method could be draw. Further experiments, involving higher contamination levels and longer contact times, should be considered, in order to test the applicability of the method to this deposit.

In general, this simple and rapid extraction methodology enabled the detection and quantification of carbofuran and chlorfenvinphos in the different types of deposits

tested, whenever the appropriate extraction percentages are taken into account. However, the fact that the developed method may be appropriate to detect and quantify the two pesticides in the deposits tested does not guarantee its suitability for use with other unknown deposits. Considering the obtained results, a calibration curve would have to be traced, for each deposit which is intended to be analyzed. This fact is a drawback of the method, since non-contaminated deposit might not be available.

3.5 References

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Chapter 4

4 Interaction of carbofuran and chlorfenvinphos with deposits from drinking water networks

4.1 Introduction

It is a fact that water distribution systems are susceptible to contamination, either intentional or accidental. If a contaminant enters the system, its path may be short or it may reach a large part of the whole distribution system. The route of the contaminant is dependent of several factors, such as: the type and quantity of the contaminant, its point of entry, the duration of the contamination, as well as the operation and design of the distribution system. Also, depending on the type of contaminant, chemical, physical or biological phenomena might occur, along with interactions with other constituents of the water, the deposits or the pipe wall itself. It is, therefore, of extreme importance, the study of the fate and transport of the contaminant in the water distribution system.

In order to better understand the interaction of the chosen model contaminants, carbofuran and chlorfenvinphos, with the constituents of a water pipeline, particularly with the deposits formed therein, adsorption studies were conducted. Up to the author's best knowledge, there are no adsorption studies published in the open scientific literature of either of these pesticides (carbofuran and chlorfenvinphos) in deposits. There is, however, a study performed by Santos, et al. [1] regarding the adsorption and desorption of paraquat pesticide onto different types of deposits from water distribution systems (one of them is the same type of deposit used in this study; S4 deposit) and a clay. Although the pesticide studied is not a carbamate or an organophosphorus pesticide, it is relevant that one of the deposits used is the same used in this study. Santos, et al. [1] verified that the adsorption process was much slower for the deposits, than for the clay, which can be related to the mineral and organic matter contents of these materials. Langmuir maximum adsorption capacities of 8.6, 5.7, 11 and 0.40 $mg.g^{-1}$ were obtained at 20 °C for clay, S2 (brown deposit), S3 (tubercle deposit) and S4 (white deposit), respectively. S2 and S3 deposits were considered good adsorbents, and possible alternatives to other expensive materials, for paraquat remediation in contaminated waters.

Due to the lack of studies regarding, particularly, the adsorption of carbofuran and chlorfenvinphos in deposits, and in order to better comprehend the type of parameters affecting the adsorption of these pesticides, other studies regarding their adsorption in several materials were considered. The studies found for carbofuran and chlorfenvinphos adsorption are summarized in Table 4.1 and Table 4.2.

The sorption of an organic chemical, like a pesticide, on a natural solid is a complex process, which is dependent upon sorbent properties, besides the physico-chemical properties of the adsorbent itself. The sorption capacity of a given sorbent might be dependent of an array of properties such as, the specific surface area, the grain-size distribution, cation exchange capacity, pH, organic matter or organic carbon content and mineral content. In most of the studies depicted in Table 4.1 it was observed that higher organic matter content of the soils enhances adsorption of pesticides, particularly of carbofuran [2-6]. A positive correlation was found between adsorption and the cation exchange capacity of a soil [2, 7, 8], which, in turn, is related with organic matter content, since organic matter contributes from 25 to 90% for the total cation exchange capacity). An important parameter is, also, the clay content of a soil, especially when organic matter content is low [4]. Higher adsorption capacities were found for the adsorption of carbofuran in soils with higher clay content [4, 7, 9, 10], probably due to the availability of large surface area/surface charge [9]. The pH of the solution was found to influence the adsorption process of carbofuran, as well. Generally, lower pH values were more beneficial to adsorption in soil samples than higher ones [7, 8, 10]. This tendency was also observed for the adsorption of carbofuran in other materials [11-17]. However, there are studies where adsorption of carbofuran was increased with the rise of the pH of the solution. Hsieh and Kao [4] reported this fact for the adsorption of carbofuran on four lateritic soils, as did Chen, et al. [18] for the adsorption on orange peel. The effect of pH of solution is, therefore, highly dependent of the pesticide physico-chemical properties, of the chemical characteristics of the surface of the adsorbent material, as well as the relation adsorbent–adsorbate. Contradictory results were also obtained for the effect of temperature in the adsorption of carbofuran. Farahani, et al. [5] and Rama Krishna and Philip [9] observed higher adsorption of carbofuran in soils when the temperature decreased, as did Gupta, et al. [13] for the

adsorption in fertilizer and steel wastes, and Chang, et al. [11, 12] for the adsorption in rice straw derived activated carbons. On the other hand, temperature was found to enhance adsorption of carbofuran on silica [19], pyrite [14], banana stalks [17] and activated carbon from coconut frond [20]. As with the pH effect, the relation between temperature and adsorption is highly dependent on the adsorbent–adsorbate pair.

Regarding the adsorption of carbofuran on soils, its interaction is frequently considered weak, allowing for the high mobility of the pesticide [5, 10, 21]. Lalah, et al. [22] assumed that the mechanism of adsorption onto soil samples involved the amino and carbonyl groups in carbofuran, which can participate in hydrogen bonding with other O- or N-atoms present in soil colloids. Carbofuran, with C=O group, may bond to soil by H-bonding between unionized functional groups of the pesticide molecule and functional groups of the soil organic matter, such as thiols (RSH) and oxygen-containing groups like carboxyls (RCOOH) and phenols (ROH), at pH values below its pK_a [22]. El M'Rabat, et al. [23] described a similar mechanism for the adsorption of carbofuran onto homoionic montmorillonite-humic acid complexes (a clay-humic acid binary association). The authors proposed that the mechanism by which carbofuran was adsorbed involved the interaction of the C=O group and exchangeable cation of the clay humic acid complex through direct bonds or, more probably, via hydrogen bonding involving water molecules surrounding exchange cation [23].

Adsorption studies of chlorfenvinphos onto soils and other sorbent materials are summarized in Table 4.2. As was observed for the adsorption of carbofuran, higher organic matter content in a soil enhances chlorfenvinphos adsorption [24-26]. Furthermore, the quality and nature of the organic carbon content is an important parameter in chemical sorption [27]. On the other hand, when the organic carbon content of a soil is low, the contribution of clay minerals in the adsorption process becomes more significant [27]. Liu, et al. [28] verified no significant variation in the adsorption of chlorfenvinphos onto graphene-coated silica, when the pH of the solution was varied in a wide range (pH≈3-11). Studies on other pesticides like diuron, chlorpyrifos, endosulfan and malathion also showed non-dependant adsorption [29]. Liu, et al. [28] discussed the adsorption mechanism, and considered that the major factors affecting adsorption should be the effect of the P atoms and hydrophobic

interactions, further concluding that the electron-donating ability of the P atom and the strong π-bonding network of benzene ring promoted the adsorption. Taha, et al. [30] also explained the mechanism of adsorption of 15 pesticides (featuring chlorfenvinphos) onto biochar and charcoal, and concluded that the binding forces for most of the pesticides tested on the studied adsorbents may involve primary π-π dispersive interactions, van der Waals forces, and H-bonding via the highly polar bond PO (in the case of chlorfenvinphos).

Regarding adsorption isotherms, Langmuir and Freundlich models were the ones that better described the adsorption of carbofuran and chlorfenvinphos in soils and in other adsorbent materials. The shapes of the isotherms were, usually, of L-type [8, 10, 20, 26-28], according to the Giles classification [31]. For the adsorption of carbofuran, there were also some studies that reported isotherms of S-type [8, 10, 23] and C-type [6]. According to Giles, et al. [31], L-type isotherms indicate that the pesticide molecules are most likely to be adsorbed in a flat position and that they do not suffer a strong competition from solvent molecules. Type-S isotherms indicate low adsorbate-adsorbent affinity, which increases with the rise in the concentration of the adsorbate in solution. The C-type curve implies conditions in which the number of sites remains constant throughout the whole range of solute concentrations (though not necessarily of equal energy); type-C curves are usually associated with low affinity of the solute for the substrate [31].

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents.

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Distribution adsorption constants (linear isotherm): Sandy soil: K_d = 0.25 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Sandy loam soil: K_d = 0.74 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silt clay soil: K_d = 1.40 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silt clay loam soil: K_d = 1.13 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silt loam soil: K_d = 1.39 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silt clay loam soil: K_d = 2.22 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Muck soil: K_d = 8.74 μg.g^{-1}.(μg.mL^{-1})$^{-1}$	- The adsorption of the pesticide was positively correlated with organic carbon content of the soils; - There was also a positive correlation between K_d and cation exchange capacity of the soil, because organic matter is known to contribute from 25 to 90% of the total exchange capacity of many soils; - There is a direct relationship between carbofuran adsorption and mobility in soil. Carbofuran mobility was inversely proportional to organic carbon content of the soils; - Carbofuran was very mobile in the agricultural soils studied.	[2] (1980)
Soil	Distribution adsorption constants (linear isotherm) at 18 °C: Clay soil: K_d = 0.1280 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silty clay loam soil: K_d = 0.6652 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Distribution adsorption constants (linear isotherm) at 25 °C: Clay soil: K_d = 0.1448 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silty clay loam soil: K_d = 0.4396 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Distribution adsorption constants (linear isotherm) at 35 °C: Clay soil: K_d = 0.2639 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silty clay loam soil: K_d = 0.7611 μg.g^{-1}.(μg.mL^{-1})$^{-1}$	- Carbofuran adsorption was higher on the silty clay loam soil, which has a higher organic carbon partition coefficient; - The dissipation rate of carbofuran in soil was increased with increasing soil temperature and moisture content in both soils.	[3] (1997)
Soil	Distribution adsorption constants range for various solution/soil ratios: Sandy loam soil: K_d = 0.047-0.19 mg.g^{-1}.(μg.mL^{-1})$^{-1}$ Clay loam soil: K_d = 0.057-0.40 mg.g^{-1}.(μg.mL^{-1})$^{-1}$ Clay soil: K_d = 0.071-0.47 mg.g^{-1}.(μg.mL^{-1})$^{-1}$ Silty clay loam soil: K_d = 0.17-97 mg.g^{-1}.(μg.mL^{-1})$^{-1}$ Freundlich adsorption constants range for various solution/soil ratios: Sandy loam soil: K_F = 0.009-0.225 mg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.2-1.623 Clay loam soil: K_F = 0.073-0.269 mg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 0.931-1.223 Clay soil: K_F = 0.061-0.366 mg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.029-1.158 Silty clay loam soil: K_F = 0.246-0.875 mg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 0.888-1.026	- The calculated K_d values for the same soil increased with the increase in solution/soil ratio and soil organic content; - The K_d values are extremely dependent on the concentration of carbofuran; - Low values of K_d implied that carbofuran had a lower affinity for the four lateritic soils; - High K_F and n values of silty clay loam soil indicated higher adsorption of carbofuran on this type of soil; - The key influencing factor for carbofuran adsorption onto untreated soils appeared to be pH. Higher pH conditions increase adsorption; - The sorption potential of mineral surfaces in surface soils is blocked by organic matter; - The extent to which clay minerals contribute to sorption depends on both the ratio of clay mineral to organic carbon fraction of the soil and on the nature of the organic sorbate. The type of soil clay becomes more important when soil organic carbon contents are low.	[4] (1998)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Freundlich adsorption constants: Ahero soil - K_F = 1.7 $mg.g^{-1}.(mg.L^{-1})^{-1/n}$; n = 1.0 Nairobi soil – K_F = 1.5 $mg.g^{-1}.(mg.L^{-1})^{-1/n}$; n = 1.0	- Carbofuran adsorbed strongly to both soil samples; - Adsorption was higher in soil samples containing more organic carbon content (Ahero, Nairobi) than in sandy soils; - The amino (—NH) and carbonyl groups (—C=O) in carbofuran can participate in hydrogen bonding with other O— or N— atoms present in the soil colloids; - Carbofuran, with C=O group, bonds to soil by H-bonding between unionized functional groups of the pesticide molecule and functional groups of soil organic matter, such as RSH, RCOOH and ROH, at pH values below its pK_a.	[22] (2001)
Soil	Distribution adsorption constants: Sandy loam soil: K_d = 0.17 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Loamy sand soil: K_d = 1.02 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Silty clay loam soil: K_d = 0.93 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Loam soil: K_d = 0.73 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Loam soil: K_d = 1.15 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Sandy soil: K_d = 1.78 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Freundlich adsorption constants: Sandy loam soil: K_F = 0.20 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.96 Loamy sand soil: K_F = 3.36 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.69 Silty clay loam soil: K_F = 2.30 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.77 Loam soil: K_F = 4.66 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.53 Loam soil: K_F = 5.34 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.61 Sandy soil: K_F = 8.12 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.61	- A positive correlation was found between the K_F constants and the soil organic matter contents; - There was no correlation with other soil variables such as clay or silt; - Organic matter is the main soil parameter that affects the adsorption of carbofuran, in the soils studied; - The values of the adsorption constants referred to the organic matter content corroborate the affinity of carbofuran for this fraction of soil.	[32] (2002)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Distribution adsorption constants for batch method: Soil I: K_d = 4.05 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil II: K_d = 2.71 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil III: K_d = 1.43 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil IV: K_d = 2.11 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil V: K_d = 3.76 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil VI: K_d = 3.28 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil VII: K_d = 2.37 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Soil VIII: K_d = 3.57 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1}$ Freundlich adsorption constants for batch method: Soil I: K_F = 4.58 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.18 Soil II: K_F = 3.29 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.14 Soil III: K_F = 1.29 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.91 Soil IV: K_F = 1.91 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.97 Soil V: K_F = 3.77 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.93 Soil VI: K_F = 2.88 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.89 Soil VII: K_F = 3.16 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.39 Soil VIII: K_F = 3.53 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.99	- The greatest value of K_d was obtained from Soil I. This may attributed to the organic carbon content of Soil I; - Sorption increases with increasing concentration of the sorbing molecule on the sorbent; - Sorption results show that carbofuran is not highly sorbed by soil, and consequently, sorption is not significantly influenced by soil physical and chemical properties in general; - Sorption coefficients (K_d, K_F, n) are correlated with soil properties, which might affect sorption of the pesticide on soils; - No statistically significant correlations were found among the whole samples. The adsorption process must be a combination of effects of different soil properties such as clay content, organic carbon and cation exchange capacity.	[33] (2005)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Freundlich adsorption constants for batch method: Soil I: K_F = 0.43 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.33 Soil II: K_F = 0.46 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.47 Soil III: K_F = 0.36 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.45 Soil IV: K_F = 0.52 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$; n = 1.64	- K_F and n values were in the same order for all soils, even though soils I and II have larger contents of clay and organic matter than soils III and IV; - The K_d values decreased while increasing initial carbofuran concentration. Adsorption was higher for the soils with higher cationic exchange capacity. For lower pH levels in soils, adsorption increases; - Carbofuran might adsorb to high-affinity sites at low concentrations (specific adsorption) and to low-affinity sites at high concentrations (non-specific adsorption); - Carbofuran under acid media has a positive charge, thus adsorption might be taking place via electrostatic interactions; - Adsorption was higher when the pH was lower. Cationic carbofuran percentage was lower at high pHs which reduces electrostatic interactions with the negative charge of the soil; - Carbofuran adsorption at different pHs was higher for soils I and II (with higher contents of clay and organic matter) than for III and IV.	[7] (2006)
Soil	Distribution adsorption constants range for 43 soils: K_d = 0.11 – 4.1 mg.kg^{-1}.(mg.L^{-1})$^{-1}$	- Only in some soils, the sorption coefficients followed the trend of organic carbon contents; - Given that carbofuran is nonionic in nature, the pH is not expected to affect the sorption directly but may have indirect effects such as by its influence on the nature of organic carbon; - Soil organic content alone is not able to adequately account for the observed variation in adsorption.	[34] (2006)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Distribution adsorption constants at 25 °C: Sandy clay soil (low organic carbon content): K_d = 1.1537 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Clay soil (high organic carbon content): K_d = 2.8003 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Distribution adsorption constants at 35 °C: Sandy clay soil:K_d = 0.9885 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Clay soil:K_d = 2.7931 μg.g^{-1}.(μg.mL^{-1})$^{-1}$ Freundlich adsorption constants at 25 °C: Sandy clay soil: K_F = 1.09 μg.g^{-1}.(μg.mL^{-1})$^{-1/n}$; n = 0.85 Clay soil: K_F = 2.82 μg.g^{-1}.(μg.mL^{-1})$^{-1/n}$; n = 0.99 Freundlich adsorption constants at 35 °C: Sandy clay soil: K_F = 1.71 μg.g^{-1}.(μg.mL^{-1})$^{-1/n}$; n = 0.83 Clay soil: K_F = 2.78 μg.g^{-1}.(μg.mL^{-1})$^{-1/n}$; n = 0.77	- The percentage of carbofuran adsorbed was higher in clay than in sandy clay soil, probably due to the higher organic content in the clay soil; - An increase in temperature caused decrease in adsorption for both soil types; - At higher concentrations, the difference due to temperature was small, this could be due to saturation of adsorption sites; - For both soils, the sorption coefficient decreased with rise in temperature, probably due to the effect of temperature on the weak binding between carbofuran and the soil particles.	[5] (2007)
Soil	Adsorption rate constants (intraparticle model): Sandy soil: k_{ip} = 0.255 mg.g^{-1}.h$^{-1/2}$ Red soil: k_{ip} = 0.5105 mg.g^{-1}.h$^{-1/2}$ Clay soil: k_{ip} = 0.765 mg.g^{-1}.h$^{-1/2}$ Compost soil: k_{ip} = 0.9266 mg.g^{-1}.h$^{-1/2}$ Freundlich adsorption constants: Sandy soil: K_F = 0.91 mg.g^{-1}.(mg.mL^{-1})$^{-1/n}$; n = 1.05 Red soil: K_F = 3.53 mg.g^{-1}.(mg.mL^{-1})$^{-1/n}$; n = 0.99 Clay soil: K_F = 8.29 mg.g^{-1}.(mg.mL^{-1})$^{-1/n}$; n = 0.93 Compost soil: K_F = 11.41 mg.g^{-1}.(mg.mL^{-1})$^{-1/n}$; n = 0.88	- The adsorption kinetics exhibited two distinct stages, a very rapid adsorption in the initial stages (within 1 hour) followed by a slow adsorption; - Kinetic results were best fitted by intraparticle diffusion model; - The organic matter content of compost soil was much higher than for the other soils. This must be the reason for high adsorption capacity of compost soil. K_F increased with organic matter content; - The mobility of pesticides also depends on the composition of clay and the nature of the major cations in the soil solution; - High clay content also increases the adsorption due to the availability of large surface area/surface charge; - Adsorption was exothermic and an increase in temperature will result in decreased sorption and favors desorption process.	[9] (2008)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Freundlich adsorption constants: Soil I: K_F = 2.51 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.01 Soil III: K_F = 1.10 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.93	- The values of K_F were higher for Soil I, which has a higher organic matter content; - The organic content of the soil is the component that most influences the adsorption for some insecticides as carbofuran; - The low adsorption coefficients and its relatively high solubility in water indicate that carbofuran is a mobile to moderately mobile compound.	[35] (2008)
Soil	Freundlich adsorption constants: Soil I: K_F = 151.36 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.72 Soil II: K_F = 114.82 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.47 Soil III: K_F = 96.60 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 1.39 Soil IV: K_F = 9.12 $\mu g.g^{-1}.(\mu g.mL^{-1})^{-1/n}$; n = 0.73	- The higher adsorption onto Soil I may be due to the greater amount of organic matter, clay content or lower pH; - n values higher than 1 indicating the non-linearity between the solution equilibrium concentration and adsorption. This may be due to specific interactions existing between compounds with polar groups and the organic matter or the mineral fraction of the soils; - Carbofuran adsorption by soils showed to be an exothermic spontaneous process, promoted by weak physical forces.	[10] (2009)
Soil	Freundlich adsorption constants: $K_F = 7.07\times10^{-5}$ $mg.kg^{-1}.(mg.L^{-1})^{-1/n}$; n = 0.40	- The low affinity in soil particles might be due to low content of soil organic matter; - Adsorption process was nonspontaneous.	[36] (2010)
Soil	Freundlich adsorption constants: Soil I: K_F = 151 $mg.kg^{-1}.(mg.mL^{-1})^{-1/n}$; n = 1.72 Soil II: K_F = 115 $mg.kg^{-1}.(mg.mL^{-1})^{-1/n}$; n = 1.47 Soil III: K_F = 97 $mg.kg^{-1}.(mg.mL^{-1})^{-1/n}$; n = 1.39 Soil IV: K_F = 9 $mg.kg^{-1}.(mg.mL^{-1})^{-1/n}$; n = 0.73	- Higher values of K_F were observed for soils with higher organic matter content; - The n values were higher than 1, which indicates the non-linearity between solution equilibrium concentration and adsorption. The lack of linearity may be attributed to specific interactions existing between compounds with polar groups and the organic matter or the mineral fraction of the soils; - There is a positive correlation between K_F and the organic carbon, clay content, clay plus silt content, and cation exchange capacity of the soils. There was a negative correlation of the constants with pH (higher pH, lower adsorption).	[8] (2011)
Soil	Freundlich adsorption constants range for 16 soil samples: K_F = 0.6 – 8.7 $\mu mol.kg^{-1}.(\mu mol.L^{-1})^{-1/n}$; n = 0.40 – 0.95	- K_F values were especially correlated with soil organic carbon; - Adsorption of carbofuran is influenced by soil organic carbon and clay. Also the irreversibility of carbofuran retention depends strongly on the organic carbon content of the soil.	[37] (2011)
Soil	----------	- Soil I (0.62% organic matter content; 8% clay content); Soil II (0.78% organic matter content; 25% clay content); Soil III (1.71% organic matter content; 17% clay content); - Percentage of adsorption of carbofuran was: Soil III>Soil II>Soil I; - Organic matter and clay content seem to influence positively carbofuran adsorption.	[38] (2012)
Soil	Freundlich adsorption constants: Soil I (clay soil): K_F = 9.2 $\mu mol.kg^{-1}.(\mu mol.L^{-1})^{-1/n}$ n = 0.99 Soil II (clay loam soil): K_F = 1.1 $\mu mol.kg^{-1}.(\mu mol.L^{-1})^{-1/n}$ n = 0.95	- Although carbofuran was not highly adsorbed by the soils, K_F values were significantly higher in the soil with high organic matter (soil I) than in the soil with low organic matter (soil II); - The irreversibility of carbofuran adsorption was higher in the soil with high organic content (soil I).	[6] (2012)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	Freundlich adsorption constants: Silty clay soil: K_F = 6.59 $mg.kg^{-1}.(mg.L^{-1})^{-1/n}$ n = 0.83	- Carbofuran tend to be adsorbed more into silty clay soil when the concentrations in solutions increased; - Carbofuran showed to be weakly adsorbed to the soil, thus is moderately mobile in the silty clay soil.	[21] (2013)
Silica	Freundlich adsorption constants at 15 °C: $K_F = 2.33\times10^{-5}$ $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 0.92 Freundlich adsorption constants at 25 °C: $K_F = 4.00\times10^{-5}$ $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 0.78	- The isotherms are S shaped at both temperatures and include two plateaus. This indicates that the leading factor in adsorption is the interaction between solute and solvent and not the solute – solid interaction; - Carbofuran adsorption tendency is rather weak, although effective; - The adsorption of carbofuran appears to increase with temperature; - Solubility limits play a role in the adsorption process, and it was demonstrated that adsorption increases close to this limits; - Carbofuran has the characteristics of a weakly fixed pesticide on soils.	[19] (1996)
Homoionic montmorillonite – humic acid complex	Freundlich adsorption constants at 15 °C: Na-M-HA: K_F = 8.84 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 1.41 K-M-HA: K_F = 15.27 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 0.91 Ba-M-HA: K_F = 2.92 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 1.37 HA: K_F = 125.46 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 1.95 Ca-M-HA: K_F = 5.38 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 0.96 Cu-M-HA: K_F = 0.30 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 1.58 Mg-M-HA: K_F = 4.97 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 1.23 Al-M-HA: K_F = 157.59 $mg.g^{-1}.(\mu g.mL^{-1})^{-1/n}$ n = 0.38	- For HA, Na-M-HA, Ba-M-HA, Cu-M-HA and mg-M-HA samples, isotherms were of S-type (n>1), indicating low adsorbate-adsorbent affinity, which increases with the rise in the concentration of adsorbate in solution; - For K-M-HA, Ca-M-HA and Al-M-HA samples, isotherms were L-type (n<1), in which the solute has such high affinity that in dilute solutions it is completely adsorbed; - The mechanism by which carbofuran is adsorbed involves the interaction of C=O group of carbofuran and exchangeable cation of the clay-humic acid complex through direct bonds or, more probably, via hydrogen bonding involving water molecules surrounding exchange cation.	[23] (2001)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Fertilizer and steel industry wastes	*Adsorbability at 25 °C:* Standard activated charcoal: 266.0 $mg.g^{-1}$ Carbonaceous adsorbent: 208.3 $mg.g^{-1}$ Blast furnace sludge: 23.0 $mg.g^{-1}$ Blast furnace dust: 13.0 $mg.g^{-1}$ Blast furnace slag: negligible Carbonaceous adsorbent (45 °C): 150.2 $mg.g^{-1}$ *For the carbonaceous adsorbent prepared from carbon slurry:* Langmuir adsorption constants at 25 °C and pH 7.5: q_{max} = 303.0 $mg.g^{-1}$; $K_L = 22.1\times10^{-3}$ $L.mg^{-1}$ Langmuir adsorption constants at 35 °C and pH 7.5: q_{max} = 277.7 $mg.g^{-1}$; $K_L = 16.3\times10^{-3}$ $L.mg^{-1}$ Langmuir adsorption constants at 45 °C and pH 7.5: q_{max} = 250.0 $mg.g^{-1}$; $K_L = 11.0\times10^{-3}$ $L.mg^{-1}$	- The carbonaceous adsorbent which has maximum surface area and porosity adsorbs pesticide to maximum extent whereas other three adsorbents having low surface area show little or negligible adsorption; - Since adsorption depends on the surface area, it is reasonable to assume that the adsorption, to some extent, is a surface phenomenon where van der Waals forces operate; - Adsorption could also be occurring through hydrogen bonding with hydrophilic sites or groups on carbon surface formed at the time of activation - Adsorption decreased with increase of the solution pH; - Adsorption increases with decrease in temperature, indicating that the process is exothermic.	[13] (2006)
Chestnut shells	Langmuir adsorption constants: q_{max} = 10.8 $mol.g^{-1}$; $K_L = 5.2\times10^{4}$ $mol.dm^{-3}$	- The sorption process is thermodynamically favorable, spontaneous and endothermic in nature for carbofuran.	[39] (2007)
Activated carbon	Langmuir adsorption constants: q_{max} = 96.15 $mg.g^{-1}$; K_L = 0.129 $L.mg^{-1}$	- Better fitting to Langmuir isotherm suggests an homogeneous distribution of active sites onto activated carbon surface; - Higher adsorption was obtained for lower values of pH. This suggests that the adsorption was dominated by the interaction between pesticide and adsorbent surface.	[40] (2010)
Banana stalks activated carbon	Langmuir adsorption constants at 30 °C: q_{max} = 156.3 $mg.g^{-1}$; K_L = 0.26 $L.mg^{-1}$ Langmuir adsorption constants at 40 °C: q_{max} = 161.3 $mg.g^{-1}$; K_L = 0.35 $L.mg^{-1}$ Langmuir adsorption constants at 50 °C: q_{max} = 164.0 $mg.g^{-1}$; K_L = 0.41 $L.mg^{-1}$	- Data was well fitted by Langmuir isotherm model, indicating an homogeneous nature of the activated carbon surface , in which the molecule/carbon adsorption had equal adsorption activation energy; - Adsorption capacity increased with the increase of temperature, indicating an endothermic process.	[17] (2010)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Rice straw agricultural waste	Adsorption capacity: Rice straw: 296.52 $mg.g^{-1}$	- Carbofuran adsorption by NaOH treated rice straw adsorbents increases with increase in initial concentration of pesticide; - Adsorption capacity decreased with increasing temperature. Maybe due to the tendency for the target molecules to escape from the solid phase to the bulk phase with an increase in temperature of the solution. - As pH increased, adsorption capacity decreased. At low pH (acidic) of the solution the carbon surface is predominantly positively charged, whereas at strongly basic pH, negative charges appear on the surface. It also suggests a weaker interaction of activated carbon surface with deprotonated carbofuran than with its neutral molecular form.	[12] (2011)
Date seed activated carbon	Freundlich adsorption constants: K_F = 13.03 $mg.g^{-1}.(mg.L^{-1})^{-1/n}$; n = 0.37	- The uptake of carbofuran by the adsorbent may involve multilayer adsorption with interactions between the pesticide molecules and the heterogeneous surface of the adsorbent; - In the range of pH studied, an increase in the pH of the solution showed no significant variation in the adsorption of carbofuran onto the adsorbent. Carbofuran is mainly non-dissociated, in the range of pH studied, being the neutral molecule the main structure in the solution. No electrostatic interactions exist between the pesticide and the surface of the adsorbent.	[41] (2011)
Hemp fibers activated carbon	Langmuir adsorption constants: q_{max} = 15.73 $mg.g^{-1}$; K_L = 25.02 $L.mg^{-1}$	- Data was well fitted by Langmuir isotherm model, indicating a homogeneous nature of the activated carbon surface.	[42] (2011)
Pyrite	Langmuir adsorption constants at 25 °C: pH = 1.77: $q_{max} = 3.68\times10^{-6}$ $mol.m^{-2}$; K_L = 317 $m^3.mol^{-1}$ pH = 2.70: $q_{max} = 2.20\times10^{-6}$ $mol.m^{-2}$; K_L = 189 $m^3.mol^{-1}$ pH = 4.01: $q_{max} = 1.05\times10^{-6}$ $mol.m^{-2}$; K_L = 78 $m^3.mol^{-1}$ Langmuir adsorption constants at 40 °C: pH = 1.77: $q_{max} = 3.90\times10^{-6}$ $mol.m^{-2}$; K_L = 410 $m^3.mol^{-1}$ pH = 2.70: $q_{max} = 2.36\times10^{-6}$ $mol.m^{-2}$; K_L = 194 $m^3.mol^{-1}$ pH = 4.01: $q_{max} = 1.35\times10^{-6}$ $mol.m^{-2}$; K_L = 87 $m^3.mol^{-1}$ Langmuir adsorption constants at 60 °C: pH = 1.77: $q_{max} = 4.20\times10^{-6}$ $mol.m^{-2}$; K_L = 511 $m^3.mol^{-1}$ pH = 2.70: $q_{max} = 2.53\times10^{-6}$ $mol.m^{-2}$; K_L = 220 $m^3.mol^{-1}$ pH = 4.01: $q_{max} = 1.50\times10^{-6}$ $mol.m^{-2}$; K_L = 92 $m^3.mol^{-1}$	- Adsorption decreased with the increase of the solution pH. When pH<4.01, carbofuran is essentially not dissociated, however, the degree of surface speciation of pyrite is different, which seems to control the carbofuran retention; - Carbofuran adsorption appears to increase with the increase of temperature, which suggests the endothermic nature of the process. Thermodynamic study of the process allowed to conclude that carbofuran adsorption is mainly an entropy driven process.	[14] (2012)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Orange peel	Langmuir adsorption constants at 30 °C: q_{max} = 84.49 mg.g^{-1}; K_L = 0.0124 L.mg^{-1}	- Data was well fitted by Langmuir isotherm model, indicating a monolayer coverage of carbofuran onto orange peel; - Equilibrium adsorption was practically achieved in 60 minutes; - Adsorption capacity increased with the increase the initial pH values. This effect can be explained considering the surface charge on the adsorbate, in the range of pHs studied. - At lower pH carbofuran molecule might have become a positively charged ion that tends to compete with other protons for positively charged sites in the adsorbent; - At higher pH, the adsorbent surface may be negatively charged, enhancing carbofuran cations through electrostatic forces of attraction.	[18] (2012)
Palm oil fronds activated carbon	Langmuir adsorption constants at 30 °C: q_{max} = 164 mg.g^{-1}; K_L = 0.236 L.mg^{-1}	- Data was well fitted by Langmuir isotherm model, indicating a monolayer coverage of carbofuran onto the adsorbent; - Equilibrium adsorption decreased slightly when the initial pH of the solution was increased. The surface charge would depend on the solution pH and the surface characteristics of the sorbent. Also, a change in pH would alter the properties of the pesticide molecules and consequently its adsorption uptake.	[16] (2013)
Walnut shells	Freundlich adsorption constants: K_F = 22.9×10^{-3} mol.g^{-1}.(mol.L^{-1})$^{-1/n}$; n = 2.63	- Adsorption percentage decreased with increasing pH. At very low pHs the surface of the walnut shell would be surrounded by hydronium ions, which may enhance the pesticide interaction with binding sites of the adsorbent; - Equilibrium adsorption was practically achieved at 30 minutes;	[15] (2014)
Humic acid copolymer	Freundlich adsorption constants for untreated humic acid: K_F = 1.12×10^{-2} mg.kg^{-1}.(mg.L^{-1})$^{-1/n}$; n = 3.51 Freundlich adsorption constants for humic acid graft copolymer: K_F = 703.75 mg.kg^{-1}.(mg.L^{-1})$^{-1/n}$; n = 0.77	- Humic acid graft copolymer has the capability to sorb more pesticide than the untreated humic acid; - The sorption ability of carbofuran, containing a hydrophobic aromatic ring, on the humic acid graft copolymer, with more phenyl rings than the untreated humic acid, will naturally increase.	[43] (2014)
Animal bone meal	Freundlich adsorption constants: K_F = 14.78 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$; n = 2.10	- n value indicate that the isotherm is favorable; - Equilibrium in adsorption was obtained at 70 minutes; - Adsorption increased with the increase in the pH of the solution.	[44] (2014)

Table 4.1 – State of the art on the adsorption of carbofuran onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Coconut frond activated carbon	Freundlich adsorption constants at 30 °C: K_F = 31.05 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$ n = 0.402 Freundlich adsorption constants at 40 °C: K_F = 38.40 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$ n = 0.365 Freundlich adsorption constants at 50 °C: K_F = 41.76 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$ n = 0.366	- The uptake of carbofuran by the heterogeneous surface of the adsorbent may involve multilayer adsorption; - Adsorption equilibrium was achieved after 4 hours; - Adsorption increased with the increase in temperature; - There was no significant variation in the amount of carbofuran adsorbed with the increase in pH, due to the nonionic nature of carbofuran (pK_a = 11.95). The surface of the adsorbent is positively charged at pH below 5.8 and negatively charged at pH above 5.8; - Highest adsorption capacity was maintained until the point of pK_a value at 11.90 and later decreased.	[20] (2014)
Rice straw derived activated carbons	Langmuir adsorption constants at 30 °C: q_{max} = 164 mg.g^{-1}; K_L = 0.236 L.mg^{-1} Langmuir adsorption constants at 30 °C: q_{max} = 164 mg.g^{-1}; K_L = 0.236 L.mg^{-1} Langmuir adsorption constants at 30 °C: q_{max} = 164 mg.g^{-1}; K_L = 0.236 L.mg^{-1}	- Adsorption of carbofuran onto the adsorbent generates a monolayer; - Equilibrium of adsorption was reached in 90 minutes; - The adsorptive capacity of the adsorbent decreased when the temperature increased, probably due to a higher tendency of the molecules to escape from the solid phase to the bulk phase with the increase in the temperature solution; - Adsorption capacity decreased with the increase in the pH of the solution; - At low pH values the surface of the adsorbent is predominantly positively charged, whereas at high pH values it is more negatively charged. At higher pH values, the interaction of the adsorbent with the deprotonated carbofuran should be weaker than with its neutral molecular form.	[11] (2014)

Table 4.2 – State of the art on the adsorption of chlorfenvinphos onto soils, clays and other adsorbents.

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Soil	----------	- Chlorfenvinphos was more persistent in the non-sterile organic soil as compared to the non-sterile sandy loam soil; - Its persistence was higher in the sterile soils as compared to non-sterile soils; - Microbial degradation played a major role in the degradation of chlorfenvinphos.	[24] (1979)
Soil	Freundlich adsorption constants for a soil: K_F = 2.7 mg.kg^{-1}.(mg.kg^{-1})$^{-1/n}$ n = 1.05 Freundlich adsorption constants range for a soil treated with organic fertilizers: K_F = 1.9 – 12.4 mg.kg^{-1}.(mg.kg^{-1})$^{-1/n}$ n = 1.01 – 1.06	- Within the same trial, there is a clear relationship between the soil organic carbon content and the insecticide adsorption coefficients. The recent organic fertilizer treatments only slightly increase the soil organic carbon content, but have a large influence onto the insecticide adsorption coefficients; - Recent organic fertilizer treatments, and fresh soil organic matter are the most able to adsorb the insecticide and increase their soil persistence, relative to the old organic fertilizer treatments and old soil organic matter.	[25] (1996)
Bentonite and kaolinite	Maximum adsorption quantity at 25 °C: Kaolinite: 163.3 µg.g^{-1} Bentonite: 758 µg.g^{-1}	- Adsorption equilibrium was reached within 2 hours; - The adsorbed quantity per mass unit of clay is lower for kaolinite than for bentonite. However, the adsorbed quantities per surface unit are 3 times higher for kaolinite than for bentonite; - Kaolinite active sites must be completely occupied, while bentonite sites must be only partially covered; - Chlorfenvinphos adsorption process was faster onto kaolinite.	[45] (1990)
Natural organic substances	----------	- From the various natural organic adsorbents tested, date stones showed the higher pesticide removal efficiency (92%), followed by olive stones (84%). The lowest chlorfenvinphos removal efficiency was obtained for *Nerium oleander* leaves (45%).	[46] (2009)
Graphene-coated silica	Freundlich adsorption constants, for chlorfenvinphos: K_F = 1.18 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$ n = 1.48 Freundlich adsorption constants for chlorfenvinphos in a mix of 11 organophosphorus pesticides: K_F = 1.72 mg.g^{-1}.(mg.L^{-1})$^{-1/n}$ n = 0.95	- There was no significant variation in the adsorption, in the range of pHs studied (pH≈3-11); - Freundlich fitted well experimental data; - The mechanism of adsorption of organophosphorus pesticides on the adsorbent is based on the electron-donating abilities of P, S, and N atoms and the strong π-bonding network of benzene rings.	[28] (2013)

Table 4.2 – State of the art on the adsorption of chlorfenvinphos onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Organic residues and soil	Freundlich adsorption constants: Soil: K_F = 0.011 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.90 2% RO1: K_F = 0.027 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.84 5% RO1: K_F = 0.060 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.84 10% RO1: K_F = 0.197 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.77 2% RO2: K_F = 0.018 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.91 5% RO2: K_F = 0.192 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.60 10% RO2: K_F = 0.371 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.59 2% RO3: K_F = 0.044 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.76 5% RO3: K_F = 0.077 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.84 10% RO3: K_F = 0.270 μg.g^{-1}.(μg.L^{-1})$^{-1/n}$ n = 0.70	- Freundlich isotherm model fitted well experimental results; - The lack of linearity of the isotherm can be due to specific interactions between polar groups of the pesticide and the organic matter of the substrate; - As the percentage of organic residue, added to the soil, increased the sorption capacity of the pesticide rose, probably because of the increase in the number of active sites; - Chlorfenvinphos was highly sorbed despite its relatively high water solubility. Other factors, beside its solubility in water, could be important for the adsorption of this pesticide, such as the presence of different functional groups of high affinity for the adsorbate; - K_F values increased with increasing organic matter content of the soil samples. Exogenous addition of organic matter resulted in increased adsorption capacity; - Organic matter content but also the nature of it plays a significant role in pesticide adsorption.	[26] (2013)
Biochar and charcoal	Distribution adsorption constants range: K_d ≈ 148 - 200 μg.g^{-1}.(μg.mL^{-1})$^{-1}$	- When comparing the properties of the different adsorbents, the surface area was found to be the most important factor in the adsorption of pesticides from aqueous solution; - Pesticides with a higher degree of hydrophobicity (ex. chlorfenvinphos) show higher adsorption ability on the studied adsorbents. Sorption of high polarity pesticides require longer adsorption contact times since water molecules compete for the polar functional groups of the sorbents; - The binding forces for most of the pesticides on the studied adsorbents may involve primary π-π dispersive interaction, van der Waals forces and H-bonding (via carbonyl group, oxygen, the nitro group oxygen, and the highly polar bonds (C – F, P=S and PO).	[30] (2014)

Table 4.2 – State of the art on the adsorption of chlorfenvinphos onto soils, clays and other adsorbents (cont.).

Adsorbent	Adsorption capacity and model parameters	Coments	Reference (Year)
Organic substances and soil	Adsorption rate constants (intraparticle model): OR1: k_{ip} = 0.192 µg.g^{-1}.min$^{-1/2}$ OR2: k_{ip} = 0.250 µg.g^{-1}.min$^{-1/2}$ OR3: k_{ip} = 0.100 µg.g^{-1}.min$^{-1/2}$ OR4: k_{ip} = 0.350 µg.g^{-1}.min$^{-1/2}$ Freundlich adsorption constants: OR1: K_F = 435 µg.g^{-1}.(µg.L^{-1})$^{-1/n}$ n = 0.49 OR2: K_F = 646 µg.g^{-1}.(µg.L^{-1})$^{-1/n}$ n = 0.55 OR3: K_F = 230 µg.g^{-1}.(µg.L^{-1})$^{-1/n}$ n = 0.56 Soil: K_F = 667 µg.g^{-1}.(µg.L^{-1})$^{-1/n}$ n = 0.54	- Freundlich isotherm model fitted best experimental data; - The similar and low sorption of chlorfenvinphos was evident for all the organic residues. The adsorption onto soil was slightly higher; - When comparing with the other pesticides studied, chlorfenvinphos showed lower values of K_F, probably due to its polar nature, and hydrophilicity; - Though the soil had the lower percentage of organic carbon content, chlorfenvinphos adsorption capacity was the highest for this adsorbent. This might indicate the contribution of soil mineral components in the adsorption.	[27] (2015)

4.2 Experimental section

4.2.1 Chemicals and reagents

Carbofuran (2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl-methylcarbamate) with a purity of 99.9%, and chlorfenvinphos standards ([EZ]-2-chloro-1-(2,4-dichlorophenyl)ethenyl diethyl phosphate) were purchased from Fluka, Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) was HPLC grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

4.2.2 Adsorbent preparation

The interaction studies of carbofuran and chlorfenvinphos were performed with S4 deposit, which is, as mentioned before, a real deposit collected from drinking water distribution systems. The preparation of the adsorbent for these studies was similar to that described previously (cf. section 3.2.4), in the development of the method for the quantification of the pesticides in deposits. The main properties of S4 deposit are described in Table 4.3.

Table 4.3 - Physical-chemical properties of S4 deposit (from [47])**.**

Deposit classification	**White**
ICP-OES analysis (wt.% of the main elements at dry basis)	Ca: 97 Fe: 1 Mg: 1
S_{BET} ($m^2.g^{-1}$)	1
pH_{pzc}, 20 °C	9.9
pH in water, 20 °C	9.0
Main components identified by XRD	Calcite ($CaCO_3$)
Organic matter content (wt.%)	0.2

4.2.3 Adsorption experiments

In the adsorption process, the effect of deposit dose (2.5 – 10 $g.L^{-1}$), initial concentration of pesticide (1 – 50 $mg.L^{-1}$), particle size of adsorbent (106.5 – 225 µm in average), stirring speed (405 – 672 rpm) and temperature (4 – 20 °C) were studied by batch experiments for a specific period of time (0 – 10080 min (7 days)).

For kinetic studies, which allowed for the determination of the equilibrium time, the experiments were performed keeping constant the adsorbent quantity (5 $g.L^{-1}$). In each experiment, 10 mL of a pesticide (carbofuran or chlorfenvinphos) solution of 20 $mg.L^{-1}$ was put in contact with the deposit. The flasks were placed inside a Lovibond thermostatic cabinet (Dortmund, Germany) at constant temperature and the solutions were continuously stirred for a certain period of time. After stirring, the solution was centrifuged at 4000 rpm for 10 min and the supernatant was recovered and afterwards analysed by high pressure liquid chromatography with diode array detector (HPLC-DAD) (as described in section 2.2). All experiments were performed in duplicate and analysed twice, in order to guarantee reproducibility of the results.

After the kinetic assays, the equilibrium time was set and the adsorption isotherms were determined, varying the adsorbent/adsorbate mass ratio. The procedure was similar to the one describe above.

The adsorption capacity (q_t in $mg.g^{-1}$) was calculated using equation 4.1:

$$q_t = \frac{(C_0 - C_e)V}{m} \qquad 4.1$$

where C_0 and C_e are the concentrations of carbofuran or chlorfenvinphos at initial and equilibrium time, respectively ($mg.L^{-1}$), V the volume of the aqueous phase (L) and m is the mass of adsorbent used (g).

4.3 Results and discussion

In order to better understand the mechanism of adsorption of carbofuran and chlorfenvinphos onto S4 deposit and the factors affecting it, kinetic and equilibrium experiments were realized. This adsorption study is of great importance to comprehend the interaction of both pesticides with the deposit, so that, in the eventuality of a contamination event, one could foresee the behaviour of these pesticides in particular. From another point of view, these experiments also help to understand if there is the possibility of utilizing such deposits as low-cost adsorbents, recovered upon maintenance operations in the water distribution systems.

4.3.1 Kinetic study of the pesticides adsorption

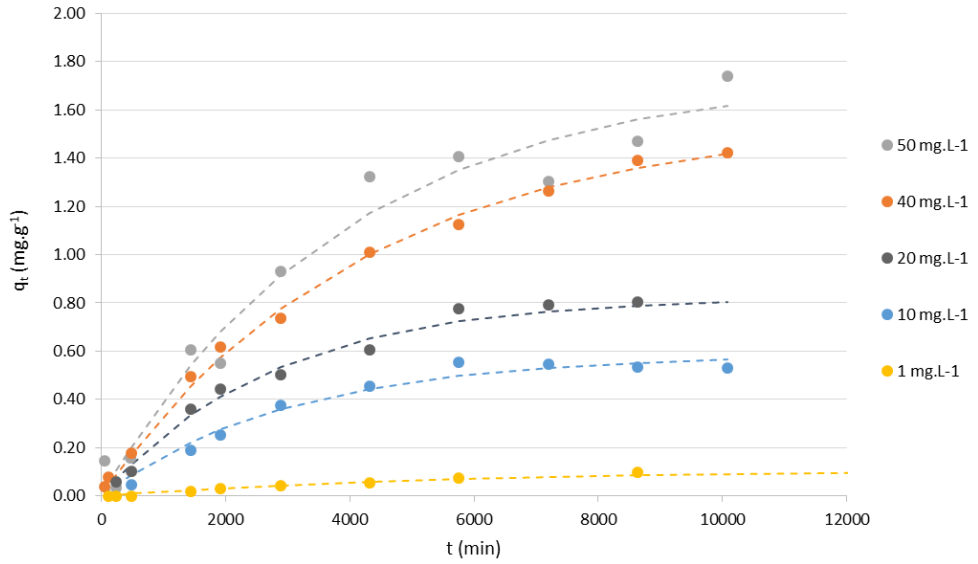
In order to understand the whole process of adsorption of carbofuran and chlorfenvinphos on S4 deposit, the effect of several parameters that might influence this process was studied. Previous experiments on the effect of adsorbent dose allowed to conclude that the quantity of S4 deposit should be set at 50 mg for the volume of 10 mL (5 $g.L^{-1}$). This adsorbent dose permitted to minimize the use of adsorbent and keep adsorption efficiency high.

4.3.1.1 Effect of contact time and initial pesticide concentration

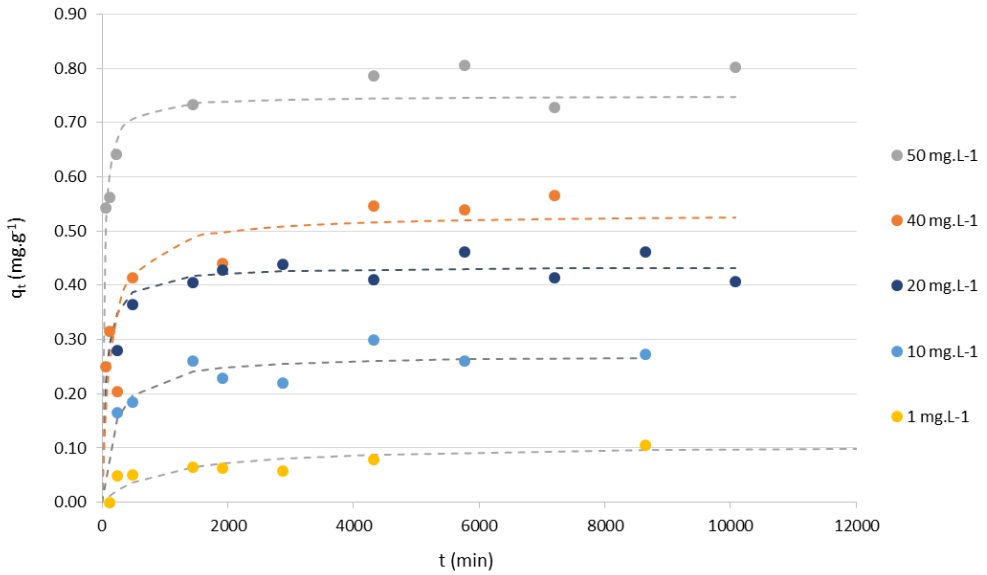
The effect of contact time and initial concentration of each pesticide on the adsorption by S4 deposit was studied.

The effect of contact time was first investigated to determine the equilibrium time for carbofuran and chlorfenvinphos adsorption onto S4 deposit at 20 °C. Note that these batch experiments were performed using single solutions of each pesticide of interest. As can be seen in Figure 4.1 (a) and (b), the adsorption capacity increased with the increase of initial concentration of pesticide (and remained constant after reaching equilibrium time, if applicable). A higher initial concentration enhances adsorption

efficiency, as it represents an important driving force to overcome mass transfer resistance between the aqueous and solid phases [18]. Also, adsorption rate was higher at the beginning of the process but slowed down with time until the equilibrium was approached/reached. This is due to the existence of a larger number of vacant sites available for adsorption at the initial stages [41].



(a)



(b)

Figure 4.1 – Effect of initial pesticide concentration (individual assays) on the adsorption of carbofuran (a) and chlorfenvinphos (b) on S4 deposit: S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, T = 20 °C, 405 rpm. Dashed lines correspond to pseudo-first order (a) and pseudo second order (b) fitted models.

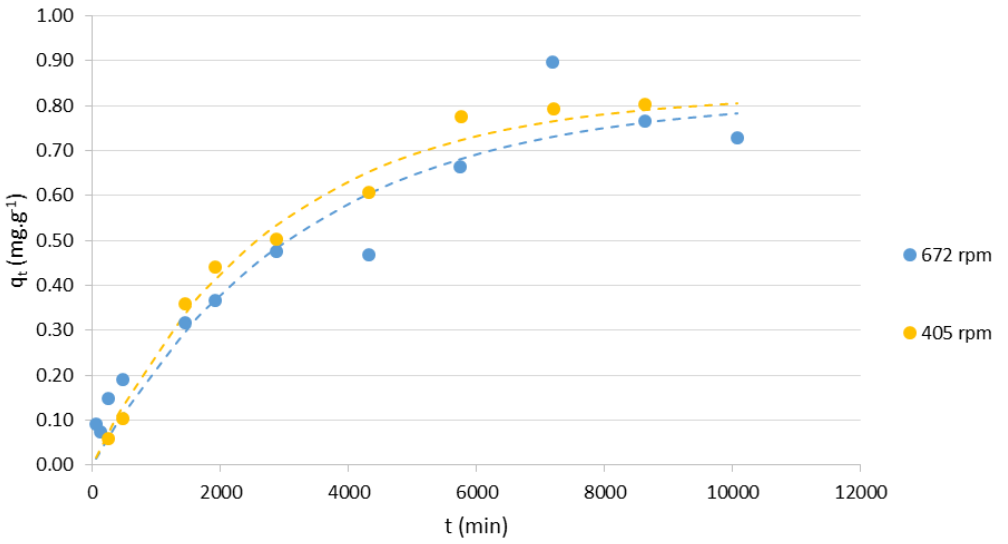
It is clear, when observing Figure 4.1 (a) and (b) that equilibrium was not reached for higher initial concentrations of carbofuran, while for chlorfenvinphos the time required for adsorption equilibrium was about 3 days (4320 min). It can be observed that, chlorfenvinphos adsorption onto the S4 deposit is faster than carbofuran, as can be confirmed by the values of the adsorption kinetic constants, presented below in Table 4.4.

In order to guarantee equilibrium, in the subsequent assays of section 4.3.2, the contact time was set at 15 days.

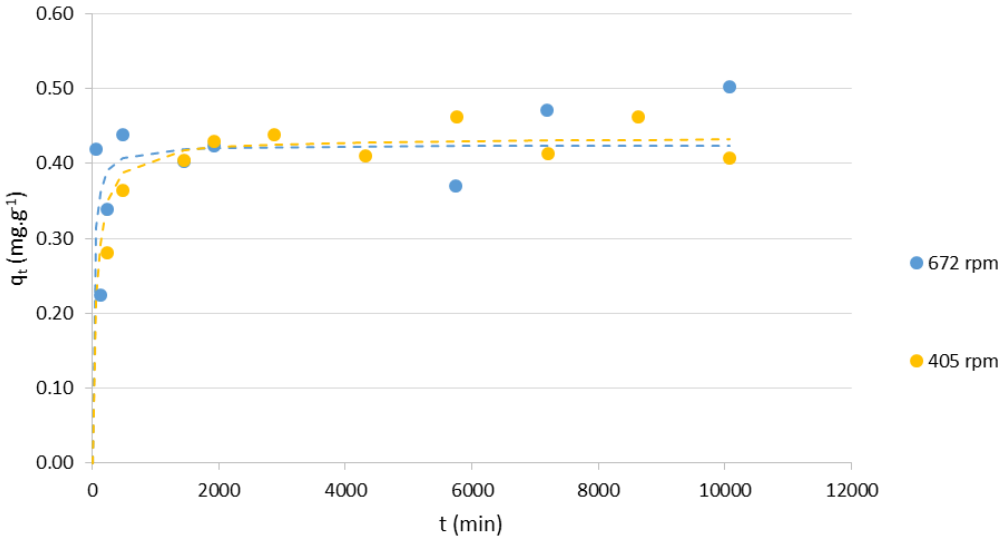
4.3.1.2 Effect of particle size and stirring speed

Stirring speed is an important parameter, as it may influence the distribution of the solute in the bulk solution and the external boundary film. The effect of this parameter in the adsorption of the two pesticides is represented in Figure 4.2 (a) and (b).

From the observation of the results obtained, it appears that, for both pesticides, stirring speed has no significant influence in the adsorption process in the range tested. The results of the adsorption kinetic constants and adsorption capacities presented in Table 4.4 confirm this fact (considering the associated errors of these parameters). This observation can be explained by the fact that the boundary layer resistance was very small and the mobility of the system was high, under the experimental conditions employed. The diffusion of the pesticide from the solution into the surface of the deposit and into the pores occurred quickly and easily. Under these conditions, external mass transfer resistances can be ignored. Therefore, the stirring speed was set at 405 rpm in further experiments.



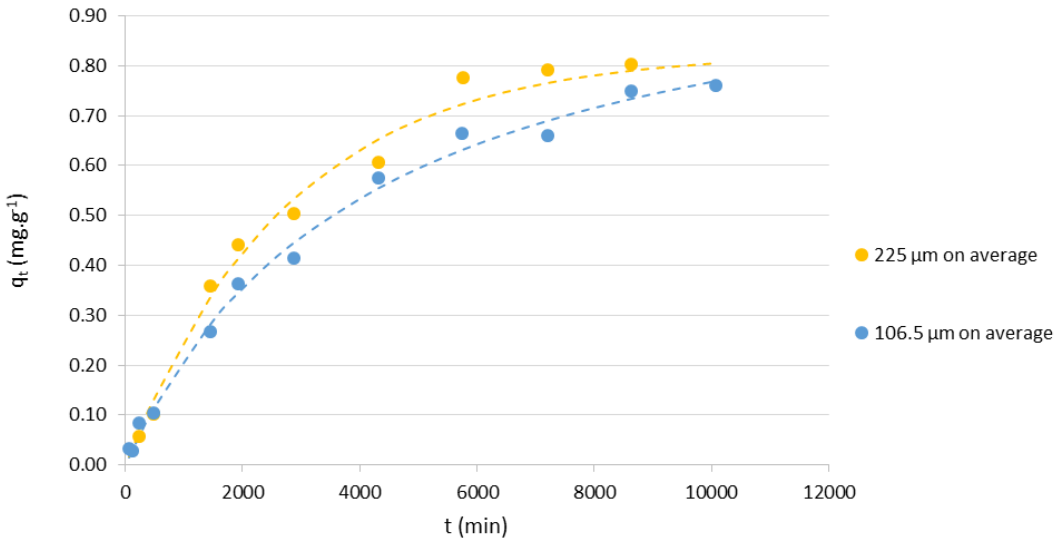
(a)



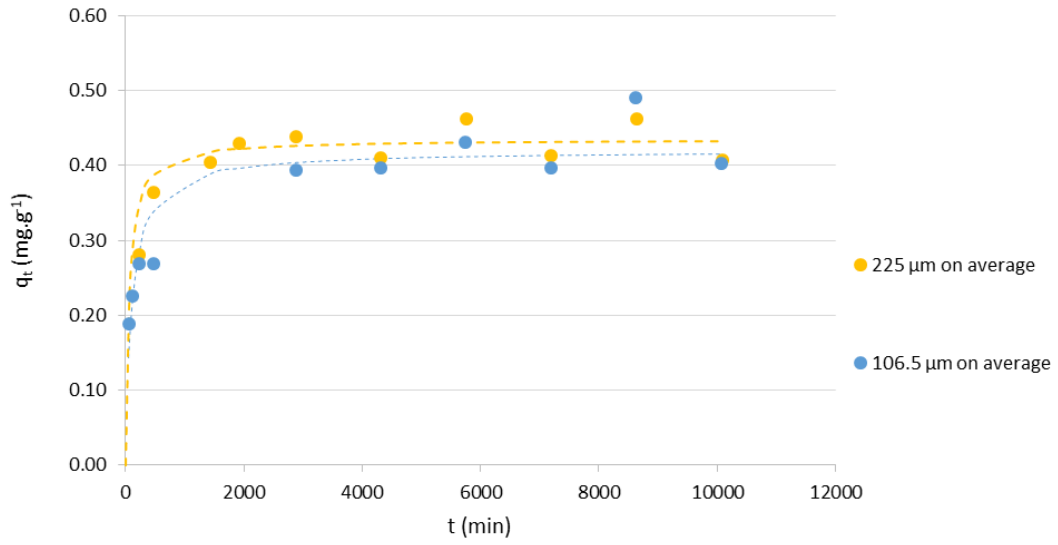
(b)

Figure 4.2 – Effect of stirring speed on the adsorption of carbofuran (a) and chlorfenvinphos (b) on S4 deposit: S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, initial concentration of pesticide = 20 mg.L^{-1}, T = 20 °C. Dashed lines correspond to pseudo-first order (a) and pseudo second order (b) fitted models.

The effect of particle size of S4 deposit in the adsorption process was also studied. Figure 4.3 (a) and (b) show the experimental results for the sorption of carbofuran and chlorfenvinphos, respectively.



(a)



(b)

Figure 4.3 – Effect of particle size on the adsorption of carbofuran (a) and chlorfenvinphos (b) on S4 deposit: S4 – 50 mg (5 g.L^{-1}), initial concentration of pesticide = 20 mg.L^{-1}, T = 20 °C, 405 rpm. Dashed lines correspond to pseudo-first order (a) and pseudo second order (b) fitted models.

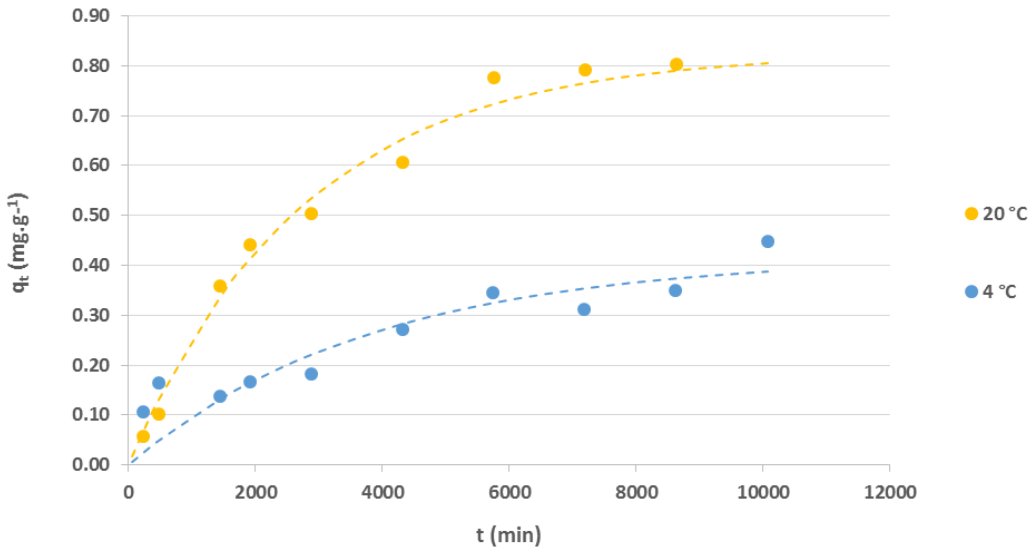
As can be seen from Figure 4.3, the effect of particle size of adsorbent is not particularly relevant, in the range studied. The kinetic curves, both for carbofuran and

chlorfenvinphos, present similar shapes and the adsorption capacity at equilibrium, as well as the apparent kinetic constants, for different particle sizes, is approximately the same (Table 4.4). These results indicate that the rate of adsorption is independent of intraparticle mass transfer effects [48].

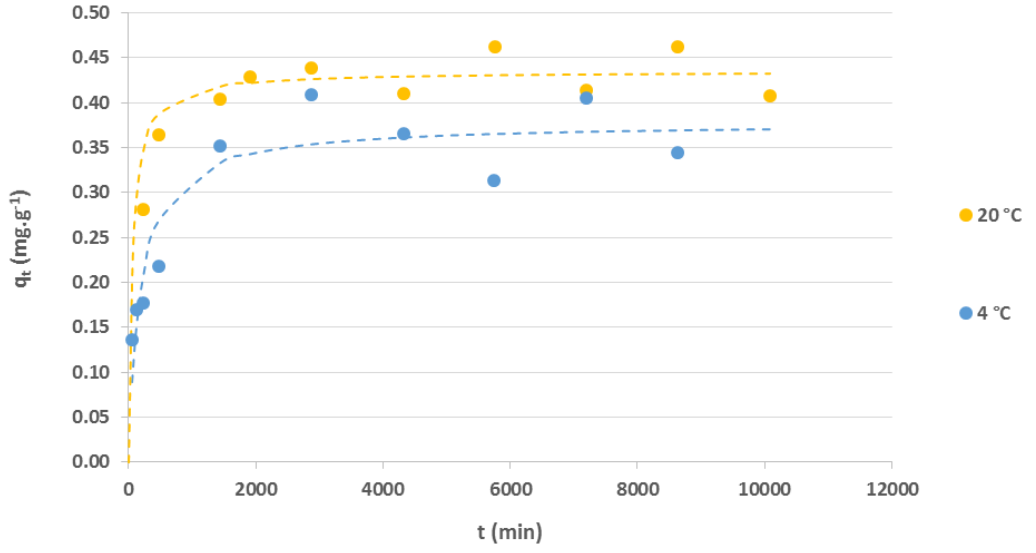
4.3.1.3 Effect of temperature

The effect of temperature on the adsorption kinetics of carbofuran and chlorfenvinphos on S4 deposit was studied. Assays were performed at 4 °C and 20 °C, in order to represent the range of temperatures typically found in drinking water distribution systems [49, 50]. In Figure 4.4 (a) and (b) are represented the results obtained for the adsorption of each pesticide.

As can be observed, the amount of each pesticide adsorbed, at equilibrium conditions, increases when temperature rises. This fact is especially noticeable for carbofuran adsorption. Indeed, the values of adsorption capacity at equilibrium for carbofuran are greater for higher temperatures, as can be confirmed in Table 4.4. Regarding the kinetic of the reaction, it seems that the rise of temperature provokes a slight increase on the adsorption rate, as can be seen by the results of the adsorption kinetic constant. Analyzing the adsorption of chlorfenvinphos, it is clear that effect of temperature on the process is less pronounced (Figure 4.4 (b)).



(a)



(b)

Figure 4.4 – Effect of temperature on the adsorption of carbofuran (a) and chlorfenvinphos (b) on S4 deposit: S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, initial concentration of pesticide = 20 mg.L^{-1}, 405 rpm. Dashed lines correspond to pseudo-first order (a) and pseudo second order (b) fitted models.

As can be confirmed from the values presented in Table 4.4, temperature does not seem to influence the adsorption capacity at equilibrium. Moreover, and similarly to

what was observed for carbofuran, the adsorption rate shows a small increase for higher temperatures, as confirmed by the values of the adsorption kinetic constant. These results are consistent with the fact that increasing the temperature is known to increase the rate of diffusion of the adsorbate molecules across the external boundary layer and in the internal pores of the adsorbent particle, owing to the decrease in the viscosity of the solution [51].

Temperature is an important parameter that seems to influence the adsorption process of both pesticides onto S4 deposit, although more pronouncedly for carbofuran.

The effect of temperature was also analyzed for the adsorption at equilibrium (section 4.3.2), where the results also indicate a positive effect of temperature on the adsorption of carbofuran, suggesting an endothermic process.

4.3.1.4 Adsorption kinetic modelling

The kinetic study enables the determination of the degree of utilization of the adsorption capacity as a function of the time of contact between the liquid and the solid. Generally, the adsorption process comprises the following consecutive steps: (i) bulk transport - mass transfer of solute from the solution to the boundary film; (ii) film transport - diffusion across the liquid film surrounding the adsorbent particles or mass transfer of solute from boundary film to surface; (iii) intraparticle transport - diffusion in the liquid contained in the pores and/or along the pore walls (internal or intra-particle diffusion); and (iv) adsorption on the active sites of the sorbate. One of these steps is the slowest and, thus, controls the rate of adsorption [52]. The overall rate of the sorption process may be controlled by any of these steps or a combination of steps.

Usually, mathematical models proposed to describe the kinetic process of adsorption can be classified as adsorption reaction models and adsorption diffusion models. In order to investigate the controlling mechanism of the adsorption process, two kinetic models were used to fit experimental data. The pseudo-first and pseudo-second order models were applied to the kinetics of adsorption of carbofuran and

chlorfenvinphos onto S4 deposit. These models are commonly used to describe adsorption data obtained from non-equilibrium conditions [27, 41, 53].

The pseudo-first order model assumes that the rate of sorption is controlled by the rate of "surface reaction", that is, the transition of solute molecules from free to adsorbed state. Also, this model gives a good description of the adsorption of contaminants at very low concentrations. Pseudo-first order model [54] is expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad 4.2$$

where q_t and q_e are the adsorption capacities at time t and at equilibrium, respectively (mg.g^{-1}) and k_1 is the apparent adsorption kinetic constant of pseudo-first order adsorption (L.min^{-1}).

After integration and applying initial condition $q_t = 0$ at $t = 0$, equation 4.2 becomes:

$$q_t = q_e\left(1 - e^{-k_1 t}\right) \quad 4.3$$

The pseudo-second order kinetic model is based on the assumption that the rate-controlling step in the adsorption process may be chemical sorption involving valence forces through sharing or exchange electrons between adsorbent and adsorbate [55]. The pseudo-second order equation [56-58] is defined as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad 4.4$$

Integrating equation 4.4 and applying the initial condition $q_t = 0$ at $t = 0$, it becomes:

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \quad 4.5$$

where k_2 is the apparent adsorption kinetic constant of pseudo-second order adsorption (g.mg^{-1}.min^{-1}).

Pseudo-first and pseudo-second order kinetic models were fitted to the experimental data by a non-linear regression analysis. In order to understand which

model better describes the experimental results two approaches/criteria were used, the method of least squares (MLS) as well as a mathematic/model selection criterion (MSC) [1, 59]. When evaluating the model fitting to experimental data by the MLS, lower values are expected for the best fitting models. The second approach, the model selection criterion (MSC), measures the relative quality of statistical models for a given set of data, taking in consideration the number of parameters of the models. Thus, higher values are expected for models with better fitting to experimental data, as well as for models with fewer parameters.

$$MSC = \ln\left[\frac{\sum_{t=1}^{m}(q_t - \overline{q_t})^2}{\sum_{t=1}^{m}(q_t - q_{t\,calc})^2}\right] - \frac{2p}{m} \qquad 4.6$$

In equation 4.6, m is the number of experimental points, p is the number of fitting parameters, $\overline{q_t}$ is the mean of the experimental pesticide concentration in the solid and $q_{t\,cal}$ is the pesticide concentration in the solid calculated by the model.

Table 4.4 provides a comparison between the adsorption kinetic parameters for pseudo-first and pseudo-second order models, for the adsorption of carbofuran and chlorfenvinphos, separately, on S4 deposit. Representation of the experimental results obtained in the kinetic studies are presented in Figure 4.1 to Figure 4.4.

Table 4.4 – Kinetic parameters for adsorption of carbofuran and chlorfenvinphos on S4 deposit.

	Parameters	Pseudo-first order				Pseudo-second order			
		q_e (mg.g^{-1})	k_1 (min^{-1})	MLS	MSC	q_e (mg.g^{-1})	k_2 (g.mg^{-1}.min^{-1})	MLS	MSC
Carbofuran	**C_0 (mg.L^{-1})[a]**								
	1	0.11±0.01	(1.6±0.4)×10^{-4}	0.0003	3.68	0.15±0.03	(9±6)×10^{-4}	0.0005	3.22
	10	0.59±0.08	(3±1)×10^{-4}	0.0300	3.17	0.8±0.2	(3±1)×10^{-4}	0.0349	2.78
	20	0.83±0.09	(3.6±0.8)×10^{-4}	0.0101	3.66	1.1±0.2	(3±1)×10^{-4}	0.0120	3.49
	40	1.6±0.1	(2.2±0.5)×10^{-4}	0.0197	4.77	2.2±0.3	(9±3)×10^{-5}	0.0182	4.85
	50	1.8±0.3	(3±1)×10^{-4}	0.1197	3.07	2.5±0.7	(8±7)×10^{-5}	0.1238	3.04
	T (°C)[b]								
	4	0.4±0.2	(3±2)×10^{-4}	0.0279	1.00	0.5±0.3	(5±8)×10^{-4}	0.0251	1.10
	20	0.83±0.09	(3.6±0.8)×10^{-4}	0.0101	3.66	1.1±0.2	(3±1)×10^{-4}	0.0120	3.49
	$\overline{d_p}$ (µm)[c]								
	106.5	0.80±0.05	(2.9±0.5)×10^{-4}	0.0053	4.80	1.1±0.1	(2.2±0.8)×10^{-4}	0.0051	4.84
	225	0.83±0.09	(3.6±0.8)×10^{-4}	0.0101	3.66	1.1±0.2	(3±1)×10^{-4}	0.0120	3.49
	Stirring speed (rpm)[d]								
	405	0.83±0.09	(3.6±0.8)×10^{-4}	0.0101	3.66	1.1±0.2	(3±1)×10^{-4}	0.0120	3.49
	672	0.8±0.2	(3±2)×10^{-4}	0.0715	2.16	1.1±0.3	(3±3)×10^{-4}	0.0676	2.22
Chlorfenvinphos	**C_0 (mg.L^{-1})[a]**								
	1	0.10±0.02	(6±1)×10^{-4}	0.0030	0.86	0.11±0.04	(1.0±0.5)×10^{-2}	0.0020	1.26
	10	0.3±0.2	(3±2)×10^{-3}	0.0051	0.53	0.3±0.2	(2±2)×10^{-2}	0.0038	0.83
	20	0.4±0.3	(7±4)×10^{-3}	0.0065	5.19	0.4±0.3	(4±4)×10^{-2}	0.0043	5.60
	40	0.5±0.4	(5±2)×10^{-3}	0.0559	0.40	0.5±0.5	(1±9)×10^{-2}	0.0363	0.83
	50	0.7±0.3	(2±1)×10^{-2}	0.0402	0.43	0.7±0.5	(5±4)×10^{-2}	0.0252	1.31
	T (°C)[b]								
	4	0.4±0.2	(3±2)×10^{-3}	0.0196	1.20	0.4±0.1	(1±6)×10^{-2}	0.0146	1.49
	20	0.4±0.3	(7±4)×10^{-3}	0.0065	5.19	0.4±0.3	(4±4)×10^{-2}	0.0043	5.60
	$\overline{d_p}$ (µm)[c]								
	106.5	0.4±0.9	(5.4±0.9)×10^{-3}	0.0269	0.80	0.4±0.2	(2±7)×10^{-2}	0.0140	1.45
	225	0.4±0.3	(7±4)×10^{-3}	0.0065	5.19	0.4±0.3	(4±4)×10^{-2}	0.0043	5.60
	Stirring speed (rpm)[d]								
	405	0.4±0.3	(7±4)×10^{-3}	0.0065	5.19	0.4±0.3	(4±4)×10^{-2}	0.0043	5.60
	672	0.4±0.2	(3±8)×10^{-1}	0.0531	-0.44	0.4±0.2	(1±8)×10^{-1}	0.0448	-0.27

[a]Experimental conditions: S4 – 50 mg (5 g.L^{-1}), $\overline{d_p}$ = 225 µm, time of contact = 10080 min (7 days), T = 20 °C, 405 rpm.

[b]Experimental conditions: S4 – 50 mg (5 g.L^{-1}), $\overline{d_p}$ = 225 µm, C_0 = 20 mg.L^{-1}, time of contact = 10080 min (7 days), 405 rpm.

[c]Experimental conditions: S4 – 50 mg (5 g.L^{-1}), C_0 = 20 mg.L^{-1}, time of contact = 10080 min (7 days), T = 20 °C, 405 rpm.

[d]Experimental conditions: S4 – 50 mg (5 g.L^{-1}), $\overline{d_p}$ = 225 µm, C_0 = 20 mg.L^{-1}, time of contact = 10080 min (7 days), T = 20 °C.

Pseudo-first and pseudo-second order kinetic models fitted well to the experimental results. Nevertheless, comparing the values of MLS and MSC for each experiment, and considering the errors associated to the calculated parameters, it can be concluded that the adsorption of carbofuran onto S4 deposit follows the pseudo-first order model, while chlorfenvinphos adsorption follows the pseudo-second order model. This observation is coherent with results found in literature both for carbofuran [6, 15, 39] and chlorfenvinphos [27, 45].

As can be observed in Figure 4.1 to Figure 4.4, chlorfenvinphos adsorption onto S4 deposit is faster than that of carbofuran. This observation is confirmed by the results of the rate constants of adsorption of chlorfenvinphos in the deposit, which are, generally, a hundred times higher than those of carbofuran. These results can also be compared to those obtained by Santos et al [1], for the adsorption of paraquat in S4 deposit and, although with different adsorption conditions, it appears that paraquat has the highest rate of adsorption of the pesticides studied (apparent pseudo-second order constants in the range of 2×10^{-1} – 4×10^{-1} $L^2.g^{-1}.min^{-1}.mg^{-1}$). Thus, as expected, the results obtained for the kinetic model constants differ depending on the combination pesticide – adsorbent.

4.3.2 Adsorption isotherms of the pesticides

In order to comprehensively understand the adsorption process for the removal of an adsorbate, it is important to establish the most suitable correlation for the experimental results. Adsorption isotherms are mathematical models that describe the distribution of the adsorbate species between the liquid phase and the solid phase when the adsorption process reaches equilibrium. The physicochemical parameters of an isotherm model, along with the underlying thermodynamic assumptions, can help understand the mechanism of the adsorption process, surface properties as well as the degree of affinity of the adsorbents [60].

4.3.2.1 Single component adsorption

Adsorption equilibrium was analyzed for both carbofuran and chlorfenvinphos, separately. The experimental data were fitted to three of the most common models: Langmuir, Freundlich and Temkin models.

Langmuir isotherm is a semi-empirical isotherm derived from a proposed kinetic mechanism. It is a model for monolayer localized physical adsorption on homogeneous surface. In the Langmuir isotherm model it is the assumed that: the surface of the adsorbent is uniform, meaning that all sites are equivalent; adsorbed molecules do not interact; all adsorption occurs through the same mechanism; at the maximum adsorption only monolayer is formed, molecules of adsorbate do not deposit on other, already adsorbed, molecules of adsorbate, only on the free surface of the adsorbent [61]. The Langmuir isotherm [62] is given by equation 4.7:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \qquad 4.7$$

where q_{max} (mg.L^{-1}) is the maximum adsorption capacity and K_L (L.mg^{-1}) is the equilibrium adsorption or equilibrium constant. The maximum adsorption capacity is an indication of the solute affinity to the adsorbent. The equilibrium constant measures how strong an adsorbate molecule is attracted onto a surface. Theoretically, when K_L is larger, the interaction between the surface of the adsorbent and the adsorbate molecules is stronger, and the adsorbent surface should be more covered with solute molecules [63].

The essential characteristics of Langmuir isotherm can be expressed by a dimensionless constant called separation factor or equilibrium parameter, RL, defined as follows [64]:

$$R_L = \frac{1}{1 + K_L C_0} \qquad 4.8$$

where C_0 (mg.L^{-1}) is the initial concentration. The parameter R_L indicates the shape of the isotherm as shown in Table 4.5:

Table 4.5 – Significance of R_L values.

Value of R_L	Type of isotherm
$R_L > 1$	Unfavourable
$R_L = 1$	Linear
$0 < R_L < 1$	Favourable
$R_L \sim 0$	Irreversible

The Freundlich isotherm [65] is an empirical formula for microporous and heterogeneous adsorbates that is represented by equation 4.9:

$$q_e = K_F C_e^{1/n} \quad 4.9$$

where K_F (mg.g^{-1}.(mg.L^{-1})$^{-1/n}$), defined as the adsorption or distribution coefficient, is related to the adsorption capacity and therefore is a measure of the affinity of the adsorbent for the adsorbate; n is a measure of the adsorption intensity and gives indication on the favourability of the adsorption. The larger the K_F and n values, the higher the sorption capacity. Generally, it is considered that for n=1 the partition between the two phases is independent of the concentration. A value for $1/n$ below one indicates a normal Langmuir adsorption isotherm while $1/n$ above one is indicative of a cooperative sorption [66].

The Temkin isotherm [67] is given by equation 4.10:

$$q_e = \frac{RT}{b} \ln(AC_e) \quad 4.10$$

where R is the ideal gas constant (8.314 J.mol^{-1}.K^{-1}), T the absolute temperature (K), A the Temkin isotherm constant (L.mg^{-1}) and b the Temkin constant related to heat adsorption (J.mol^{-1}). Temkin isotherm equation assumes that the heat of adsorption of all the molecules in the layer decreases linearly with coverage due to adsorbent – adsorbate interactions, and that adsorption is characterized by a uniform distribution of the binding energies, up to some maximum binding energy [67].

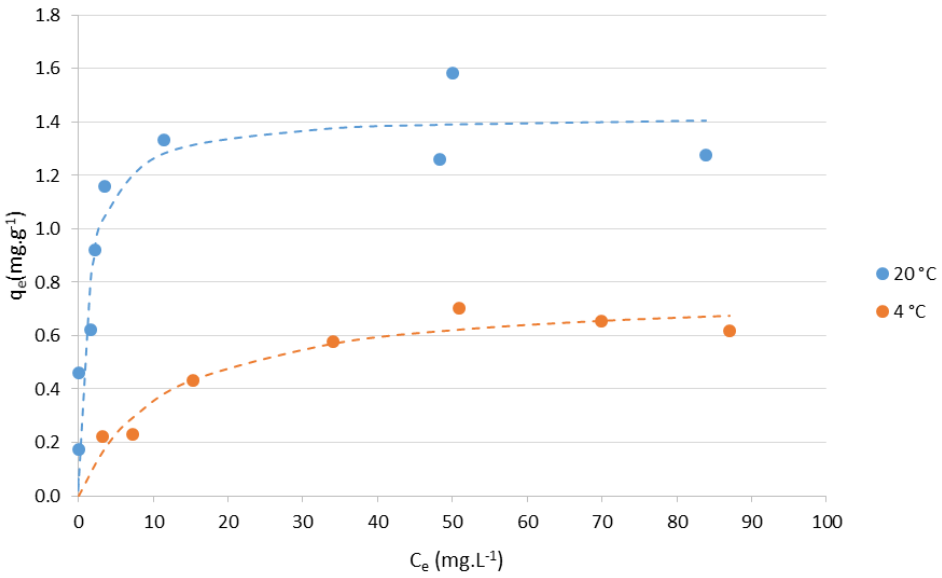
With the purpose of better comprehending the interaction between the pesticides and the deposit, and to explore its utilization as an alternative adsorbent, adsorption equilibrium experiments were executed. Isotherm adsorption assays were performed at

4 and 20 °C for carbofuran and chlorfenvinphos, separately. The Langmuir, Freundlich and Temkin isotherm models were fitted to the experimental data in a non-linear form. The parameters calculated by each isotherm model along with the values obtained by the method of least squares (MLS) and the model selection criterion (MSC) [59] are summarized in Table 4.6.

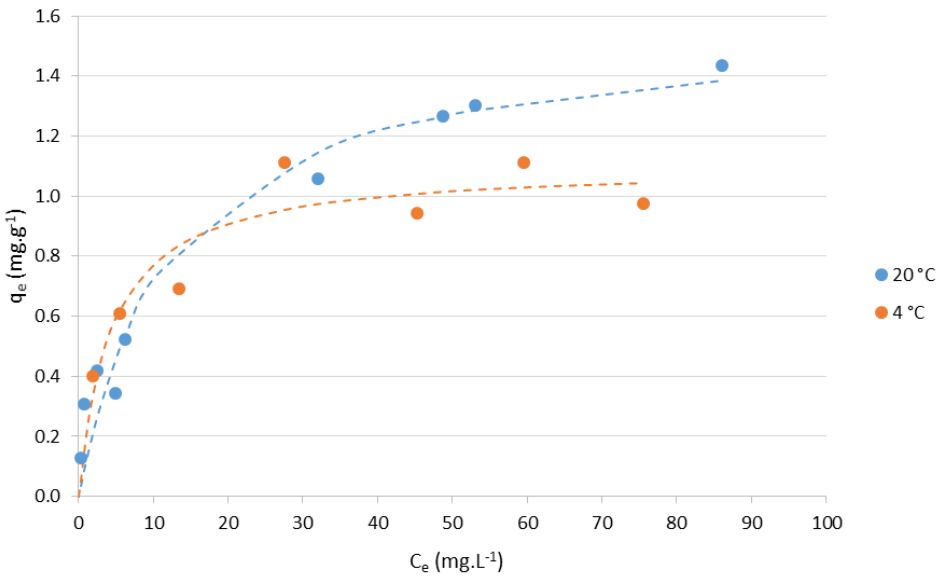
Table 4.6 – Isotherm parameters for the adsorption of carbofuran and chlorfenvinphos on S4 deposit. S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, initial concentration of pesticide = 20 mg.L^{-1}, time of contact = 15 days (21600 min), 405 rpm.

		Langmuir Isotherm				Freundlich Isotherm				Temkin Isotherm			
		q_{max} (mg.g^{-1})	K_L (L.mg^{-1})	MLS	MSC	K_F (mg.g^{-1}.(mg.L^{-1})$^{-1/n}$	n	MLS	MSC	*A* (L.mg^{-1})	*b* (J.mol^{-1})	MLS	MSC
Carbofuran	T (°C)												
	4	0.8±0.2	0.09±0.06	0.0163	2.28	0.2±0.1	3±2	0.0314	1.63	1±1	(15±6)×10^3	0.0224	1.97
	20	1.4±0.3	0.8±0.9	0.3285	1.22	0.8±0.2	6±4	0.3623	1.13	(0.3±1)×10^3	(17±7)×10^3	0.3245	1.24
Chlorfenvinphos	T (°C)												
	4	1.1±0.2	0.2±0.2	0.0664	1.46	0.4±0.2	4±3	0.0867	1.20	5±11	(13±6)×10^3	0.0706	1.40
	20	1.6±0.3	0.08±0.06	0.1013	2.56	0.26±0.07	2.5±0.5	0.0437	3.40	3±3	(10±3)×10^3	0.1926	1.92

The effect of temperature in the adsorption isotherms, for each pesticide, is presented in Figure 4.5.



(a)

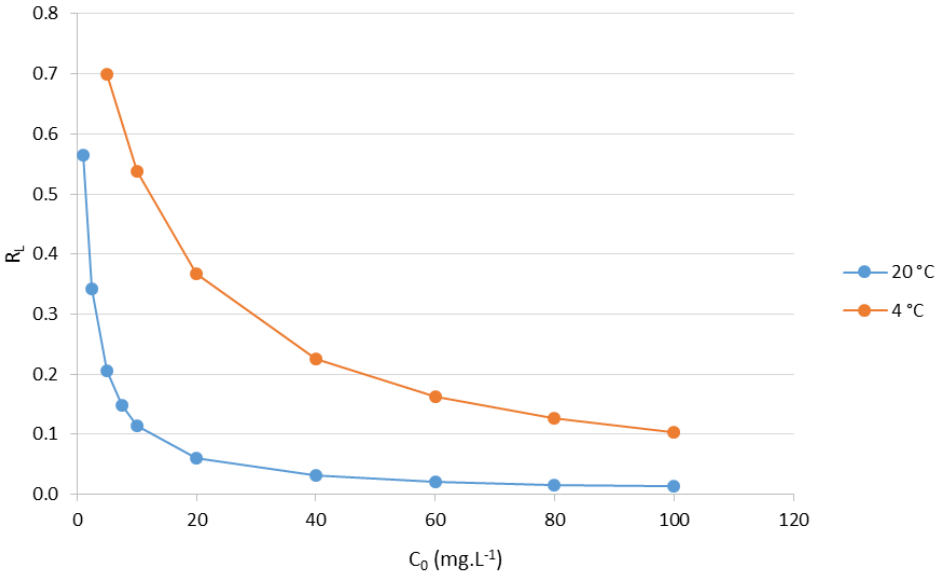


(b)

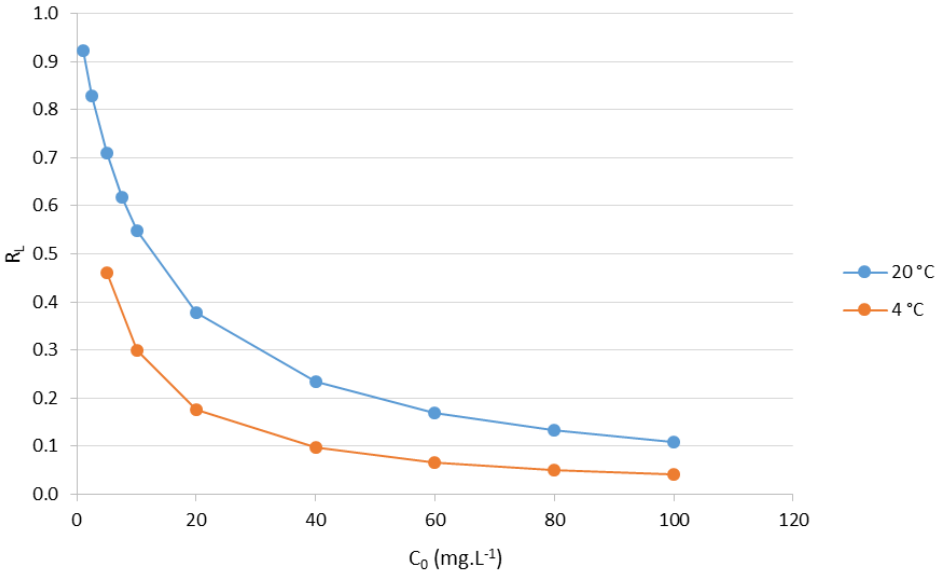
Figure 4.5 – Adsorption isotherms, at 4 °C and 20 °C, for carbofuran (a) and chlorfenvinphos (b) on deposit S4. S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, time of contact = 21600 min (15 days), 405 rpm. Dashed lines correspond to Langmuir fitted model.

Isotherm models fitted well the experimental results, for both pesticides. Nevertheless, as the results in Table 4.6 show, Langmuir model was slightly better overall, since it provided the higher values for the model selection criterion (MSC) or lower values of the method of least squares(MLS) (except for chlorfenvinphos at 20 °C). The better fitting of Langmuir model points towards a homogeneity of the adsorbent. The results agreed with the works carried out by previous researchers which reported that Langmuir model gave better fit on the adsorption of carbofuran onto activated carbon [40], banana stalks activated carbon [17], fertilizer industry waste and steel industry wastes [13], hemp fibers activated carbons [42], orange peel [18], palm-oil-fronds activated carbon [16] and rice straw-derived activated carbons [11]. Langmuir model was also successfully fitted to adsorption data for the adsorption of chlorfenvinphos onto sunflower seed shells, rice husk, composted sewage sludge and soil [27].

The separation factor, or equilibrium parameter R_L, was calculated through equation 4.8 for each initial concentration, at 4 and 20 °C, and the plot is presented in Figure 4.6.



(a)



(b)

Figure 4.6 – Effect of initial concentration of carbofuran (a) and chlorfenvinphos (b) on separation factor R_L at 4 and 20 °C.

The obtained values of R_L parameter for each pesticide, at different temperatures, ranged between 0 and 1, indicating that the adsorption of carbofuran and chlorfenvinphos onto S4 deposit was of the favourable type at the conditions being studied. Nonetheless, it can be seen that the R_L values decreased as the pesticide

concentrations increased, indicating that the adsorption was more favourable at higher initial concentrations [51]. Accordingly, the obtained values of the *n* constant of the Freundlich model were higher than 1 (thus, *1/n* < 1), for both pesticides at both temperatures, which is in agreement with the convex, curved downward Langmuir isotherm. According to Giles, et al. [31], the L-type (or Langmuir-type) isotherms reflects a relatively high affinity between the adsorbate and the adsorbent. This type of isotherms usually indicate the existence of chemisorption [68]. However, no general trend can be described because L-type isotherms may be obtained with extremely different solute/sorbent systems.

When comparing the values of maximum adsorption capacity, $q_{max,}$ obtained for the adsorption of carbofuran and chlorfenvinphos (Table 4.6) onto S4 deposit, with the values presented in Table 4.1 and Table 4.2, for the adsorption of carbofuran and chlorfenvinphos onto other adsorbents, it is noted that the obtained values of the monolayer adsorption capacity are lower. Thus, by comparison, one can say that this deposit in particular, does not have a great potential to be used as a low cost alternative adsorbent. Furthermore, the affinity of these two pesticides for the S4 deposit appears to be low, thus, the risk of contamination of the deposit is minor, consequently diminishing the risk of late water contamination, as a result of subsequent desorption of the contaminants.

In Figure 4.5 it is observable that the effect of temperature is more relevant for the adsorption of carbofuran, while for chlorfenvinphos adsorption the influence is minor. This fact is also confirmed by the values of the Langmuir constants K_L (Table 4.6), which increase with the temperature for carbofuran, but have a slight decrease with temperature rise for chlorfenvinphos. Generally, adsorption capacities decrease with increasing temperature. This behaviour was observed by Santos et al [1] for the adsorption of paraquat in the same type of deposit. So, the results obtained are opposite to the effect usually observed for the adsorption of a single component on a solid. Nevertheless, there is evidence that endothermic chemisorption process has been observed [69] (endothermic physical adsorption has never been reported) [70]. A few examples have been reported of an increase in the amount adsorbed with temperature rise, for several compounds [69-75]. As stated in the literature, the influence of

temperature on pesticide adsorption depends on the adsorbent/adsorbate pair [75]. Depending on the adsorbent, there are studies that report an increase of the adsorption capacity of carbofuran with the decrease of temperature [5, 11, 13], while other studies report the opposite effect [17, 19]. A possible elucidation for this occurrence is based on the assumption that adsorption is comprised of a two component system, which includes the single component and the hydrated species of the single component adsorbing simultaneously on the surface of the adsorbent. Therefore, it is assumed that adsorption is influenced not only by the adsorbate-adsorbent interactions, but also by the solute-solvent interactions, which are significantly affected by temperature [19, 69, 75]. Furthermore, the enhancement in the adsorption capacity might be due to the chemical interaction between adsorbates and adsorbent or the creation of new adsorption sites [51].

In sum, it was observed that adsorption of carbofuran and chlorfenvinphos onto S4 deposit is a slow process (as demonstrated by the low adsorption kinetic constants). As such, in the case of a contamination of the drinking water distribution systems with carbofuran and/or chlorfenvinphos, it can be affirmed that no significant contamination of the deposit (white type) attached to the pipes would occur. Here, the surface area available for adsorption and the time of contact of the contaminant with the deposits should be low, therefore, it is unlikely that any of the pesticides would adsorb, unless there is fluid stagnancy for a large period of time.

4.3.2.2 Multi component adsorption

When considering the case of a threatening event such as a deliberate contamination of drinking water distribution systems, there is the strong possibility of more than one chemical being used with the intent of causing harm. Hence, the multi component adsorption was also considered in this study.

Adsorption equilibrium was analysed for carbofuran and chlorfenvinphos, simultaneously, at the same conditions than mono component adsorption. The competitive adsorption of both pesticides was evaluated, at 20 °C, at different initial

concentrations of each pesticide in the same solution (mix). The comparison between mono and binary adsorption isotherms, for each pesticide, is represented in Figure 4.7.

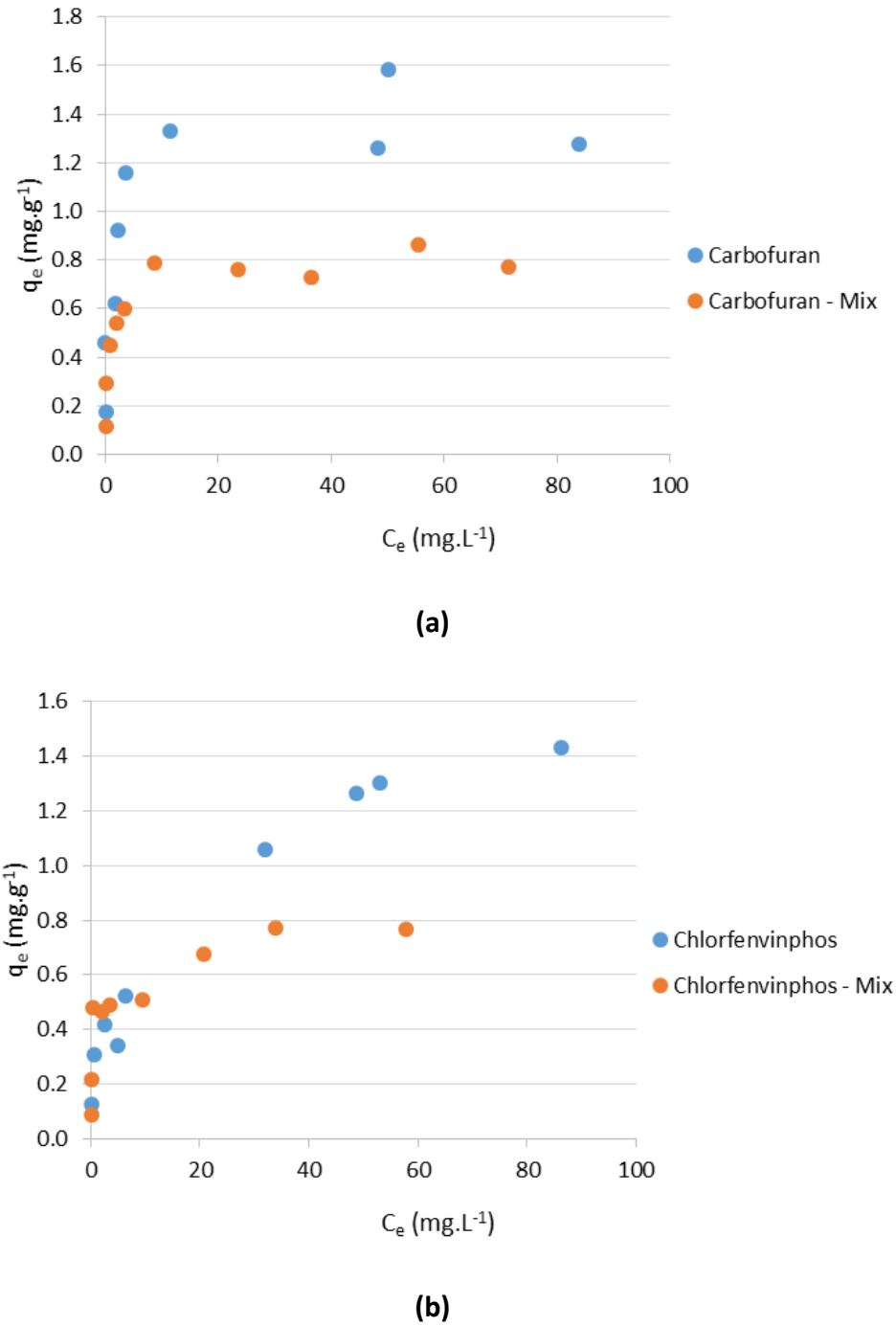


Figure 4.7 - Adsorption isotherms, at 20 °C, for carbofuran, mono and binary adsorption (a) and chlorfenvinphos, mono and binary adsorption (b) on deposit S4. S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, time of contact = 21600 min (15 days), 405 rpm.

Competitive adsorption of carbofuran and chlorfenvinphos onto S4 deposit is represented in Figure 4.8.

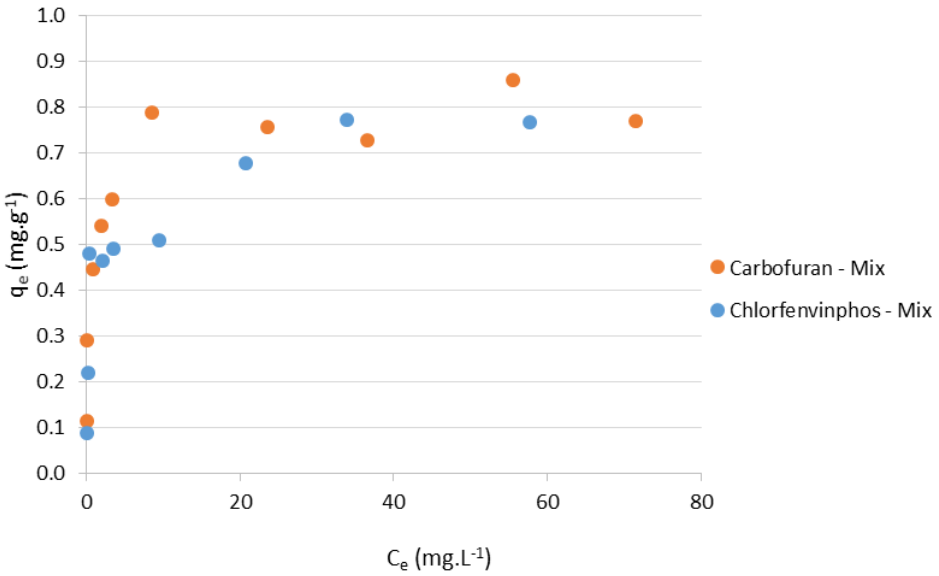


Figure 4.8 - Adsorption isotherms for the multi component system (mix), at 20 °C, on deposit S4. S4 – 50 mg (5 g.L^{-1}), average particle diameter = 225 µm, time of contact = 21600 min (15 days), 405 rpm.

As can be observed in Figure 4.7, the behaviour of each pesticide in the presence of the other compound is similar. At the equilibrium, the adsorption capacities diminished compared to the adsorption in the mono component system. Furthermore, Figure 4.8 shows that, at equilibrium, the adsorption capacities of carbofuran and chlorfenvinphos, for the binary adsorption system, are almost identical. This might indicate that there is no predominance of none of the pesticides in the adsorption process, which leads to the even distribution of the two compounds between active sites.

The comparison of the equilibrium adsorption capacities for both adsorbates in mono and binary solutions is presented in Table 4.7.

Table 4.7 – Comparison of adsorption capacities q_e (mg.g^{-1}), in mono component and binary systems.

Initial concentration (mg.L^{-1})	Carbofuran	Chlorfenvinphos	Carbofuran/Chlorfenvinphos
	q_e bin/q_e mono	q_e bin/q_e mono	q_e bin/q_e bin
1	0.7	0.7	1.3
3	0.6	0.7	1.3
5	0.7	1.2	0.9
8	0.6	1.4	1.2
10	0.5	0.9	1.2
20	0.6	n.d.	1.5
40	n.d.	0.6	1.1
60	0.6	0.6	0.9
80	0.5	n.d.	n.d.
100	0.6	0.5	1.0

n.d.: not determined.

These results substantiate what was observed in Figure 4.7 and Figure 4.8. The ratio of adsorption capacities of carbofuran and chlorfenvinphos, in the binary system, is close to 1.2 for the whole concentration range. In return, for both adsorbates, there is over-all a decrease of the adsorption capacities when compared to the mono component solutions. The apparent lack of competition effects may be attributable to the comparable solubilities of carbofuran and chlorfenvinphos in water. The presence of the other compound does not seem to induce major alterations in the solubility of the first compound, at most, there is a similar decrease of solubility of each pesticide, when in presence of the other. A similar behaviour was observed by Faur, et al. [76] for the binary adsorption of atrazine and simazine onto activated carbon fibers. The authors studied the competitive adsorption of three binary systems and observed that when there is a strong difference of solubility between the compounds (such as between atrazine and deethylatrazine), the adsorption of the adsorbate with the lower solubility (atrazine) is favoured and the presence of a co-adsorbate has no influence on the process. Contrarily, the adsorption of the highly soluble compound (deethylatrazine) decreases. As mentioned, in the study of the adsorption of atrazine and simazine (compounds with similar solubilities in water) the authors did not observe relevant competition effects [76].

The existence of more than one compound in a solution can cause interference and competition for the available adsorption sites. Thus, several models have been proposed in order to describe the multi-component adsorption behaviour. In this study, the experimental data was fitted by two multi component adsorption isotherm models, namely, the extended Langmuir and the extended Freundlich models.

The extended Langmuir model (also known as non-modified competitive Langmuir model), first introduced by Butler and Ockrent [77], is still one of the most widely used models to describe the adsorption of multi component systems [76, 78-81]. This model predicts the amount of adsorbed component i in a mixture of N components, as follows:

$$q_{e,i} = \frac{q_{max,i} K_{L,i} C_{e,i}}{1 + \sum_{j=1}^{N} K_{L,j} C_{e,j}} \quad 4.11$$

where $q_{max,i}$ and $K_{L,i}$ can be estimated from the fitting of the experimental data by the corresponding individual Langmuir isotherm equations. This model implies that there is a homogeneous surface with respect to the energy of adsorption; there is no interaction between the adsorbed species; and all adsorption sites are equally available to all adsorbed species. Also, its thermodynamic consistency requires the monolayer capacities (q_{max}) of each compound to be the same [82].

The extended empirical Freundlich model, derived from the mono component Freundlich isotherm, was developed by Fritz and Schluender [83], The extended Freundlich model is used to correlate binary adsorption data and is described as:

$$q_{e,1} = \frac{K_{F,1} C_{e,1}^{n_1 + x_1}}{C_{e,1}^{x_1} + y_1 C_{e,2}^{z_1}} \quad 4.12$$

$$q_{e,2} = \frac{K_{F,2} C_{e,2}^{n_2 + x_2}}{C_{e,2}^{x_2} + y_2 C_{e,1}^{z_2}} \quad 4.13$$

where $K_{F,1}$, $K_{F,2}$, n_1 and n_2 can be estimated from the correspondent mono component Freundlich isotherms, and the other six parameters (x_1, y_1, z_1 and x_2, y_2, z_2) are the multi component Freundlich adsorption constants of the first and second compounds.

In order to understand which model better describes the experimental results, the average relative error (ARE) between the experimental and calculated q_e values was calculated.

The obtained parameter values for the fitting of the two multi-component isotherms to the experimental results are summarized in Table 4.8.

Table 4.8 – Estimated binary adsorption isotherm model parameters.

Parameters and % Average relative error (ARE)	Carbofuran	Chlorfenvinphos
Extended Langmuir model		
q_{max}	1.4±0.3	1.6±0.3
K_L	0.8±0.9	0.08±0.06
% ARE	57.0	82.3
Extended Freundlich model		
K_F	0.8±0.2	0.26±0.07
n	6±4	2.5±0.5
x_i	-6.44	-3.02
y_i	1.33	0.56
z_i	-0.18	-0.61
% ARE	30.6	37.0

It is worth noting that the parameters used in the extended Langmuir model were the ones obtained in the single component equilibrium adsorption experiments. This model simply predicts the multicomponent adsorption based on the single component adsorption parameters. It can be used without performing any multicomponent adsorption experiments. The extended Freundlich model uses the K_F and n values, obtained in the single component equilibrium adsorption experiments, along with three other parameters, that are calculated considering the multicomponent equilibrium adsorption experimental results.

The binary adsorption isotherms, obtained through the fitting of the experimental data to each model, are represented in Figure 4.9.

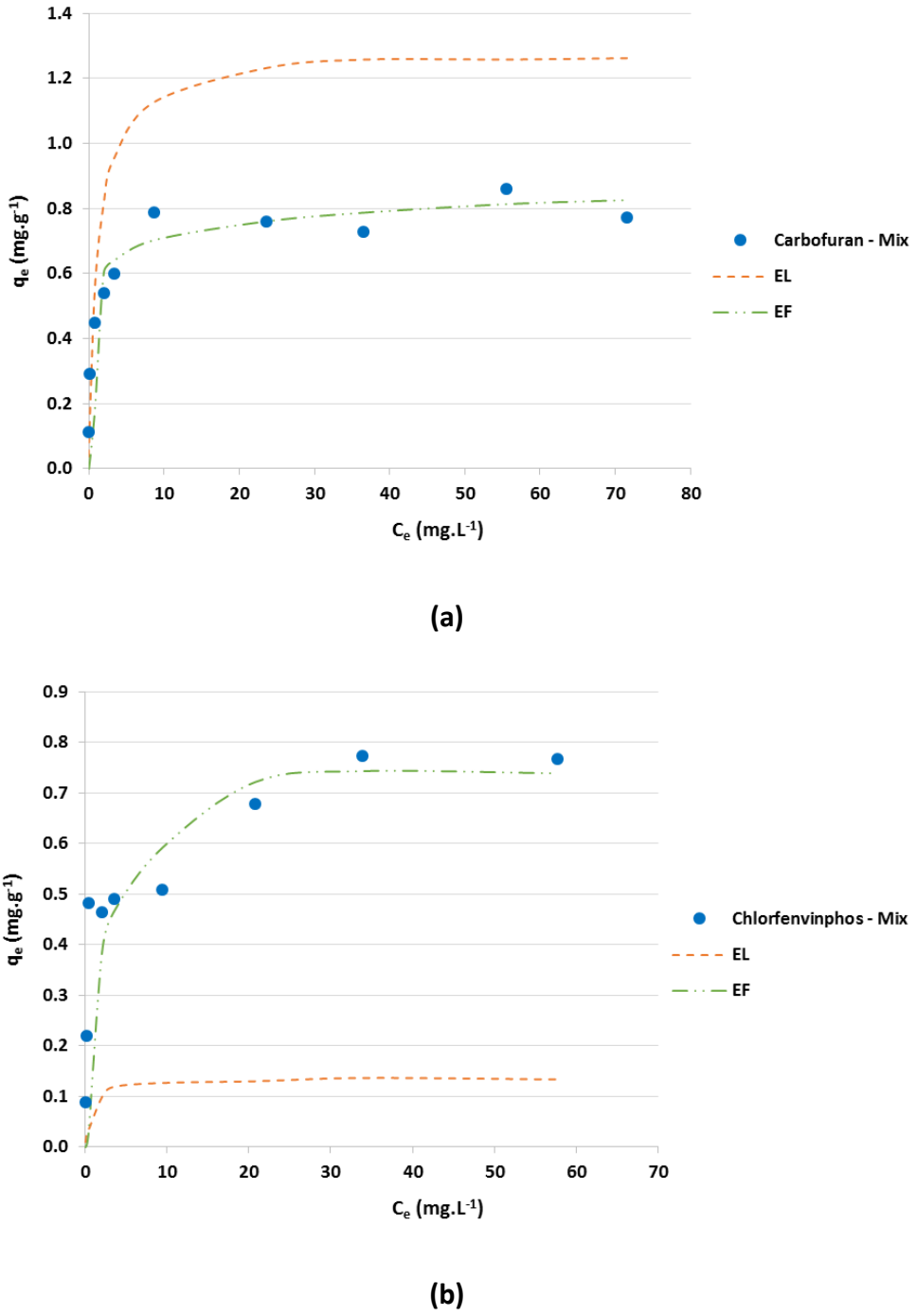
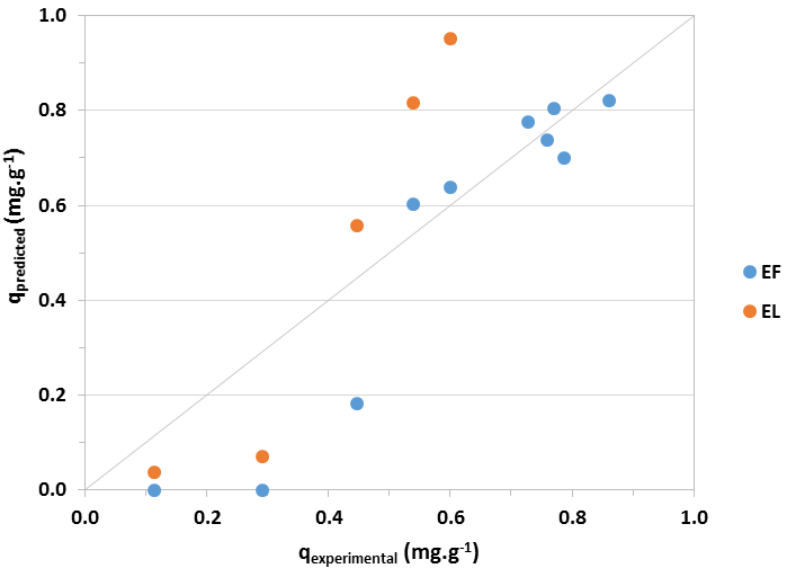
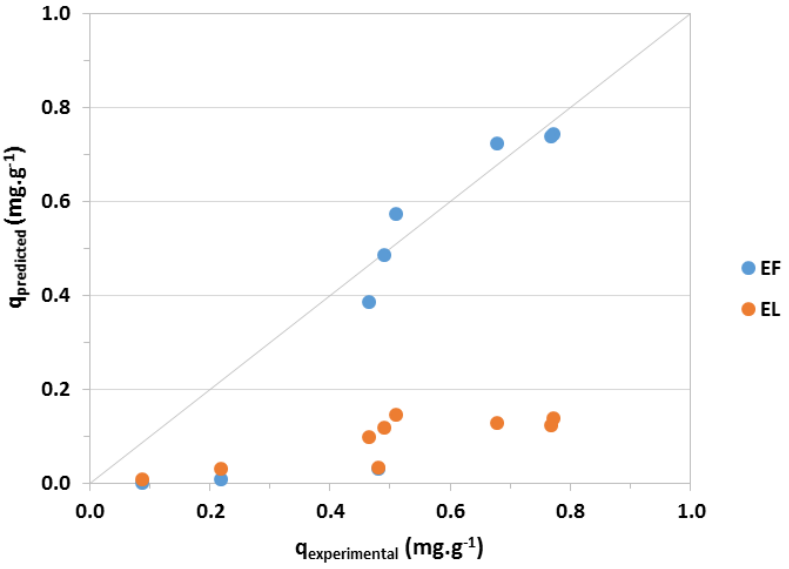


Figure 4.9 – Modelling results of binary adsorption of carbofuran (a) and chlorfenvinphos (b) pesticides with extended Langmuir (EL) and extended Freundlich (EF).

Also, the comparison between the experimental and the calculated values for the adsorption capacities (q_e), for each pesticide, are depicted in Figure 4.10.



(a)



(b)

Figure 4.10 – Comparison of the experimental and calculated q_e values of carbofuran (a) and chlorfenvinphos (b) in a binary mixture, by extended Langmuir (EL) and extended Freundlich (EF) models.

Evaluating Figure 4.10, it is obvious that the values predicted by the extended Freundlich model are in better agreement with the experimental data, since most of the data points fall around the 45° line (diagonal in the parity plot). The values predicted by the extended Langmuir model are more scattered and, thus, the model fails to predict the experimental values. The comparison between the two binary models is better

observed in Figure 4.9. The extended Langmuir model clearly overestimated the adsorption of carbofuran, when in presence of chlorfenvinphos. This model also fails to predict the adsorption of chlorfenvinphos, this time by underestimating its adsorption, in the presence of the other compound. The poor fit of the EL model is furthermore confirmed by the extremely high percentages of average relative error (% ARE of 57.0 and 82.3%, for carbofuran and chlorfenvinphos, respectively), for both pesticides (Table 4.8). As stated before, in order to maintain its thermodynamic consistency, the extended Langmuir model requires the monolayer capacity of the molecules in solution to be the same [82]. Thus, the lack of fit of the EL model to the experimental data might be related to the difference between the values of monolayer capacity (q_{max}) of each pesticide. As evidenced by Figure 4.9 and Figure 4.10, the extended Freundlich model presented the better fit to the experimental data of both pesticides. Moreover, Figure 4.10 shows a very poor fit of the EF model for the lowest equilibrium concentrations, but a good agreement for the higher equilibrium concentrations. Overall, the extended Freundlich model provided the better fit for the experimental data. The average relative errors for each pesticide confirm this observation, since the values obtained (%ARE of 30.6 for carbofuran, and 37.0% for chlorfenvinphos) were significantly lower than those obtained for the fitting with the EL model. It seems that the extended Freundlich model is able to account for the different interactions that exist between pesticides and among molecules of the same pesticide, and adsorbent interactions with the adsorbate. Therefore, the simultaneous adsorption of carbofuran and chlorfenvinphos onto S4 deposit can, satisfactorily, be represented by the extended Freundlich isotherm model.

In sum, the results indicate that there is no predominance of carbofuran nor chlorfenvinphos in the process of adsorption onto S4 deposit (white deposit). Considering the extreme case of a contamination, with both compounds, in stagnant water, it can be assumed that the adsorbed amount of each pesticide in white deposit would be very similar.

4.4 Conclusions

The adsorption of carbofuran and chlorfenvinphos onto S4 deposit is an effective, but slow process. The kinetic study showed that the adsorption of carbofuran onto S4 deposit follows a pseudo-first order model, whereas the adsorption of chlorfenvinphos is best described by a pseudo-second order model. It was also observed that the adsorption capacity of carbofuran increased with the rise of temperature, indicating that the adsorption of this pesticide onto the S4 deposit is an endothermic process. Nonetheless, a thermodynamic study on the adsorption of each pesticide onto the deposit could help clarify the nature of the process, and the importance of temperature in it. The three isotherm models tested were able to successfully fit the experimental results, of each pesticide; yet, Langmuir model was slightly better overall.

The simultaneous adsorption of carbofuran and chlorfenvinphos onto S4 deposit was also studied. The obtained results showed no significant competition effects between the two pesticides. Furthermore, it was shown that the extended Freundlich model is able to satisfactorily describe the simultaneous adsorption of carbofuran and chlorfenvinphos onto the S4 deposit.

Furthermore, the affinity of these two pesticides for the S4 deposit appears to be low, thus, the risk of contamination of the deposit is minor, consequently diminishing the risk of late water contamination, as a result of subsequent desorption of the contaminants.

4.5 References

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Chapter 5

5 General Conclusions and Future Work

5.1 Main conclusions

The work developed aimed to contribute to the definition of an approach, intended to minimize the public impact of a contamination event (deliberate or accidental), which could affect public water infrastructures. Such approach was the main goal of the European project SecurEau. Carbofuran and chlorfenvinphos were the two model chemical compounds chosen as case studies.

Two analytical methods for the simultaneous analysis of carbofuran and chlorfenvinphos in water were developed and validated. The first method, consisting on the direct injection and analysis by LC-DAD, intended to be a rapid way of detecting a possible contamination, at high concentration levels, in water. LODs of 4 and 11 $\mu g.L^{-1}$ were obtained for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed, in four different matrices at three levels of concentration, and average recoveries of 99% and 95% were obtained for carbofuran and chlorfenvinphos, respectively. These results show that there were no relevant interferences in the matrices. A second method, involving the preconcentration of water samples by DLLME and analysis by GC-MS was also developed. The main objective of this methodology was to enable the rapid and reliable identification and quantification of carbofuran and chlorfenvinphos, up to the levels required by European legislation. LODs of 0.04 and 0.02 $\mu g.L^{-1}$ were achieved for carbofuran and chlorfenvinphos, respectively. Recovery assays were performed in different matrices, such as river, tap, plain and sparkling water. Except for the results obtained for the sparkling water (recoveries ranging from 58-85%), the recoveries for the extraction in the other matrices were in the range of 75-120% (average value of 98.2% was reached). Given the results obtained, it can be assumed that no relevant matrix effect was observed when applying this method. Both methods showed wide linearity (DI-LC-DAD: 0.05-100 $mg.L^{-1}$; DLLME-GC-MS: 0.1-250 $\mu g.L^{-1}$ for carbofuran and 0.1-200 $\mu g.L^{-1}$ for chlorfenvinphos), good repeatability and high recoveries in several matrices, within a short analysis period. Therefore, the two

methods present the advantages of speed, simplicity, ease of operation and low consumption of sample volume and solvents.

When considering a contamination event in a water supply system, it is important to consider, not only the presence of the contaminant in the water, but also the possibility of interaction of the contaminant with the deposits formed at the inner surface of the pipes (and that may exist therein or in suspension, upon being released). The possibility of contaminants being accumulated in the deposits should not be discarded. Thus, in order to account for the possibility of contamination of the deposits, there was the need to develop methods for the quantification of carbofuran and chlorfenvinphos in these materials. The developed methodology, consisting of ultrasonic extraction with acetonitrile of chlorfenvinphos from clay, was intended to allow the detection and quantification of a possible contamination, in a fast, simple and reliable way. The obtained limits of detection and quantification were of 0.35 and 1.05 $mg.g^{-1}$, respectively. Also, high average recoveries were obtained for the three contamination levels tested, with values between 101 and 106%. This method was also tested in the extraction of chlorfenvinphos from two other types of deposits: S3, a tubercle deposit, and S4, a white deposit. Detection limits of 0.23 and 0.11 $mg.g^{-1}$ were obtained for the extraction of S3 and S4 deposits, respectively. However, since the trend line obtained for the extraction of S3 deposit crosses the x axis at 0.54 $mg.g^{-1}$, this extraction procedure is not appropriate for the analysis at levels below this value. Additionally, the method was tested for the extraction of carbofuran from clay, S3 and S4 deposits, and the obtained results indicate that it can be used to the quickly detect and quantify carbofuran in clay and S4 deposit, if the extraction percentages are considered. Since no contamination was achieved for the S3 deposit, no conclusions about the applicability of the extraction method could be draw. Thus, this simple and rapid extraction methodology enabled the detection and quantification of carbofuran and chlorfenvinphos in different types of deposits, whenever the appropriate extraction percentages are taken into account.

In order to better understand the interaction of our model contaminants, carbofuran and chlorfenvinphos, with the deposits that exist in the water pipelines, adsorption studies were conducted. Kinetic and equilibrium experiments were performed, only

with S4 deposit. The adsorption of carbofuran and chlorfenvinphos onto S4 deposit is an effective, but slow process. The kinetic study showed that pseudo-first and pseudo-second order kinetic models fitted well to the experimental results. Nevertheless, that the adsorption of carbofuran onto S4 deposit follows a pseudo-first order model, whereas the adsorption of chlorfenvinphos is best described by a pseudo-second order model. At equilibrium, the three isotherm models tested were able to successfully fit the experimental results, of each pesticide; yet, Langmuir model was slightly better overall.

The simultaneous adsorption of carbofuran and chlorfenvinphos onto S4 deposit was also studied. The obtained results showed no significant competition effects between the two pesticides. Also, it was verified that, at equilibrium, the extended Freundlich model is able to satisfactorily describe the simultaneous adsorption of the pesticides onto the S4 deposit.

In summary, it was verified that the developed analytical methods are appropriate for the detection and quantification of carbofuran and chlorfenvinphos in water and various kind of deposits. Additionally, the interaction studies between the two pesticides and one type of deposit revealed that, in the case of a contamination event in drinking water distribution systems, the adsorption of the pesticides onto white deposits in the conducts should be low. However, the possibility of contamination in places where there is long contact time between contaminated water and deposits (e.g. stagnant regions, or during prolonged low consumption rates), should not be discarded.

Finally, it can be considered that the work presented is an important contribution for the development of contamination warning strategies in drinking water distribution systems.

5.2 Future Work

This work consisted on the development of analytical methodologies for the detection and analysis of carbofuran and chlorfenvinphos in water and deposits, as well as on the study of the interaction of the two pesticides with pipe deposits. Several variables, affecting the process, were studied and optimized, and some parameters, that allow the understanding of the type of interaction between the pesticides and the deposits, were estimated. There are, however, some aspects that ought being explored and should be considered for future work:

- Test higher levels of contamination of carbofuran, in order to allow its extraction from the deposits;
- Perform matrix-matched calibration for the simultaneous extraction of carbofuran and chlorfenvinphos from clay, S3 and S4 deposits, using the developed extraction methodology;
- Complete the study on the simultaneous adsorption of the two pesticides with the S4 deposit (kinetics, effect of temperature);
- Perform interaction studies between the other types of deposits (clay and S3 deposit) and the two pesticides, separately and simultaneously;
- Study the interaction of the two pesticides with biofilms;
- Extend the study to other types of pesticides or to other chemical compounds;
- Extend the study to other types of deposits.