Universidade do Minho
Escola de Engenharia

Beatriz Azevedo Carneiro

Supported Covalent Organic Frameworks for the monitoring of pharmaceutical pollutants.

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Beatriz Azevedo Carneiro

Supported Covalent Organic Frameworks for the monitoring of pharmaceuticals pollutants

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Drª Begoña Espina
Drª. Luciana Pereira

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STATEMENT OF INTEGRITY

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RESUMO

Estruturas orgânicas covalentes para a monitorização de poluentes farmacêuticos

A qualidade de água tem sido comprometida pela poluição causada por atividades antropogénicas, o que põe e causa a função ecológica dos habitats e constitui uma ameaça para a saúde animal e humana. Contaminantes emergentes, tais como produtos farmacêuticos, têm sido detetados em águas naturais em pequenas concentrações (desde μg/L até ng/L). A incapacidade das estações de tratamento de águas residuais para a remoção destes compostos promove a poluição do meio ambiente. No entanto, as consequências da exposição por longos períodos a águas contaminadas são desconhecidas. Este trabalho teve como objetivo preparar compósitos de estruturas orgânicas covalentes (COFs), e testar a sua eficiência de adsorção de contaminantes farmacêuticos para a aplicação destes como sensores de monitorização de águas naturais, estudando a sua eficácia à escala laboratorial em matrizes de água relevantes.

Para esta investigação foi analisada a eficiência da adsorção de fármacos (tetraciclina, diclofenac e sulfapiridina) por diferentes COFs (TpBD-$(CF_3)_2$, TpBD-Me_2 e TPB-DMTP) e um compósito (NPS@TPB-DMTP). Foram avaliados vários parâmetros, dentro dos quais a cinética de adsorção e desorção, isotermas, influência do pH, possibilidade de reciclagem e reutilização dos COFs.

Todos os COFs testados mostraram boas eficiências de adsorção dos produtos farmacêuticos testados, no entanto, verificou-se que a adsorção da tetraciclina por TpBd-Me_2 foi mais eficiente a pH 6, o qual mostrou grande potencial para a sua aplicação em águas naturais. Na adsorção do diclofenac, o COF TPB-DMTP demonstrou ter a maior capacidade de adsorção e destacou-se por ser ideal para capturar contaminantes farmacêuticos, portanto, foi selecionado para preparar o compósito que facilitará a sua aplicação em cartuchos. Para o compósito de TPB-DMTP, é necessária mais investigação, no entanto, com base no seu bom desempenho atual de adsorção estes ensaios preliminares, parece promissor.

Palavras-chave: Estruturas Orgânicas Covalentes, Adsorção, Monitorização ambiental, Contaminantes emergentes, Tetraciclina, Diclofenac, Sulfapiridina.

ABSTRACT

Supported Covalent Organic Frameworks for the Monitoring of Pharmaceutical Pollutants

Water quality has been compromised by pollution caused by anthropogenic activities, which jeopardises the ecological function of aquatic habitats and poses a threat to animal and human health. The inability of wastewater treatment plants to eliminate these contaminants at such small concentrations contributes to environmental pollution. However, the consequences to the long exposure to contaminated waters is still unknown. The aim of this work was to prepare cartridges based on high-performance covalent organic frameworks (COFs) as well as testing their efficiency in adsorbing pharmaceutical pollutants to apply it as sensors that monitor natural waters, and then study their effectiveness on a laboratory scale in relevant water matrices.

For this investigation it was analysed the adsorption efficiency of pharmaceutical compounds (tetracycline, diclofenac, and sulfapyridine) by differents COFs (TpBD-$(CF_3)_2$, TpBD-Me_2, and TPB-DMTP) and a composite (NPS@TPB-DMTP). Different parameters were evaluated, including adsorption and desorption kinetic parameters, isotherms, influence of pH, possibility of recycling and reusing COFs.

All tested COFs showed good adsorption efficiencies of the tested pharmaceuticals, however, the adsorption of tetracycline by TpBd-Me_2 was more efficient at pH 6, showing potential for application of this COF in natural waters. For the adsorption of diclofenac, the COF TPB-DMTP showed the highest adsorption capacity, and it stood out for being ideal to capture pharmaceutical pollutants. For the composite, further investigation is necessary, however, based on its current good adsorption performance in these preliminary tests, it looks promising.

Keywords: Covalent Organic Framework, Adsorption, Environmental monitoring, Emerging contaminants, Tetracycline, Diclofenac, Sulfapyridine

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CHAPTER 1. INTRODUCTION

1.1 Context and Motivation

The most fundamental resource for the development and survival of human life on earth is water [1]. However, due to the exponential population growth accompanied by industrial and technological expansion, there has been a greater demand for water from different types of consumers, from a domestic to the industrial point of view, which menaces the water quality and increases wastewater discharges [2,3,4,5]. Conventional treatments are not designed to remove recalcitrant pollutants from wastewater effluents, such as pharmaceuticals, and are expensive. In this sense, various alternative and/or complementary processes for the more efficient and complete treatment of effluents is being widely studied and proposed [6,7,8].

Pharmaceutical products are considered contaminants when they reach the environment through the pharmaceutical industry or through anthropogenic action [5,9,10]. Several pharmaceutical compounds are detected in analyses of different water matrices, due to the ineffectiveness in eliminating or removing pharmaceutical compounds from the conventional wastewater treatment plants (WWTP) [11]. The existence of a gap in the legislation on this topic means that there is no control of WWTP for this type of contaminants. However, this implies a big concern due to uncertainty of what consequences of continuous exposure to low concentrations of pharmaceutical compounds will have on humans and the environment [5, 12].

Therefore, it is crucial to invest in the study of monitoring pharmaceuticals using selective, efficient, and recyclable materials, for *in situ* extraction and pre-concentration, making it possible to detect these contaminants quickly and accurately at the smallest concentrations [5,9,10].

1.2 Main Objectives

The main objective of this work is to select highly efficient COFs for the adsorption of certain pharmaceutical pollutants present in water samples, for their integration into cartridges that allow simpler, more sensitive, and efficient monitoring of these compounds.

In this study, on a first stage, the adsorption efficiency of pharmaceutical compounds by the pre-chosen COFs was studied, namely the adsorption kinetic parameters, the isotherms, the influence of pH, desorption kinetics, recycling, and reuse potential of COFs. On a second stage, the COFs that have proven to be more efficient are used to develop extraction cartridges and prototypes of solid phase extraction

(SPE) columns. In the third, and final stage, it was intended to test the laboratory-scale performance of the developed prototypes, to validate the possible use of these devices in the extraction and *in situ* pre-concentration of contaminants, and thus facilitate the process of monitoring pharmaceutical pollutants in water. The performance of these prototypes needs to be compared with conventional sample preparation techniques, namely conventional SPE, to verify its efficiency, and imply a significant improvement. However, it was not possible to do and to include in this work.

1.3 General Outline of the Thesis

The structure of the thesis consists of five chapters. Chapter 1 refers the context, the scope, the main objectives and context, and the thesis format. Chapter 2 includes the state of the art, theoretical knowledge on wastewater treatment, water pollution, pharmaceutical compounds, and covalent organic frameworks. On the Chapter 3, the material, methods, and procedures used on the experimental work are reported. On the Chapter 4, the results from this study are presented and discussed. The last one, Chapter 5, comprises the conclusions from this investigation and suggestions for future works.

CHAPTER 2. STATE OF THE ART

2.1 Background

Overall growth of population and climate change incited a reflection on our daily life and how irresponsible use of water resources has an impact in the ecosystem [3,4,5,13,14]. Currently, the world recognizes that hydric resources are finite due this high demand, so water consumption is faster than renewal capacity, which promotes hydric stress for potable water [2,3,4,5]. Hydric resources are irreplaceable [9] and it is important that they are managed rationally and efficiently [6], without instigating economic and public health issues, guaranteeing the water quality [2,3,4,5]. Despite this, water quality has been compromised by pollution from anthropogenic activities that threaten the safety of drinking water if not properly treated [15,16]. Furthermore, if this water is discharged into water resources, polluting them, they will lose their economic value and become a hazard to the ecosystem [17]. Therefore, WWTP are crucial for treating these effluents and avoid that fate.

Wastewater effluents can be categorized into four different groups: 1) domestic effluents, which are a mixture of waste transported by water and liquids ejected from houses and commercial buildings; 2) industrial effluents, containing waste produced by industrial activities, 3) stormwater effluents, which are a result of storms and rainfalls; and 4) sewage effluents which occur due to surface or ground water

entering the sewer system through direct and indirect ways like cracks, leaking conjectures and porous walls [17,18,19]. WWTP are infrastructures delegated to treat wastewater effluents aiming to reduce any negative impact on environment and public health and diminish water pollution by producing a stream of clean water that is safe to be released into the ecosystem [18,20,21,22,23]. The waste from wastewater comes from daily activities, such as dirt, paper, proteins, food particles, organic and inorganic compounds, oil and grease and they are evaluated in milligrams of pollutants per litre of water (mg/L) or parts per million (ppm) [17,23]. In the case of micropollutants, like pharmaceuticals, they can be found in the range of μg/L or even ng/L [11,24,25]. So, the processes for the removal of this type of pollutants may be focused on eliminating a few milligrams per litre of a pollutant, and monitoring removal involves measuring techniques with sensitivities to detect very low concentrations [23].

The steps of the treatment process that occurs on a conventional WWTP can be observed on the Figure 1. The first step on WWTP is the *Preliminary treatment* where large debris or pollutant materials like twigs or rags are eliminated from the received effluent, and bigger particles are grinded, suspended solids are gritted, and it culminates with the removal of grease and oils through flotation [23]. This step enables a smoother treatment for the next phases of the process. The residual effluent proceeds to the primary decanters, where it suffers the *Primary treatment*, which involves removing suspended solids through sedimentation, and eliminates 50% of pollutants, although it's not enough, so the effluent is forwarded to the *Secondary treatment* [22,23]. On this step, the process can be done aerobically or anaerobically, and it consists of a biological treatment that removes organic matter and reduces the quantity of solids [22,23]. Under aerobic conditions, the conventional process uses activated sludge, a mixture culture of microorganisms in aeration tanks, in constant agitation and with regular supply of oxygen. The microbial community removes organic matter and synthesizes new microbial cells [22,23]. On the physical phase, the secondary sludge that consists of microbial aggregates resulted from the excess of sludge produced suffers a sedimentation through the secondary decanter which boosts the clarifier action on the aerated effluent [22,23]. Efficiency of this process can be evaluated through suspended solids removal, and in terms of Biochemical oxygen demand (BOD) [22,23]. On some scenarios, with only these mentioned treatments, the effluent can be considered safe to be released into aquatic environment [22,23]. The treated effluent must comply with certain parameters defined by legislation, which depends on where it will be disposed off, some of which are more complex than others, so it may or may not be necessary to undergo a disinfection step at the WWTP [22,23]. Disinfection of residual effluents is only mandatory in cases where water is released into the environment downstream, where water is captured or being reused in several anthropic activities and go back to the ecosystem [22,23]. Tertiary treatment is an additional

step to more specific and demanding standards to protect the environment. It is very effective to eliminate nutrients like nitrogen and phosphorus, suspended solids (by filtration), persistent pollutants, and to disinfect (microorganisms, pathogens) with ozone, UV or chlorine [22,23]. Nutrient removal is done, when necessary, with the goal of achieving the allowed limit in residual waters due to nitrogen and phosphorus being two very important resources to microbiota culture [26,27,28].

Chemical disinfection via UV lights results on degradation of pollutants through radiation and is very crucial that WWTP do this type of treatment on effluents that will be reused, even if the costs of this treatment are higher [28,29]. Another type of chemical disinfection consists of adding a strong chemical oxidant such as chlorine or ozone [22,23]. Chlorine is the most common chemical used in WWTP, however due to producing halogenated compounds when in contact with organic matter, and the demand for large quantities of this chemical to have an effective disinfection, it is not the most appealing process [28,30]. Ozone is preferable due to its efficiency in less time, which is related to ozone's strong potential in diminishing odours, clearer waters, and higher degradability of organic pollutants [28,30,31]. Although, when ozone is in the presence of bromide it reacts and synthetizes toxic bromide compounds [20,29,31].

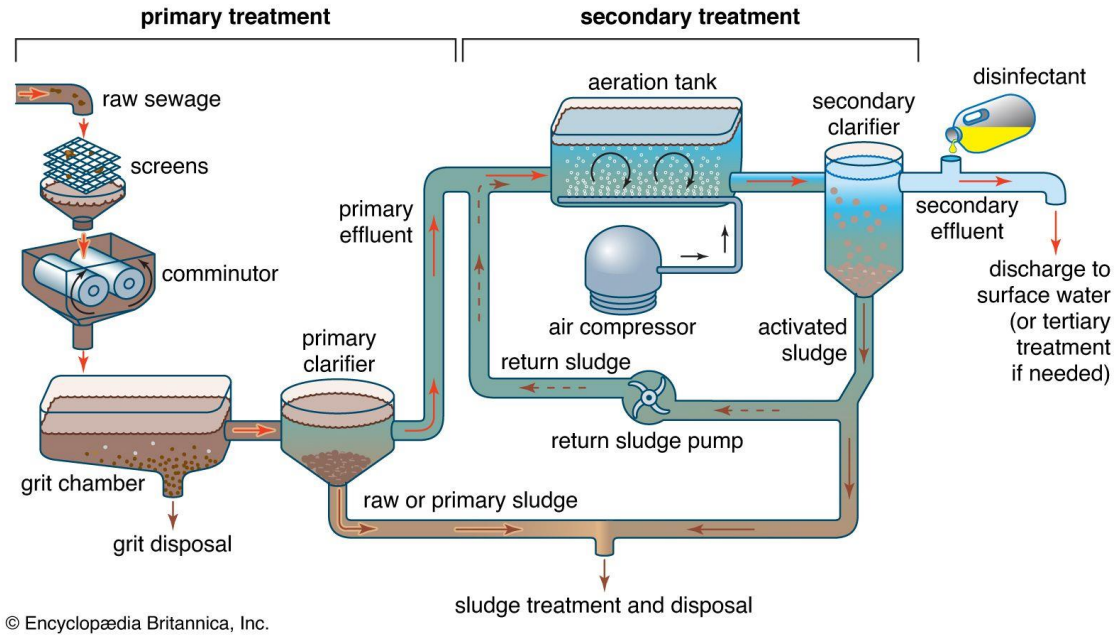

Figure 1 - WWTP treatment phases reproduced from (https://www.britannica.com/technology/wastewater-treatment/Clustered-wastewater-treatment-systems#/media/1/666611/19281)

2.2 Water pollution

The future of earth's ecosystem depends on environmental constituents as water, and the sustainability of resources to assure life for every organism, so when a part of the components is contaminated, eventually the whole system will be affected [32]. That is the major concern regarding water pollution, the contamination of hydric resources that makes water unsafe for drinking, cleaning, cooking, swimming and for other economic sectors to apply it in their industries [32, 33]. This is because contaminated water can contain dangerous or toxic substances from the discharge of diversified chemical compounds, and/or disease-causing bacteria from dispersion of pathogens from anthropogenic activities [32,34,35].

All types of pollution eventually become a part of the water's components, such as trash, bacteria, parasites, and chemicals. Air pollution settles onto lakes and oceans, soil pollution passes through groundwater and then to a river and, eventually, into the ocean, which affects the whole system and jeopardises water quality for future generations [32,33,36]. Water pollution is responsible for diminishing the ecological function of aquatic habitats and for being the leading cause of diseases and in worst cases, death in some regions of the world [35,36]. The origin of water pollution can be attributed to industrialization, urbanization, population growth, pesticides, fertilizers, plastics, domestic sewage, industrial sewage, mining, eutrophication, agro-chemical wastes, radioactive waste thermal pollution, acid rain, oil spillage, disruption of sediments, climate change and much more [32,37]. Water contamination can be categorized as biological due to viruses [38], coliforms [39], protozoan parasites [40], or chemical due to toxic metals [41], pesticides [42], fertilizers [43], polycyclic aromatic hydrocarbons [44], polychlorinated biphenyls [44], arsenic [45] and per-chlorate [46]. Likewise, water pollutants can be characterized into seven categories of different types of pollutants: organic, inorganic, suspended solids, pathogens, radioactive, thermal, nutrients and agricultural [11]. Organic pollutants consist of oxygen, carbon, hydrogen, nitrogen, and sulphur and are originated from urban wastewater, industrial wastewater, agricultural waste, and sewage [11, 47]. These types of pollutants are not only present in water, but also in soil and sediments, and may lead to toxicity, bioaccumulation, mutagenicity, and carcinogenicity, all having an impact on the ecosystem and the most concerning of all, may enter the food chain, which is a menace to public health [48,49,50,51]. Inorganic pollutants can be defined as harmful chemicals, for example nitrogen pollutants (nitrate, ammonium, nitrite), phosphates and heavy metals [11,47]. The first two mentioned are related to water draining of agricultural fields which induces health problems [11]. Suspended solids can be categorized into three different categories: sand and organic content, and suspended solids in the waste effluent [11]. Pathogens pollutants are defined as microbes that develop

diseases, such as viruses (ex.: Hepatitis), bacterial infection (ex.: by Salmonella), fungal infection (ex.: by Candida) and parasites infection (Ex.: by Cryptosporidium and Schistosoma) [11]. Radioactive pollutants are the most dangerous to the planet and human beings, they can come from nature, from earth's crust and dissolve into drinking water, or from anthropogenic radioactive material released from nuclear power plants [11]. Thermal pollution induces temperature changes in water bodies and can be caused by discharges from thermal power plants, and global warming [11]. The rise in temperature of hydric systems poses a serious threat for the flora and fauna living on those habitats [11]. Agricultural pollutants are related to fertilizers used in agriculture, such as heavy metals or ammonium, salts of phosphate, potassium, and nitrate [35]. The fertilizer industry contributes to the release of heavy metals like Cd, Hg, Cu, As, Pb and Ni to the environment by being applied into the soil, which then will contaminate groundwater and are absorbed by plants, therefore, it can contaminate both the food chain and water supply [11].

Lately, the topic of emerging contaminants (EC) has become increasingly relevant in the study of water quality, although EChave been detected in some analysed water from the environment only at low concentrations (µg/L to ng/L levels), side effects on human health are still unknown [11,24]. Emerging water pollutants include pesticides, herbicides, personal care products, endocrine disrupting compounds and pharmaceuticals, which are by-products of globalization and our modern lifestyle [11,52]. The continuous use of chemicals in our daily basis and the inadequacy of wastewater treatment to effectively remove these contaminants causes them to end up in environment, threatening the water quality of river and lakes, endangering flora and fauna, and human health [11].

Advances on the medical field and on the health care system induced an easier access to pharmaceuticals at a worldwide level [24,53]. Recent data declares that human beings consume 1 lakh ton of pharmaceuticals per year, which means the average consumption at worldwide level is 15 gm/capita/annum [53]. There is a variety of 12,000 approved human pharmaceuticals available, and 850 of them are active compounds [53]. The global market of pharmaceuticals has had reached a revenue of 1072 billion dollars in 2015 [36]. The market offers a diverse range of medications to the public such as antipyretics (reduce fevers), analgesics (painkillers), antiseptics, antidepressants, contraceptives, hormone replacements, mood stabilizares, cytostatics and statins [36].

Pharmaceuticals have been added to the list of candidates Emerging Contaminants due to their detection in water courses, all over the world, and therefore their potential health effects, ecotoxicity and occurrence, that might need regulation of their concentration in the future [5,28,36,54,55,56]. Currently there isn't regulation for discharge of pharmaceutical residues [5,56,57,58], but it was established a

maximum acceptable concentration of pharmaceuticals in natural waters of 10 ng/L to prevent a harmful impact on human health and the environment [10]. However, pharmaceuticals can evoke biological effects at very low concentrations, so the detection of quantitative and accurate data of these contaminants in natural waters is still a challenge due to the low analyte concentration and the complex matrix they are constituted [5,59]. Therefore, for the monitoring of pharmaceutical contaminants it is necessary to purify the samples, concentrate and remove any potential effects from the natural water matrices [5,59].

Pharmaceutical bioactive compounds can be originated from animals, plants, or microbes, or by chemical synthesis, and they are very common in human medicine as well as in veterinary (Figure 2), with a very frequent use every year [36, 60, 61] and those molecules have been appearing in various effluents of the aquatic environments [62]. This problematic is of high concern because pharmaceuticals released through water, soil, and food, can have undesirable effects on human and animal organisms, and therefore may harm fauna and flora, even if those compounds are present in very low concentrations [63,64,65,66]. Furthermore, in the case of antibiotics, it might contribute to an increase in cases of multi-resistant bacteria.

Water contamination by pharmaceuticals can occur via human and animal excretion due to not all the pharmaceuticals being metabolized by the body and the intact bioactive compounds and/or their metabolites will be excreted [24,53,67,68]. In case of livestock animals, those excretions create manure storage tanks and lagoons, which without any treatment will contaminate the soil and aquatic environment (Figure 2). The same happens in aquaculture, that uses pharmaceuticals on fish and is a form of direct pollution of the aquatic environment [69]. The pharmaceutical industry has had a fast and enormous expansion due to the high public demand for medicines [28,53]. This poses as a threat to the environment because those factories might dispose their effluents after a partial treatment from WWTP [28,53].

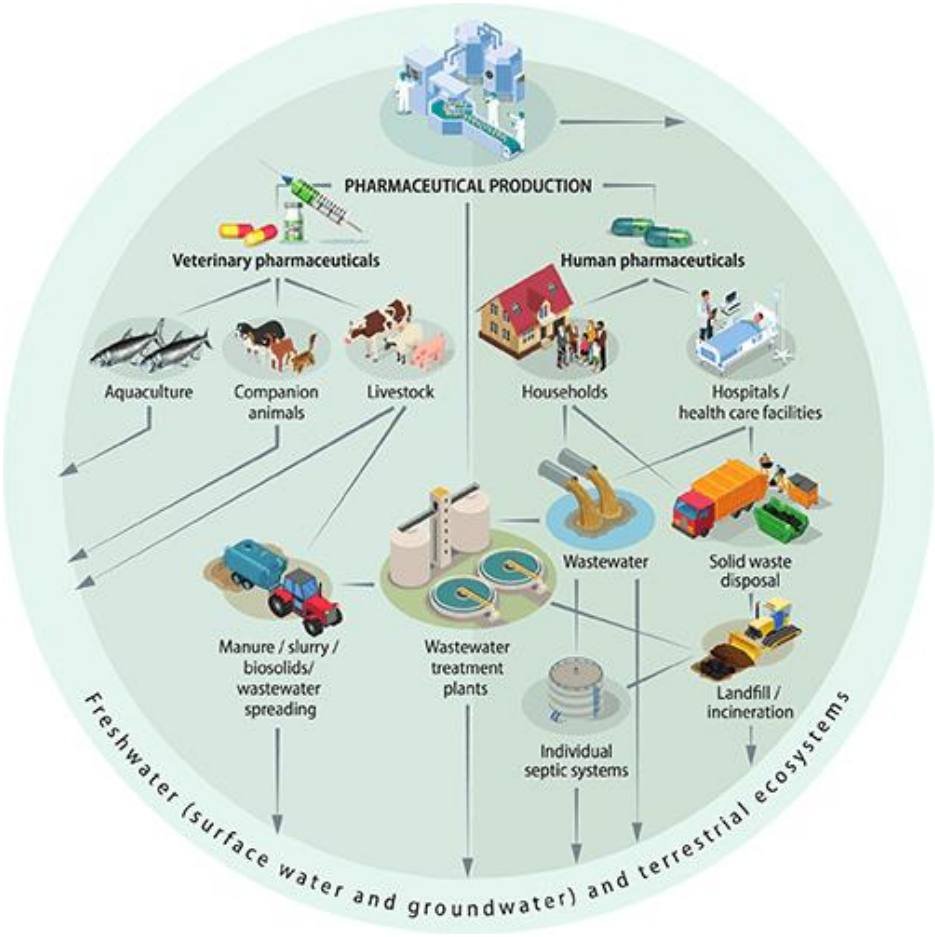

Figure 2 - Infographic - major pathways of release of human and veterinary pharmaceuticals into the environment reproduced from OECD [70].

Another pathway of pharmaceutical pollution to be considered is the general incompliance of the population to recycle expired or unused medicines, therefore they are discarded in toilets which rises the flux of these contaminants [24,53,68]. Promoting the recycling of unused and expired drugs could prevent this route of pollution [24,68] because even after the medicines expire, it can contain at least 90% of the active compound [68].

The contamination of the soil, as well as the surface water and groundwater, is a direct or indirect way of contamination of human beings [68]. The direct contact originates from drinking tap water, which comes from Water Treatment Plants (WTP) [68]. However, indirect contact can happen through the food chain, due to the water used to irrigate agriculture fields when it comes from WWTP without proper treatment, so it still is contaminated due to lack of development of wastewater treatment on removing pharmaceutical contaminants [36,68]. As a result, the contaminated crop products will be consumed by humans and livestock animals and, eventually, those contaminated animals will also be eaten by humans [36,69]. A big concern is the use of antibiotics in anthropogenic activities such as aquaculture and agriculture, which creates an environment resistant to antibiotics and diminishes their ability to act on any bacteria [36,69].

These drugs end up in WWTP and, as they are not effectively treated [24, 71], they are discharged through the treated effluent and through biosolids, causing pollution of the aquatic environment and the soil that encounters groundwaters [60, 68]. This exacerbates the problem, increasing the toxic effects, cumulative and/or synergistic, when in contact with same and other micropollutants that have direct impacts [68,72,73].

Removal of pharmaceuticals in conventional WWTP depends on the chemical composition of the pharmaceuticals, and their metabolites, and the methods applied in the WWTP [24,71]. Therefore, when a pharmaceutical active compound (PhAC) reaches the WWTP it can be divided into four courses of action [74,75,76]:

1. The active compound can be altered into a lipophilic derivative of the original compound;
2. The active compound can be mineralised through various processes and organic matter is transformed into simpler inorganic compounds;
3. Polar and stable compounds do not suffer any alteration during the wastewater treatment and that results into them entering the aquatic ecosystem completely intact;
4. The compounds might not be degraded due to their characteristics, such as physicochemical proprieties that enables the compound to connect to the solid phase and it leads to a potentially accumulation onto the sludge.

The pollution from pharmaceuticals tends to accumulate into different organisms and their environment, which is an ecological menace due to compounds being very stable [7,71,77,78]. Even though, in nature compounds can be degraded through hydrolysis, photodegradation and biodegradation, depending on the chemical composition of the compound and the environment conditions [71]. These alterations to the compound may diminish their pharmacological effect, but this results in the formation of products that might have the same impact and be more stable than the parent compound [79].

It is still unknown the exact impacts of life-long exposure to random combinations of low levels of pharmaceuticals compounds on human's health and the environment but it shouldn't , and it mustn't be considered harmless [24,71,80].

The study of the chemical compounds used to produce medicines, and the changes they may undergo, difficulties their monitoring and identification on water matrices [36,53,80,81,82]. Therefore, there is a need for an investment on water quality investigations, regulation on the dispersion of pharmaceutical compounds into water, development of methods to detect and remove these pollutants efficiently from water in WWTP and avoid the continuous introduction of pharmaceutical pollutants to the aquatic environment [7,28,36,71].

2.3 Pharmaceutical compounds

2.3.1 Antibiotics

Antibiotics use has been dramatically increasing each year at worldwide scale due to their abilities to destroy or inhibit the bacterial infection [83,84,85]. Antimicrobial agents are divided into two categories based on their in vitro effect on bacteria: bacteriastatic and bactericidal [86,87]. The first one blocks the multiplying of bacteria, and the second one kills the bacteria, by interfering with either the formation of the bacterial cell contents or cell wall [86,87].

Antibiotics are applied in human and veterinary medicine, agriculture and aquaculture, to prevent (prophylaxis) or treat bacterial infections [88,89]. Despite these medicines requiring prescription by a doctor, overuse and misuse of antibiotics can lead to antibiotic resistance, which consists of no reaction from the bacteria after being in contact with the antibiotic, due to improving their defences [87]. To make matters worse, this transfer of resistance is passed on to new generations [90]

These drugs can be divided into categories according to their spectrum of activity, mechanism of action, action spectrum, chemical structure, and the route of administration [83,91]. There are broad-spectrum antibiotics, which are less specific, and narrow-spectrum antibiotics, which are more specific and effective against certain types of bacteria [87]. The preferred method to identify groups of antibiotics is the mechanism of action and the most common ones are: monobactams, β-lactams, carbapenems, glycopeptides, aminoglycosides, polyenes, polypeptides, macrolides, quinolones, fluoroquinolones, chloramphenicol, lincomycin, rifamycin, sulfonamides and tetracyclines [91].

2.3.1.1 Tetracycline

Currently, the most common antibacterial used is Tetracycline due to their robust efficiency, high quality, and low cost [83,92]. In the 1940's, the isolation of various *Streptomyces* species was done, allowing for the first medication from the tetracycline family, which has been commercialized ever since, with successful clinical results [82, 93]. The general structure of Tetracycline can be observed on the Figure 3. The elementary structural component is a tetracyclic ring system with many methyl, hydroxyl, keto and dimethylamino functional groups [94]. The tetracycline family can be categorized into three classes based on their nature, dosage, and elimination time [92]. With the development of antibiotics, it has been proved that the third generation of tetracyclines is more effective [82]. Tetracyclines are powerful because they inhibit protein synthesis in the bacteria by attaching to the 30S ribosomal subunit of bacteria [82,95,96].

It has also been concluded that they have a broad-spectrum activity capable of restraining the active of most Gram-negative and Gram-positive strains [82,97].

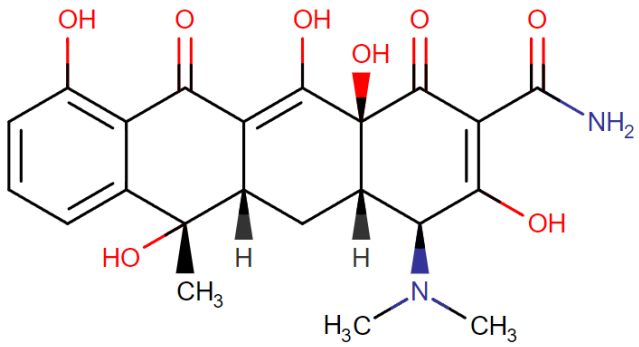

Figure 3 - Representation of the chemical structure of Tetracycline. Reproduced from Drugbank [98]

Therefore, tetracyclines are used to treat diverse bacterial infections, for example on the urinary tract, intestines, skin, genitals, lymph nodes, respiratory tract, among other body systems [92]. It can also be used to heal severe acne, sexually transmitted diseases such as gonorrhoea, chlamydia, or syphilis [92]. Although, overusing tetracycline is related to bacterial resistance, allergic reactions in humans, serious harmful alterations in environmental microflora [92].

This pharmaceutical compound is very difficult to deteriorate in humans and animals, which leadsto 50% to 80% of tetracyclines being excreted into the ecosystem [99]. Tetracyclines are very difficult to degrade in nature by oxidation due to their stability. They have the potential to bind to Ca^{+2} and other ions, and are sensitive to extreme pHs, which poses as a threat to ecological equilibrium [9, 92, 100,101,102]. There is still not any regulation for specifically analysing the presence of this pharmaceutical compound and for routine sampling of water resources [103,104].

Tetracycline has a moderate solubility in water (231 mg/L at 25 °C), consonant the logP, it is hydrophilic (-1,3) and, has a logS value of -3.12, being considered slightly soluble (Table 1). The pka value indicates that Tetracycline is a weak acid with a value of 3.3 at 25 °C [98].

Property	Value	Source
Melting point (°c)	172.5 dec °C	Physprop
Water solubility	231 mg/L (at 25 °C)	Yalkowsky,sh & dannenfelser,rm (1992)
logP	-1.30	Hansch,c et al. (1995)
logS	-3.12	Adme research, uscd
pKa	3.3 (at 25 °C)	Serjeant,ep & dempsey,b (1979)

Table 1 - Experimental properties of Tetracycline [98].

2.3.1.2 Sulfapyridine

Sulfapyridine is a sulfonamide antibiotic (SAs), very effective, and has been vigorously synthesized and consumed worldwide [105]. May and Baker discovered sulfapyridine in 1938, and it could cure bacterial pneumonia, which revolutionized the treatment of streptococcal infections [106]. However, the introduction of penicillin diminished the demand for SAs medicine [106]. Sulfonamide antibiotics are a type of synthetic antimicrobial agents that consist of the sulfonamide group, and that includes sulfadiazine, sulfasalazine, sulfamethoxazole, sulfisoxazole, sulfamethizole, sulfadoxine, sulfacetamide, sulfanilamide, and sulfapyridine [107,108]. SAs contains p-aminobenzenesulfonamide into their structure, they are derivatives of ammonia benzene sulfonic group [107,109]. The molecular structure of sulfapyridine can be seen on Figure 4 and it has in its constitution a sulfonphthalamide group, a benzene ring, and a para-amino group [107]. Sulfapyridine method of action consists of acting as a competitive inhibitor of the bacterial enzyme dihydropteroate synthetase [110]. This process will block the organisms from producing folic acid which is crucial for cells to synthetize nucleic acids such as DNA, or RNA, therefore the cells can't divide and replicate [110].

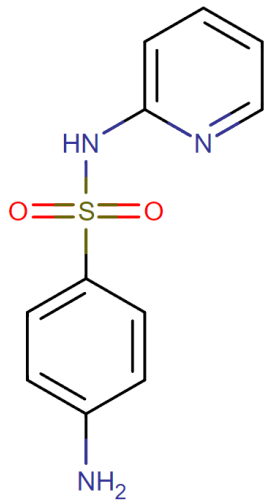

Figure 4 - Chemical structure of sulfapyridine reproduced from Drugbank [110].

Sulfapyridine is applied in medicine to treat benign mucous membrane pemphigoid, pyoderma gangrenosum and dermatitis herpetiformis [109,110].

Sulphonamides are frequently consumed which justifies their repeatedly detection in different water matrices [107,109]. Their strong hydrophilicity makes them more prone to enter any water environment through drainage,rainwater and bioaccumulate in its intact form [107,111]. SAs residues are hazardous to ecosystems and humans, when reaching the food chain [107 (61)]. They promote the development and spread of antibiotic resistance genes, as well as impact the growth of fauna and flora [105,107,113]. Sulfapyridine has a moderate solubility in water (268 mg/L at 25 °C), the logP is 0,35 which indicates it is more lipophilic (Table 2). As for aqueous solubility, the logS has a value of -2,7 which indicates it is slightly soluble, and according to the pKa (8,43) it is a weak base [110].

Property	Value	Source
Melting point (°c)	192 °C	Physprop
Water solubility	268 mg/L (at 25 °C)	Yalkowsky,sh & dannenfelser,rm (1992)
logP	0.35	Hansch,c et al. (1995)
logS	-2.7	Adme research, uscd
pKa	8.43	Perrin,dd (1965)

Table 2 - Experimental properties of sulfapyridine [110].

2.3.2 Non-Steroidal Anti-Inflammatory medicines

Currently, there has been a growing concern with the wildly growth of analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) consumption [114,115,116]. These types of medicine are the most prescribed class of pharmaceuticals and can also be easily obtained without a medical prescription [116,117]. NSAIDs are used for inflammatory reduction, as analgesic agents and as antipyretic [114,116,118, 119]. So, they are applied to treat arthritic conditions, muscle pain, migraines, pyrexia, dysmenorrhea, gout and, on more severe cases, they can be used as opioid-sparing agents [120, 121]. NSAIDs are organized based on their selectivity and chemical structure: propionic acids (naproxen, ibuprofen), naphthylalanine (nabumetone), enolic acids (meloxicam, piroxicam), anthranilic acids (meclofenamate, mefenamic acid), selective COX-2 inhibitors (celecoxib, etoricoxib), acetylated salicylates (aspirin), non-acetylated salicylates (diflunisal, salsalate), and acetic acids (diclofenac, indomethacin) [120,122]. The anti-inflammatory analgesic medicines come from plants extracts that contain salicylate, one example of that is the bark of the willow tree (Salix alba as other constituents of the Salix species) [122].

2.3.2.1 Diclofenac

Diclofenac (2-(2,6-dichloroanilino) phenylacetic acid) is one of the most consumed NSAID medicine throughout the world, it can be purchased as a topical gel or as oral tablets [123,124,125]. Diclofenac sodium was first synthesized by Alfred Sallmann and Rudolf Pfister and then in 1973, it was launched by Ciba-Geygy, now known as N Novartis AG, Basel, Switzerland [126,17]. Diclofenac belongs to the family of phenylacetic acids, and their general structure can be seen on the Figure 5 [124,128]. The structure constituents consist of a phenylacetic acid group, a secondary amino group, and a phenyl ring containing chlorine atoms, which potentiates the maximum twisting of the ring [129].

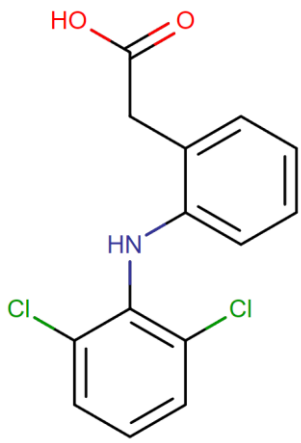

Figure 5 - Chemical structure of diclofenac reproduced from Drugbank [130].

Diclofenac is used to treat and manage chronic and acute pain associated with inflammatory conditions, especially the ones associated with the musculoskeletal system [124,128]. Examples of this are problems related with joints, muscles and bones which constitute symptoms of ankylosing spondylitis, rheumatoid arthritis, and osteoarthritis [124,128,130].

Being one of the most consumed compounds, Diclofenac is also one of the most detected pharmaceuticals in rivers, sludges, sediments, and WWTP [123,124]. Diclofenac administration is done orally or topically, and it suffers almost full biotransformation in the human body [125]. When orally consumed, 65% to 70% is usually excreted in urine and 20% to 30% in faeces, as the parent compound or as metabolites [125]. However, the dermal absorption was found to only be 6% to 7%, due to possibly being attached to clothes, or even washed off the skin [125].

WWTP are not efficient on eliminating Diclofenac from wastewater due to high consumption, high persistence, and low biodegradability [123,124,125]. Diclofenac has been detected in municipal wastewaters at concentrations between 0.44 to 7.1 μg/L, with an average of 0.11 and 2.3 μg/L, which leads to bioaccumulation in water effluents [123,125]. The growing concern is associated with the detection of Diclofenac in drinking water sources, and what consequences it can have on a variety of organisms that consume that water with significant concentrations [124].

As presented in Table 3, diclofenac has a very low solubility in water (0,0047 mg/L at 25 °C), according to the logP, as in molecular lipophilicity this compound is more lipophilic with a value of 4.26. As for aqueous solubility, the logS has a value of -4.8, so it is considered insoluble. The pka value indicates Diclofenac has two pka, which means it has two different ionisable functional groups, where the one with a pka of 4 is a weak acid and the one with a pka with a value of -2.1 is a strong acid [130].

Property	Value	Source
Water solubility	0.00447 mg/mL	alogps
logP	4.98	alogps
logS	-4.8	alogps
pKa (strongest acidic)	4	chemaxon
pKa (strongest basic)	-2.1	chemaxon

Table 3 - Experimental properties of diclofenac [130].

2.4 Treatment of emerging contaminants in water

Conventional WWTP are not efficient on removing emerging recalcitrant compounds. However, there has been a development on the sector of technologies to remove emerging contaminants that include pharmaceuticals, and they can be divided into three categories, natural attenuation, conventional and advanced treatment methods [131]. The natural attenuation methods consist of biological, chemical, and physical processes, such as volatilization, biodegradation, photolysis, dilution, sorption, and many others, which are simple, low cost and efficient [131]. Conventional treatment technologies are inefficient on removing ECs and they include adsorption with activated carbon, membrane filtration, among others [131]. Advanced treatment technologies consist of bioelectrical systems, constructed wetlands, enzymatic processes, oxidation methods, and much more [131]. Notwithstanding, even with all these possibilities to treat contaminated water with ECs, it still is not satisfactory due to being dependent on the properties of ECs and the treatment conditions [131]. WWTP can be effective on eliminating some ECs, however they aren't designed to remove them at low concentrations, which constitutes as a big concern [131]. Therefore, WWTP should be considered as primary barrier against the spread of ECs, therefore, it's necessary to improve the treatment methods to target ECs at the ng/L concentrations to protect the environment [131].

2.5 Monitoring of emerging contaminants in water

The biggest obstacle on the analysis of pharmaceutical contaminants is the scarcity of methods to quantify them at low concentrations, in the range of ng/L level or even below that [6]. For an appropriate risk assessment, and a successful monitoring of the water quality, is necessary to develop processes that can achieve that precise range of quantification [6].

Analytical methods for each group of ECs may have high selectivity and sensitivity, especially for very complex matrices, for example, wastewater effluents [6]. Preparation of samples by cleaning and pre-concentrate is very important, and generally the best option is solid-phase extraction (SPE) incorporated into a column system with a resin capable of retaining several different types of ECs [1]. The most used techniques for the detection of EC are metal analysis, spectroscopic techniques and chromatographic [132]. The preferred analytical process for detection and identification of several compounds in all types of samples is chromatography [132]. It includes gas chromatography (GC) and liquid chromatography (LC) [132]. The first one focuses on volatile, nonpolar compounds and thermostable ECs, such as pesticides, filters, and flame retardants, the second one analyses non-volatile, polar compounds and thermolabile ECs [132].

2.5.1 Solid-phase Extraction

SPE is a fast and efficient technique designed for selective sample preparation and purification before the chromatography analysis [133,134,135]. With SPE, it is possible to eliminate one or more compounds from interfering with a liquid sample by extracting/partitioning, and or adsorbing onto a solid stationary phase [133,134,135] or even concentrate analytes of interest into the sample through the material embedded with a compound of interest in the solid phase to function as an absorbent and concentrate the sample, while not retaining the interfering analytes, then the solvents elute the sample and it is collected to be studied [133,134,135].

The application of SPE refines the original sample, to a simpler sample, that consequently is more appropriate for analytical chromatography, which simplifies and improves the final quantitative and qualitative assessment [134]. Ideally, a great SPE process eliminates interferences that would induce misleading peaks, poor sensitivity, and high background [134]. SPE allows the concentration of analytes, samples matrices to be changed to be more specific to the target chromatographic method, and the extraction method to be automated [134].

2.5.2 Liquid chromatography

Chromatography is a scientific technique that separates constituents of a mixture [136,137]. This process is used to separate nucleic acids, proteins, or small molecules in complex mixtures [138].

Liquid chromatography (LC) consists of the separation of molecules in a liquid mobile phase with a solid stationary phase and it can be used for preparative or analytical purposes [138]. This process requires

an injector to apply a certain amount of the sample and a pump to create the flow of the liquid, then the separation can take place on the column [139]. The results will be recognised by detectors from the equipment [139].

The criteria for columns (stationary phase) selection are based on the compounds that we want to detect, the dimensions of the column and the packing material [139]. LC originally uses alumina or silica due to having on their surface a good portion of hydroxyl groups, which makes the columns polar, and this is denominated as the normal phase [139]. The surface of silica can be modified by adding hydrocarbons, which makes it a non-polar surface, and that is denominated as the reverse phase [139]. Examples of a variety of common phases are: C18, C4, Phenyl-Hexyl and Cyanopropyl [139].

There are three types of LC, LC and high-performance liquid chromatography (HPLC) are the standard analytical method, and the ultra-high-performance liquid chromatography (UHPLC) is an innovative process [132]. HPLC can be applied in a wide group of ECs, if they constitute as non-volatile, and are polar compounds [132].

UHPLC has been the preferred method to use because it allows high pressures, enabling efficient separation more quickly [132]. UHPLC uses columns with particles smaller than 1.7 μm, and almost always carries out the reversed phase mode mostly applying the C18 stationary phase [132]. The reversed phase for the separation of ECs usually applies acidified water, acetonitrile, or methanol as organic solvents for the mobile phases. On studies with pharmaceutical compounds, silica packing is the most common used stationary phase, amongst the LC modes, reversed phase (RP) with octadecyl C18-bonded or octyl C8-bonded [132].

When LC is complemented with mass spectrometry (MS), it amplifies the detection efficiency by having greater selectivity and sensibility to larger molecular weights, thermally unstable compounds, and very polar compounds [132]. This is possible because MS is an analytical instrument to determine the mass-to-charge ratio (m/z) of one or several molecules from a sample [140]. The results can be applied in calculus to obtain the exact molecular weight of the sample constituents [140].

MS is an interesting technique for the quantification of compounds or residues in the environment. Tandem MS (MS/MS) has been the chosen method due to their selectivity and sensitivity in complex matrices [132]. MS/MS is a process capable of fracturing selected ions, which are the precursor ions, into fragments, or the product ions from the splitting [141]. Usually, this technique monitors a selected MS/MS transition from the precursor to product of the elected compounds. Because of this detailed analysis, it is ideal for quantification of environmental samples at trace levels [132]. Although, MS/MS removes the untargeted species, by removing the matrix background, it will only detect predetermined

species [132]. To avoid this limitation, high-resolution mass spectrometers (HRMS) allow for a vast unlimited number of species by using the full-scan mode and accurate mass measurements [132]. Therefore, by using LC-HRMS it will be able to identify targeted and untargeted compounds, metabolites, or transformation products [132].

2.5.3 Passive sampling.

Passive sampling is a monitoring method based on the unassisted molecular diffusion of analytes through a diffusive surface onto an adsorbent [142,143]. This method can be applied to detect a vast range of compounds in different matrices, such as air, soil, and water [142,143]. Usually, the passive sampling device has a filter, diffusion gradient and a sorbent with a strong affinity to the target molecule as the receiving phase [142]. This method is established in the target analyte passing through the sampling device's receiving phase from the sampling matrix [142]. It is possible to do *in situ* extraction and pre-concentration of analytes over longer periods of time (days, weeks, or months), and afterwards the concentration of the analyte is quantified through time weighted average (TWA) [142,144]. After the sampling is complete, the adsorbed molecules are desorbed from the surface via thermal desorption or by a solvent [143]. This technique has numerous advantages, such as being simple, portable, compact, less labouring, and low cost, these characteristics allows passive sampling to do multiple sampling locations, it can detect average pollution levels for extensive periods of time, it doesn't need power supply to be operated, it doesn't require supervision, and it is less vulnerable to contamination during transportation, when compared with studies that handle bigger sample volumes [142,143,144].
Passive sampling being applied to monitor ECs is a very promising method and it may even be interesting to use COFs as an adsorbent on the device, especially if the adsorbent shows great promise for adsorbing pharmaceutical compounds on natural waters.

2.5.4 Alternative methods to monitor emerging contaminants.

Currently there are two types of alternative methods to monitor ECs, the electrochemical based detection, and the optical based techniques [145,146]. The first one consists of manipulating reactions at the interface of an electrode and a solution by exploiting the transfer of electrons [145,146]. It contains three dominant measurements techniques, amperometry-based sensing, voltammetry, and potentiometry, which are most used to detect ECs [145,146]. These processes stand out due to being fast, simple, low-

cost, portable, and have good selectivity and sensitivity, interesting characteristics for on-site monitoring with a wide range of ECs [145,146].

Electrochemical research had an important role in the expansion of various original working electrodes, examples of that are glassy carbon, carbon paste, carbon nanofibers, mesoporous carbons, carbon composite, nanoporous gold wires, boron-doped diamond electrodes, and thin-film electrodes modified with nanomaterials and polymers specific to analytes of interest [145]. The electrodes can be produced from nanomaterials such as quantum dots, multi-walled carbon nanotubes, graphite, graphene oxide, gold, silver, zinc, nickel, and copper, because they have a big efficient surface area, mechanical strength, strong electrical conductivity, improved chemical stability, and striking structural orientation [145]. However, the use of single nanomaterials to produce electrochemical sensors has had some complications, due to the aggregation of quantum dots, the difficulty in obtaining a uniform dispersion, as colloidal metal nanoparticles tend to gather and lamellar graphene to twist [145]. Aiming at improving electron transfer kinetics, conductivity, and the electrocatalytic performance of the electrode catalyst it is possible to add one or more nanomaterials, co-doped in conjunction with composites, and that has proved to be very effective [145]. Another process to create a prepolymerization complex is molecular imprinting (MIP), that joins monomers acting as a template, as for example polystyrene ethylene dioxythiophene, polypyrrole, polypyrenes, 6-mercaptonitic acid (MCN), 4-aminobenzoic acid, cyclodextrin, or diethyl aminoethyl methacrylate, with the analyte, through electro and a bulk polymerization technique [145]. After polymerization, the template separates from the polymer matrix and that frees the binding holes to act as recognition site to target molecules [145].

MIP is very interesting for creating devices for monitoring of biological and chemical contaminants and it's a non-destructive method [145,146].

Optical biosensors are preferred because of their advantages when compared to other analytical processes, they are easy to comprehend and analyse, of low cost, high specificity, sensitivity, capable of monitoring multiple analytes with different optical properties at the same time [145,146]. The general optical sensors consist of a recognition element and a transducer, theoretically, it is applied a beam of light to excite the electrons in specific molecules and then measuring to the corresponding optical property changes detection [146]. The signal obtained by optical techniques is proportional to the concentration of the analyte [145]. The digital signal is produced on the sensors by a recognition-free mode of measurement via interaction with the transducer and the analyte or by using generated optical signals through recognition elements [145,146]. These recognition elements can be antibodies, nucleic acids, enzymes, proteins, a functionalized surface, or a receptor that interacts with analytes through a

hydrophobic interaction, enzymatic reaction, hydrogen bonding or electrostatic interaction (Van der Waals), and whose connections are detected by transducers [145,146]. This reaction induces physical or chemical alterations such as raman scattering, absorbance, resonance light scattering, refractive index colorimetric, and fluorescence [145,146]. This allows transducers to convert those changes into electrical signals to quantify the concentration of the analyte [145,146].

2.6 Adsorption

Adsorption is a technique where solid materials can attract aqueous or gases solutions to their surface [147]. The solids used to adsorb are denominated as adsorbents, the adsorbed solutions are denominated as adsorbate [1,147,148]. The adsorption can occur on the external surface or on the internal surface of adsorbents and it can also happen by a chemical or physical reaction [147]. Chemical adsorption consists of gases adhering to the solid surface of adsorbents through specific chemical forces between the gas and the surface [147]. This process needs an activation energy and usually takes longer to achieve the equilibrium [147,148]. Physical adsorption is like condensation of gases in liquids, which depends on the van der Waals forces of attraction between the adsorbent and the adsorbate [147,148]. This process can emerge if the gas pressure is high, and the temperature is low and has no correlation with chemical specificity [147,148].

The efficiency of adsorption is determined by the adsorbent particle size, surface area and the interactions between adsorbent and target compound [28]. Among different water treatments, the adsorption technique stands out due to the simplicity, minimal operational cost, high efficiency, and, in some cases, the adsorbent can be reutilized, diminishing secondary pollution, besides having wide applicability [1,28,80,]. In addition to remove contaminants from water, adsorption allows their pre-concentration, which makes it easier to detect pollutants even with less sensitive equipments and, thus, facilitates their monitoring [1,80,148]. Although, adsorbents must be able to reverse the interaction with the adsorbate molecules so that the recovery of contaminants can be achieved, allowing the identification of these adsorbed contaminants and their concentrations, as well as giving them a destination (degradation or reuse/recovery) [1,148]. This characteristic also allows the recycling of the adsorbent, which diminishes the environmental impact and promotes sustainability [1,148]. The most desirable forces for an adsorption of water contaminants are the weak non-covalent interactions [1,148].

2.6.1 Porous materials

Growing interest in developing the functional properties and structure of porous materials has contributed to extensive research in this area in recent studies [1]. Porous materials include organic and inorganic material such as silica, activated carbon and zeolites, or hybrid materials from inorganic-organic such as MOFs, or even crystalline COFs [1]. The most relevant aspects from these materials to consider are their shape, pore size, stability, surface area and geometry, which are determinant to the performance of the porous material [1]. Those aspects can be compared on the Table 4 between the most interesting adsorbent materials considered for the adsorption studies. The covalent organic framework was the chosen one as it had the most advantages and was more specific for this investigation.

Adsorbent material	Advantages	Disadvantages
COFs (covalent organic frameworks)	Crystalline materials High surface area Controllable topologies and pore size Stability in water Recyclable material	Synthesis is not sustainable
MOFs (metal-organic frameworks)	Crystalline materials High surface area Tunable pore structure	Not stable in water
Activated carbon	High surface area High availability in the market Low cost	Low selectivity High primary energy demand Synthesis is not sustainable

Table 4 - Adsorbents used in adsorption studies with their characteristics [1,28,131].

2.6.1.1 Covalent organic frameworks

COFs are porous crystalline materials from light elements with two- or three-dimensional geometry (2D, 3D) [1,28,80]. Literature has reported interesting properties of these materials, such as high surface area, ultra-low density, controllable topologies, periodic skeleton, controllable pore size, and environmentally friendliness [28,80,149]. Their functionalities are very interesting for a variety of fields, such as ion conduction, optoelectronics, chemical sensing, catalysis, gas storage, gas separation, isolation of industrial compounds, carriers for drug delivery, and for water monitoring and treatment, as they have shown great adsorbent capacity for mercury, organic dyes, lanthanides, radionuclides, and pharmaceuticals [28,80,149].

COFs are built by self-assembly from a carefully selected well-defined organic building blocks via strong covalent bonds, by applying the principles of reticular chemistry [1,28,80]. This process is done through dynamic covalent chemistry by stitching small units together [80] and with atomic precision, which enables access to materials that have determined pore size and surface and that could promote selectivity for target materials [15,80,149]. The COFs linked with boronic esters and boroxines have poor water stability, because of the high reversibility of the dehydration reactions, therefore it will induce COF decomposition when they are present in an aqueous system [15,149]. Notwithstanding, imine-linked COFs have exhibited substantially higher chemical stability in the presence of water, acids, and alcohols, with retention of COF crystallinity in comparison to the boronic ester COFs [15]. The hydrolytic stability was improved further by preventing water molecules from getting in direct contact with the imine bonds due to bulky substituents next to the linkage [15,150]. Moreover, the addition of a hydroxyl group into the α-position of the reacting aldehyde as an intralayer hydrogen-bonding interaction with the imine nitrogen has proved to increase the water stability of COFs [15,150]. This technique potentiated the development of an impressive chemical stability in water under harsh conditions, such as alkaline or acid solutions [9, 49]. These types of COFs have in their composition triformylphloroglucinol (Tp), which renders the resulting materials with high hydrolytic stability, a critical component for environmental applications [129]. COFs can be modified with diverse functional groups that have affinity for water pollutants, and an example of this is addition of hydrophilic functional groups that will increase the affinity of COFs to polar molecules; however, if a hydrophobic functional group is added it will boost the COF's affinity to non-polar molecules [125, 136]. The COFs pore sizes can be changed to selectively capture molecules with different sizes, the ones with larger pores can capture the larger organic molecules, and the narrow pores can capture smaller ones [106]. Furthermore, these materials stand out for their recyclability and reusability,

which are very attractive characteristics for large-scale water treatments [145]. However, the application of COFs in water treatment has still some challenges to overcome and methods to optimize to guarantee their effectiveness. Figure 6 shows the structure of COFs used in this work to monitor pharmaceutical compounds, which will be described below.

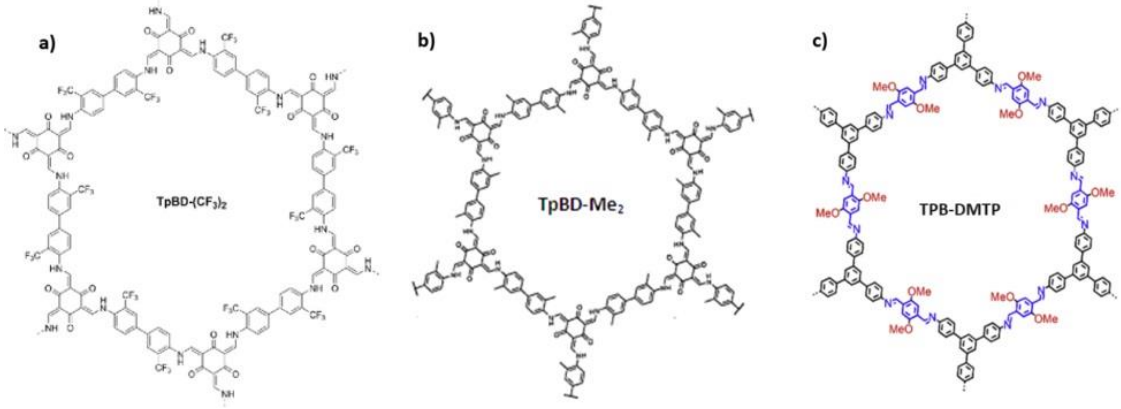

Figure 6 - Structure of COF TpBD-(CF3)$_2$ (a), of COF TpBD-Me$_2$ (b), of COF TpB-DMTP (c), reproduced from Mellah et al, Zhuang et al [151,152]

2.6.1.1.1 COF TpBD-$(CF_3)_2$

COF TpBD-$(CF_3)_2$ has a lipophilic surface designed to target lipophilic pharmaceutical contaminants, and it was prepared from 3,3'-bis(trifluoromethyl)benzidine (BD-$(CF_3)_2$) and Tp [1,28] as it can be seen on the Figure 6. The Tp-series of COFs have been reported as to have a strong feature of high stability in water, which is crucial for environmental applications, even under acidic or basic conditions [28]. The fluorine has low polarizability and high electronegativity, which affects the material properties, enhancing light and air stability. Moreover, CF_3 groups are known to improve lipophilicity of molecules [1].

The N_2 sorption measurements at 77 K provided a type I isotherm with Brunauer–Emmett–Teller (BET) surface area of 1018 m^2/g and a pore volume of 0.44 cm^3/g (Table 5). The pore size distribution is calculated through quenched solid density functional theory (QSDFT), which shows a large micropore contribution with a pore size in between 0.5 to 1.2 nm [151]

Pore size	0.5-1.2 nm
Pore volume	0.44 cm^3/g
BET surface area	1018.0 m≤/g
Surface charge	Slightly negative
Point of zero charge	3.86

Shape	Rod-shaped

Table 5 - COF TpBD-$(CF_3)_2$ experimental characteristics.

2.6.1.1.2 COF TpBD-Me_2

TpBD-Me_2 was firstly reported by Banerjee and co-workers [151]. This COF is a part of Tp-series of COFs that have been showed to have high stability in water, which is very important for studies of water quality [28,151]. Due to the absence of fluorine, the lipophilicity of TpBD-Me_2 is lower as compared with TpBD-$(CF_3)_2$[1]. Their chemical structure can be seen on Figure 6.

The N_2 sorption measurements at 77 K provided a type I isotherm with Brunauer–Emmett–Teller (BET) surface area of 575.9 m^2/g and a pore volume of 0.387 cm^3/g (Table 6). The pore size distribution is calculated through quenched solid density functional theory (QSDFT), which shows a large micropore contribution, with a pore size from 1.1 to 1.5 nm [151].

Pore size	1.1-1.5 nm
Pore volume	0.387 cm^3/g
BET surface area	575.9 m≤/g
Surface charge	Neutral surface
Shape	Rod-shaped

Table 6 - COF TpBD-Me_2 experimental characteristics.

2.6.1.1.3 COF TPB-DMTP

The COF TPB-DMTP consists of TpB (tris(4aminophenyl)benzene) – DMTP (dimethoxyterephthalaldehyde) and it has been reported to act as an effective adsorbent (to remove sulfamerazine from an aqueous solution), having a robust structure, large specific surface area, high porosity, and crystallinity (Figure 6) [152]. These elements make it ideal for adsorption studies because it has proved to have great adsorption capacities with a rapid adsorption rate, it reaches the equilibrium in shorter periods, and has potential to be reused [152]. The adsorption capacity increases when COF TPB-DMTP is present in natural pH 6 to 7, which is good for natural waters applications [152]. However, this COF specific surface is hydrophobic, which is interesting for environmental remediation which hasn't been completely explored and it should be investigated [152].

The N_2 sorption measurements at 77 K provided a type I isotherm with Brunauer–Emmett–Teller (BET) surface area of 2176.5 m^2/g and a pore volume of 1.74 cm^3/g (Table 7). The pore size distribution is calculated through quenched solid density functional theory (QSDFT), which shows a large micropore contribution, with a pore size of 3.3 to 3.6 nm [152].

Pore size	3.3 to 3.6 nm
Pore volume	1.74 cm^3/g
BET surface area	2176.5 m≤/g
Surface charge	Negative surface tension [60]
Shape	Rod-shaped

Table 7 - COF TpB-DMTP experimental characteristics.

2.6.1.1.4 Composite NPS@TPB-DMTP

The composite was based on a recent approach that consists of applying polystyrene (NPS) spheres and support COFs as a porous functional shell for liquid chromatography, and it was developed to guarantee the independent effect of the COFs on the separation [153]. NPS@TPB-DMTP was prepared by applying TPB and 2,5-dimethoxy-1,4-benzenedicarboxaldehyde DMTP as monomers through *in situ* polymerisation on the surface of NPS [153]. NPS are a recent generation of chromatographic stationary phases with surface porous chromatographic fillers core and a surface non-porous layer [153]. This material is considered very efficient, rapid and with a back-flow performance, which is highly attractive and why they have been widely used [153].

The surface is rough and the NPS are covered with COFs, as it can be observed on the Figure 7 from the SEM images. The thickness of the COFs layer connected to the NPS surface is about 98 nm [153].

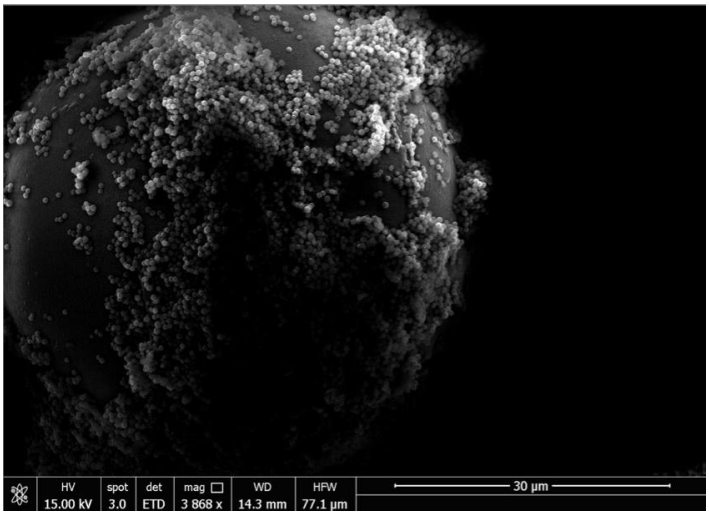

Figure 7 - SEM capture of NPS spheres with COF TpB-DMTP on the surface.

The N_2 sorption measurements at 77 K provided a type I isotherm with Brunauer–Emmett–Teller (BET) surface area of 177.1 m^2/g (Table 8). The pore size distribution is calculated through quenched solid density functional theory (QSDFT), which shows a large micropore contribution, with a pore size of 2.7 nm [153].

Pore size	2.7 nm [153]
Particle size	1.6 μm [153]
Shape	Spheric

Table 8 - NPS@TPB-DMTP experimental characteristics.

CHAPTER 3. MATERIAL AND METHODS

3.1 Reagents and chemicals

T The reagents and chemicals used in the experimental work include Ultrapure water (18.2 mΩ cm^{-1} at 25 °C); Pure standard Tetracycline (Sigma–Aldrich) at 99.9%; Pure standard Diclofenac (Sigma–Aldrich) at 99.9%; Pure standard Sulfapyridine (Sigma–Aldrich) at 99.9%; Metanol >99,8% 1 L; Acetone 99,5% (Honeywell purity); Acetonitrile; HPLC grade 99.9 % (Sigma–Aldrich); Isopropanol 1 L 99,9% (Carlo Erba Reagents); borate buffer at pH 9 (10 mM); Hydrochloric Acid and Potassium Chloride buffer at pH 2 (10 mM); phosphate buffer at pH6 (10 mM); acetate buffer at pH 4 (10 mM).

3.2 Materials

The material used in the experimental work include magnetic stirrers (Fisher bioblock scientific); Magnetic bars; Laboratory glassware; Thermo Scientific ™ NanoDrop ™ 2000/2000c Spectrophotometer; Centrifuge Hettich Mikro 220r Refrigeree – 2205; UV-Vis Thermo Scientific™ NanoDrop™ 2000c; Eppendorf Thermomixer comfort, 2 mL Block; quartz cuvette (Suprasil® 300 quartz, 10 mm optical path); Ultrasound Bath Elmasonic P70h with Heating; Micropipette Research® plus Eppendorf; Micropipette Kit: 4 Pipettors (0.5-10µl; 10-100µl; 100-1000µl); 0.22 µm polysulfone syringe filter; 50 to 100 mL Schott flasks Fisherbrand™; 50 to 100 mL beaker flasks Fisherbrand™; test tube rack, plastic test tube.

3.3 Synthesis of COFs

The COFs used in these studies were synthesized by Joana Araújo at the INL.

The procedure for the synthesis of COF TpBD-$(CF_3)_2$ used in this work was performed as it is described in the literature by *Mellah et al.*, to check the synthesis, and characterization, see the Experimental Section (synthesis of COF TpBD-$(CF_3)_2$) [28].

The procedure for the synthesis of COF TpBD-Me_2 used in this work was performed as it is described in the literature by *Chandra et al.*, to check the synthesis, and characterization, see the Experimental Section (Synthesis of TpPa-1, TpPa-2, TpPa-NO2, TpPa-F4, TpBD, TpBD-$(NO_2)_2$, TpBD-Me_2, and TpBD-$(OMe)_2$) [154].

The procedure for the synthesis of COF TPB-DMTP used in this work was performed as it is described in the literature by *Zhuang et al.*, to check the synthesis, and characterization, see the Material and Methods (2.2. Synthesis of TPB-DMTP-COF) [152].

The procedure for the synthesis of NPS@TPB-DMTP composite used in this work was performed as it is described in the literature by *Xie et al.*, to check the synthesis, and characterization, see the Supporting information (Experimental section) [155].

3.4 Adsorption procedures

3.4.1 Calibration curve

A stock solution of 50 mL of Tetracycline was prepared at 200 mg/L with ultrapure water. For the calibration curve, various samples at decreasing concentrations of Tetracycline were prepared as is represented in Figure 8 (a). To achieve those concentrations, it was done a serial dilution on 2 mL microtubes and with ultrapure water. For diclofenac, a stock solution of 50 mL was prepared, at 1 g/L, in methanol. For the calibration curve, various samples at decreasing concentrations of diclofenac were also prepared. To achieve those concentrations, a serial dilution on 15 mL test tubes was done. This process is exemplified in Figure 8 (b). The measurements of the samples for the calibration curve were done in the Nanodrop 2000c through UV-vis spectrometry to determine the linear range equation for the determination of pharmaceuticals concentrations at the time of the adsorption tests.

For sulfapyrdine, it was used the calibration curves from a previous study done in INL.

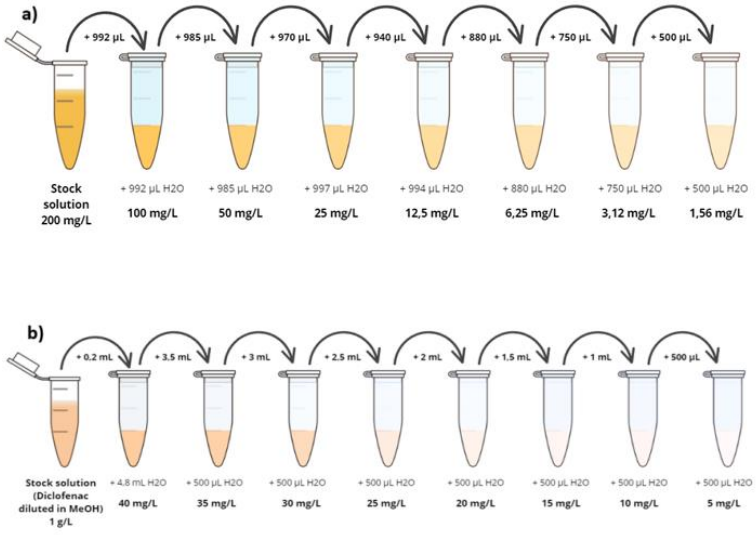

Figure 8 - Calibration curve of tetracycline (a) and diclofenac (b).

3.4.2 Adsorption test of pharmaceutical compounds

It was studied the adsorption behaviour and mechanism of COFs TpBD-$(CF_3)_2$, TpBD-Me_2, and TPB-DMTP with different concentrations of tetracycline and diclofenac, as well as sulfapyridine with a final concentration of 15 mg/L by composite with a final concentration of 1 mg/mL and 2 mg/mL. The COF final concentration used for all the tests was of 1 mg/mL, while for tetracycline and diclofenac, various final concentrations were tested: 100, 75, 50, 40, 35, 30, 25, 20, 15, 12.5, and 10 mg/L. To achieve an initial concentration of 2 mg/mL of a dispersion of COF TpBD-$(CF_3)_2$, TpBd-Me_2, and TPB-DMTP and 4 and 2 mg/mL of the composite, the powder had to be weighted, and added to ultrapure water. Which were subjected to ultrasounds (37 kHz 19°C) for 1 to 5 minutes to boost COF and composite dispersion into the water. Pharmaceutical solutions at different concentrations were prepared from the initial stock solutions (200 mg/L tetracycline, 1 g/L diclofenac and 100 mg/L sulfapyridine**.**

From the prepared COF or composite dispersion, 500 µL was split into 1.5 mL microtubes, and 500 µL of the pharmaceutical compound was added to each test tube, in duplicate. The blank sample consisted of 500 µL of COF or composite dispersion with 500 µL of ultrapure water without the pharmaceutical compound, and the control sample used was 500 µL of pharmaceutical compound with 500 µL of ultrapure water.

The samples were incubated in the Thermomixer at 1400 rpm at 19 °C for 4h. Afterwards, the samples were centrifuged at 15000 rpm for 15 minutes, or until the pellet was fully separated from the supernatant. Then, the supernatant was removed into clean microtubes, and the pellets were stored at the fridge at 4 °C for desorption studies. Supernatants were measured in a Nanodrop 2000c in terms of UV-vis spectrometry, and the maximum adsorption peak was documented.

3.4.2.1 Isotherms

To carry out the study of the adsorption isotherms, adsorption tests were always carried out with a COF ratio of 1 mg/mL and the adsorption was evaluated at different concentrations of the tetracycline or diclofenac, following the methodology previously mentioned in the Adsorption section. The determination of adsorbed analyte quantity was calculated using Equation 1:

$$q_e = \frac{(C_0 - C_e)V}{m}$$

Equation 1 - Adsorption capacity at equilibrium.

Where:

q_e is the adsorbed quantity by adsorbent amount at equilibrium (mg/g)

C_0 is the initial concentration of the species (mg/L)

C_e is the contaminant concentration in solution at equilibrium (mg/L)

V is the solution volume (1 mL)

m is the adsorbent mass (1 mg)

For this work, two models of isotherms were applied as parameters of evaluation, Langmuir and Freundlich, using the software Origin 9.0. The Langmuir adsorption isotherm interprets the equilibrium between the adsorbate and adsorbent system, in which the adsorption is a homogenous method where the adsorbate is limited to adsorption of one molecular layer onto the adsorbent [156,157]. The Langmuir equation (Equation 2) demonstrates how the adsorbed quantity of solute increases with concentration on the liquid phase but reaches a plateau at high concentrations [158]. This can happen due to saturation of two available adsorption sites on the surface of the adsorbent [158].

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

Equation 2 - Langmuir isotherm.

Where:

q_e is the adsorbed quantity by adsorbent amount at equilibrium (mg/g)

q_m is the maximum quantity of species absorbed per unit of adsorbent (mg/g)

C_e is the concentration of the species on the equilibrium (mg/L)

K_L is the empirical constant of Langmuir (L/mg)

The Freundlich adsorption isotherm is a model (Equation 3) that explains a reversible and heterogeneous adsorption used on multilayer adsorption with an equal distribution of selectivity and enthalpy [159]. This indicates that the adsorption locations aren't uniform on the affinity [159].

$$q_e = K_F C_e^{1/n}$$

Equation 3 - Freundlich isotherm.

Where:

q_e is the adsorbed quantity by adsorbent amount at equilibrium (mg/g)

C_e is the concentration of the species on the equilibrium (mg/L)

K_F is the empirical constant of Freundlich ($mg^{1-1/n}L^{1/n}/g$)

3.4.3 Adsorption kinetics

The study of adsorption kinetics was carried out by doing the adsorption experiment by incubating the samples at different times. It was studied the kinetics adsorption of tetracycline with a final concentration of 12.5 mg/L by COF TpBD-$(CF_3)_2$, TpBD-Me_2, and TPB-DMTP with a final concentration of 1 mg/mL, as also the kinetics adsorption of diclofenac with a final concentration of 10 mg/L by COF TpBD-$(CF_3)_2$, and 12.5 mg/L by COF TpBd-Me_2, and TPB-DMTP with a final concentration of 1 mg/mL. Mixtures of prepared solutions were incubated at 19 °C under constant shaking at 1400 rpm on the Thermomixer, with incubation times of 30 seconds, 1, 2, 5, 15, 30 minutes, 1, 2, 4 hours, and overnight. The supernatants were isolated by centrifugation, except for the smaller periods of time, such as 30 seconds, 1, 2, and 5 minutes, which were separated by filtration. Subsequently, the samples were collected in a 2 mL syringe and filtered with 0.22 filters µm pore size. The supernatants were collected to quantify the concentration of tetracycline and diclofenac to determine the equilibrium time (time at which the Ce is attained). For each time, two replicates were used. The protocol proceeded as described on topic 3.4.2. The determination of the amount of analyte adsorbed on the COF, was calculated using Equation 4:

$$q_t = \frac{(C_0 - C_t)V}{m}$$

Equation 4 - Determination of adsorption capacity over time.

Where:

q_t is the amount of the adsorbed molecule in time t by adsorbent unit (mg/g)

C_0 is the initial concentration of the contaminant (mg/L)

C_t is the concentration of contaminant in solution at a given time (mg/L)

V is the solution volume (1 mL)

m is the adsorbent mass (1 mg)

For the assessment of adsorption kinetics studies, it's necessary to analyse the performance of the adsorbent and examine the adsorption mass transfer procedures. On this work, it was applied Lagergren,

Ho and Mckay, and Elovich with software Origin 9.0. The pseudo first order model from Lagergren is a simplified approximation to better describe the kinetics of adsorption on heterogeneous environments [160]. In cases where adsorption develops multiple layers of adsorbate on a solid surface, it is not indicated to use this model because it might not be sufficient to describe this process [161]. The equation for this model consists of the Equation 5.

$$q_t = q_e(1 - e^{-k_1 t})$$

Equation 5 - Lagergren pseudo first order kinetic model.

Where:

q_t is the amount of the adsorbed molecule in time t by adsorbent unit (mg/g)

k_1 is the empirical constant from the pseudo-first order (1/min)

t is the time (min)

The pseudo second order model from Ho and McKay is a kinetic model used to describe the adsorption of molecules into solid surfaces [162] The equation for this model is described on the Equation 6.

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

Equation 6 - Ho and McKay pseudo first order kinetic model.

Where:

q_t is the amount of the adsorbed molecule in time t by adsorbent unit (mg/g)

k_2 is the empirical constant from the pseudo-second order (g/mg.min)

t is the time (min)

The model from Elovich consists of the assumption that adsorption is a process where there's an interaction between the adsorbate and the adsorption sites on the solid surface [163]. The equation for this model is described on the Equation 7. As time moves forward, the abt tends to zero and the adsorption is closer to saturation [163]. The a coefficient is related with the initial adsorption velocity and it represents the availability of adsorption sites on the surface [163]. The b coefficient is associated with

activation energy of adsorption, and it represents the difficulty of adsorbate to connect to the solid surface through adsorption [163].

$$q_t = \frac{\ln(abt)}{b}$$

Equation 7 - Elovich kinetic model.

Where:

q_t is the amount of the adsorbed molecule in time t by adsorbent unit (mg/g)

a is an empirical constant (mg/g.min)

b is an empirical constant (g/mg)

t is the time (min)

3.4.4 Adsorption test with different pHs

Firstly, it was conducted three calibration curves of tetracycline and four calibration curves of diclofenac with each buffer. The calibration curves were prepared with the pharmaceutical stock and diluted with pH 2, pH 4, pH 6 and pH 9 buffer. The calibration curve tests followed the method described on the topic 3.4.1.

It was studied the influence of pH on the adsorption of tetracycline and diclofenac with a final concentration of 12.5 mg/L by COF TpBD-Me_2, and TPB-DMTP with a final concentration of 1 mg/mL. To achieve a concentration of 2 mg/mL of COF dispersion it had to be weighted and added into ultrapure water. The tests were done with a final concentration of pharmaceutical compound of 12.5 mg/L from prepared solutions of 25 mg/L of pharmaceutical compound diluted in each buffer. Afterwards, 500 µL of the COF dispersion was divided into 1,5 mL microtubes and 500 µL of pharmaceutical compound were added to each test tube, having duplicates of all samples. The blank sample consisted of 500 µL of COF dispersion with 500 µL of each buffer and the control sample used was 500 µL of pharmaceutical compound with 500 µL of each buffer. The samples proceed with the protocol as it is described on topic 3.4.2.

3.5 Desorption procedures

3.5.1. Desorption tests the COFs and composite

The desorption studies used the pellets from the adsorption kinetics test of tetracycline by COF TpBd-Me_2 and from the adsorption kinetics test of diclofenac by COF TPB-DMTP. To better understand what the best solvent to effectively desorb tetracycline or diclofenac, it was applied three buffers based on previous studies, which were isopropanol, pH 2, and pH 9 buffer [10,80]. Therefore, it was necessary to do three calibration curves of tetracycline (isopropanol, pH 2, and pH 9 buffer) and two calibration curves of diclofenac (isopropanol, and pH 9 buffer) with each buffer. The calibration curve tests followed the method described on the topic 3.4.1.1 for each buffer.

For the desorption studies of sulfapyridine by the composite with two concentrations study, it was used the pellets from the adsorption tests, and the only solvent used was isopropanol.

Afterwards, 1 mL of each solvent was added to the COF pellets in the microtubes and to the blank pellets was also added 1 mL of each solvent. This test was executed using duplicates of all the samples. Subsequently, each sample was applied to the vortex for 30 seconds and 1 minute on the ultrasounds (37 kHz 19°C), and then the samples proceeded to the Thermomixer for an overnight period with 1400 rpm at 19 °C. On the next day, the samples were centrifuged, and the supernatants were collected to quantify the concentration of the desorbed pharmaceutical compound and determine which buffer promoted the most efficient desorption. The pellets were stored at the fridge at 4 °C for recycling studies. The measurements of the supernatants were done in the Nanodrop 2000c through UV-vis spectrometry to register the maximum desorption peak.

3.5.2 Desorption kinetics

The desorption kinetics studies used the pellets from the adsorption test of tetracycline with a final concentration of 12.5 mg/L by COF TpBd-Me_2 study. After concluding the most effective solvent to desorb tetracycline from the COFs TpBd-Me_2 was isopropanol, it was applied to pellets that achieved the 4-hour period of incubation and had higher adsorption rates. Afterwards, 1 mL of isopropanol was added to the COF pellets in the microtubes and to the blank pellets was also added 1 mL of isopropanol. This test was executed using duplicates of all the samples. Subsequently, each sample was applied to the vortex for 30 seconds and 1 minute on the ultrasounds (37 kHz 19°C), and then the samples proceeded to the Thermomixer for 8 periods of time (5, 10, 15, 30 minutes, 1h, 2h, 4h and overnight) with 1400 rpm at

19 °C. Afterwards, the samples were centrifuged, and the supernatants were collected to quantify the concentration of tetracycline. The measurements of the supernatants were done in the Nanodrop 2000c through UV-vis spectrometry to register the maximum desorption peak.

3.6. Recycling

3.6.1. Recycling test after desorption

The stored pellets on the microtubes from the adsorption kinetics test of tetracycline by COF TpBD-Me_2 were centrifuged to eliminate any residual supernatant from the previous desorption experiments. Then, the supernatants were removed, and the pellets were ready to be tested with a second cycle of adsorption. The tests were done with a final concentration of 12.5 mg/L of tetracycline from a prepared solution of 25 mg/L of tetracycline diluted in ultrapure water. Afterwards, 500 µL of ultrapure water was added to the COF TpBD-Me_2 pellets and 500 µL of tetracycline were added to the microtube, having duplicates of all samples. The blank sample consisted of 500 µL of COF pellets with 1 mL of ultrapure water and the control sample used was 500 µL of tetracycline with 500 µL of ultrapure water. The samples followed the protocol described in the point 3.4.2.
After the desorption of these samples, this process was repeated two more times.

3.7 Compounds quantification by means of UV-Vis spectroscopy

The equipment used to measure the absorbance of the studied pharmaceuticals was a Thermo Scientific™ NanoDrop™ 2000c UV-Vis spectrophotometer equipped with 700 µL quartz cuvettes (Suprasil® 300 quartz, 10 mm optical path). The absorbance of the analytes was measured directly from an aliquot of the corresponding supernatant (adsorption or desorption) against the control (ultrapure water for the adsorption supernatant and the corresponding solvent for the desorption supernatant), performing a scan between 200-600 nm. An external calibration method was used for the quantification, with a calibration curve made for each experiment. The individual concentration of the pharmaceutical compounds was obtained by interpolation of the corresponding calibration curve by determining the calibration curves in each condition for each analyte (Table 9).

Compound	Solvent	Maximum wavelength (nm)
tetracycline	ultrapurewater	275
tetracycline	pH 2 buffer	275
tetracycline	pH 6 buffer	275
tetracycline	pH 9 buffer	275
tetracycline	isopropanol	275
diclofenac	ultrapurewater	277
diclofenac	pH 2 buffer	277
diclofenac	pH 4 buffer	277
diclofenac	pH 6 buffer	277
diclofenac	pH 9 buffer	277
diclofenac	isopropanol	277
sulfapyridine	ultrapure water	260
sulfapyridine	isopropanol	270

Table 9 - Maximum light absorption peaks for each pharmaceutical in each condition for each analyte.

To measure the concentration of the analytes in the samples, it was essential to resort to a measurable relationship between the properties of the analyte and its concentration, which consists of the Beer-Lambert law (Equation 8).

$$A = \varepsilon \mathrm{Cl}$$

Equation 8 - Beer-Lambert law.

Where:

A is the absorbance

ε is the absorption coefficient, specific for each analyte and measurement conditions (L·cm/mg)

C is the concentration of the analyte in the measured sample (mg/L)

l is the optical path (cm)

This equation represents a straight line with the general form of Equation 9, where the slope (m) is equal to εl, y is the detected absorbance and x is the real concentration (mg/L).

$$y = \mathrm{mx} + \mathrm{b}$$

Equation 9 - Slope-intercept form of a straight line.

The Equation 9 is obtained through the calibration curves of the analysed pharmaceuticals, with their theoretical concentrations and the measured absorbance. Therefore, with the Equation 6 is possible to calculate the real concentration of the supernatant, which corresponds to the amount of non-adsorbed pharmaceutical compound by the COF. However, it was necessary to eliminate the influence of the COF, what was done by applying the Equation 10 on the results of the calculated concentration of the supernatant.

$$C_{na} = C_s - C_{bs}$$

Equation 10 - Determination of the concentration of non-adsorbed pharmaceutical compound.

Where:

C_s is the concentration present on the supernatant (mg/L)

C_{bs} is the concentration of the blank sample (mg/L)

To determine the adsorbed concentration of the pharmaceutical compounds by the COF, it was applied the Equation 11 on the results of non-adsorbed pharmaceutical compounds by the COF without the COF influence.

$$C_{ads} = C_{na} - C_{cs}$$

Equation 11 - Determination of the concentration of adsorbed pharmaceutical compound.

Where:

C_{ads} is the concentration of the adsorbed pharmaceutical compound (mg/L)

C_{na} is the concentration of the non-adsorbed pharmaceutical compound (mg/L)

C_{cs} is the concentration of the control sample (mg/L)

With this data, it was possible to calculate the adsorption efficiency of the COF as well as the desorption efficiency of the solvent, with Equation 12 and 13, respectively.

$$Adsorption\ (\%) = \frac{C_{ads}}{C_{cs}} \times 100$$

Equation 12 - Determination of the efficiency of adsorption of pharmaceutical compound.

Where:

C_{ads} is the concentration of the adsorbed pharmaceutical compound (mg/L)

C_{cs} is the concentration of the control sample (mg/L)

$$Desorption\ (\%) = \frac{C_{ads}}{C_{dsp}} \times 100$$

Equation 13 - Determination of the efficiency of desorption of the pharmaceutical compound.

Where:

C_{ads} is the concentration of the adsorbed pharmaceutical compound (mg/L)

C_{dsp} is the desorbed concentration of the pharmaceutical compound on the supernatant (mg/L)

All the results were applied with a standard deviation error (SD) to better evaluate the variability of the samples. The calculation of a SD of a sample is an estimate of the variability of the population from where the sample was drawn. The determination of the SD was done with the Equation 14.

$$\sigma = \sqrt{\frac{\sum_i^n = 1\ (x_i - \bar{x})^2}{n - 1}}$$

Equation 14 - Determination of the standard deviation error.

Where:

σ is the standard deviation error

x_i is the value of the ith point in the data set

$\bar{x}$ is the mean value of the data set

n is the number of data points in the data set

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Tetracycline

4.1.1 Adsorption test of tetracycline by three types of COFs

It was conducted a study to compare the adsorption capacity of tetracycline between different types of COFs: TpBD-Me_2, TpBD-$(CF_3)_2$ and TPB-DMTP. As it can be observed on the Figure 9, among the tested COFs, the COF TPB-DMTP had the lowest value of adsorption capacity 5.22 mg/g. The adsorption capacity by the other two COFs was almost the double. These results could be associated with the fact that tetracycline is a hydrophilic compound, and this COF is hydrophobic [28,152]. The polar molecules have a strong attraction for other polar molecules, or free ions, which leads to them to form a sturdy link between the two molecules [164]. However, when they are present in an environment with non-polar molecules, they only present a slight attraction, preferring and pursuing polar molecules or ions instead [164]. Moreover, COF TPB-DMTP has the biggest surface are as well as the bigger pores, therefore, the affinity with tetracycline is probably very low.

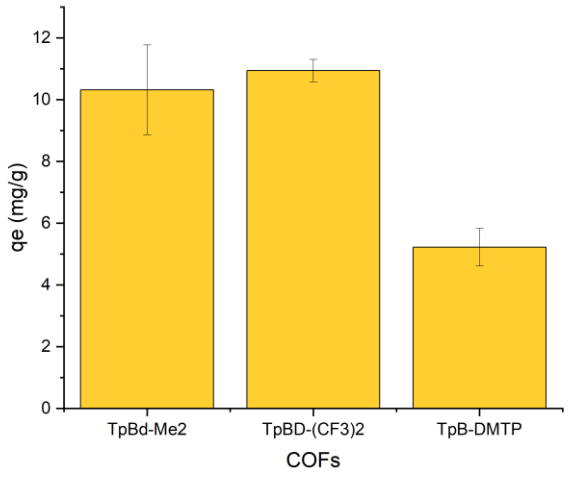

Figure 9 - Adsorption capacity of 12.5 mg/L of tetracycline with 3 types of COFs. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

Notwithstanding, the best results occurred with the COFs TpBD-Me_2 and TpBD-$(CF_3)_2$ with similar values of capacity of adsorption (10.32 mg/g and 10.94 mg/g, respectively), which showed great potential for studies of adsorption. This may be explained by tetracycline being present on ultrapure water, and it is at

its neutral state, therefore there are less electrostatic repulsions between the pharmaceutical compound and the COFs. Furthermore, there's not only one mechanism of adsorption, the structure of tetracycline might allow for π-π interactions with the COF, that also has those electrons on its structure. However, this couldn't be checked on this study due to the lack of bigger samples to do a more detailed characterization of the relation between the two elements of adsorption.

Similar results have been described in literature, for example, diclofenac, a lipophilic molecule had an adsorption capacity of 18 mg/g with COF TpBD-$(CF_3)_2$ which corresponded to 77% of the added amount, therefore it indicated that the COF pore surfaces has potential to be designed to target specific contaminants, and to monitor their presence in water [28]. COF TpBD-Me_2 showed a slightly decrease of the capture efficiency, that may be explained to the higher lipophilicity of the fluorine-bearing in TpBD-$(CF_3)_2$, and by the lower surface area of TpBD-Me_2 compared to TpBD-$(CF_3)_2$ [28,80].

Based on these preliminary results, the study of tetracycline adsorption by COFs TpBd-Me_2 and TpBD-$(CF_3)_2$ was continued to better understand which one would be the more specific and effective for the monitoring of tetracycline.

4.1.2 Adsorption isotherms of tetracycline by different COFs

4.1.2.1 TpBD-$(CF_3)_2$

Four different concentrations (100 mg/L, 50 mg/L, 25 mg/L, and 12.5 mg/L) of tetracycline were incubated with a COF TpBD-$(CF_3)_2$ dispersion at 1mg/mL. It is observed in Figure 10 that the adsorption efficiency is higher when tetracycline concentrations are lower while maintaining the same concentration of COF. The saturation of the COF might explain these results.

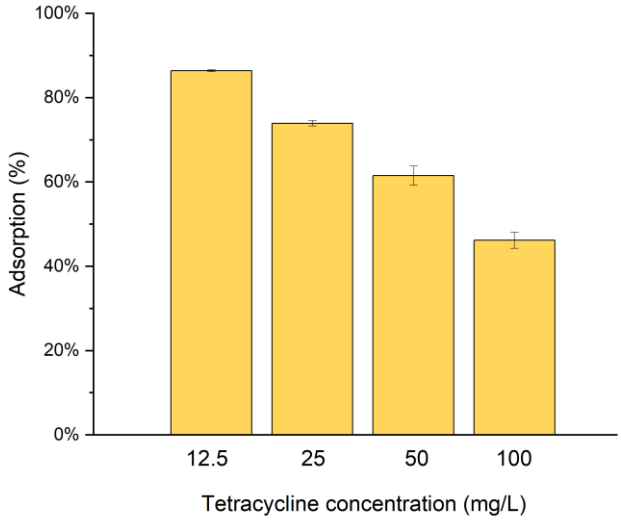

Figure 10 - Adsorption efficiency of tetracycline with final concentration of 100 mg/L, 50 mg/L, 25 mg/L and 12.5 mg/L with TpBD-$(CF_3)_2$. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

A similar study, which investigated the adsorption of trimethoprim antibiotic by activated carbon in distilled and natural water, showed that increasing the concentration of the antibiotic, without changing the concentration of the adsorbent, causes an increase of pharmaceutical particles in the solution [166]. This is due to competition for the attachment of those particles to the fixed available sites available for adsorption, consequently the adsorption is not as efficient for very high concentrations, where the availability of adsorbate is greater compared to the adsorption sites of the adsorbent [166].

As can be observed in figure 11 the adsorption capacity of TpBD-$(CF_3)_2$ reaches a plateau at the highest concentration tested. Langmuir model fits perfectly (R^2= 0.998) the isotherm (figure 11), indicating that the adsorption occurs through relatively weak forces such as van der Waals. This test achieved a high concentration of q_m 328 mg/g, which indicates that there's a strong affinity between TpBD-$(CF_3)_2$ and tetracycline. Notwithstanding, with the Freundlich model, a coefficient of correlation (R^2) of 0,967 was obtained, which is a very high value, and it means that both mechanisms fit the data obtained experimentally.

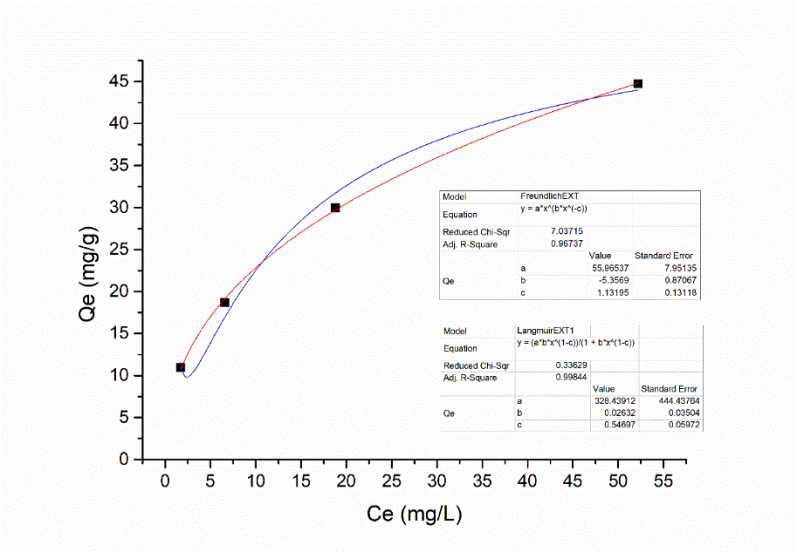

Figure 11 - Isotherm of tetracycline adsorption by TpBD-$(CF_3)_2$ fitting Freundlich and Langmuir models.

4.1.2.2 TpBD-Me_2

After analysing that COF TpBD-$(CF_3)_2$ and COF TpBD-Me_2 had similar adsorption results for tetracycline, it was decided to pursue the investigation with the second COF. This decision was based on the COF TpBd-Me_2 being a functional nanoporous material less harmful for the environment as it does not contain fluorinated groups, which is a big concern in this study when considering the applications, it could have in the future of water treatment.

The isotherm does not fit very well any of the most common tested models, but the highest R^2 (0.715) was obtained with Freundlich as it can be observed in the Figure 12. Based on the Figure 12, it seems to be indicating that the adsorption occurs on a heterogeneous surface and that the adsorption is possibly caused by surface precipitation of the solute on the surface of the adsorbent.

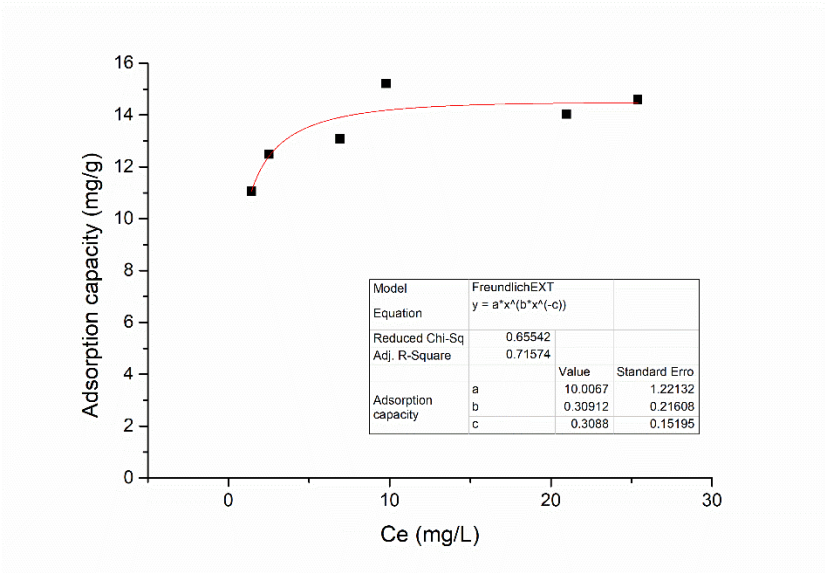

Figure 12 - Isotherm of tetracycline with final concentration of 12.5; 15; 20; 25; 30; 35 and 40 mg/L by COF TpBD-Me₂ adjusted to Freundlich.

4.1.3 Kinetics adsorption of tetracycline by various COFs

4.1.3.1 TpBD-$(CF_3)_2$

The kinetics study is crucial when considering the treatment is for aqueous effluents through adsorption, because with that method it is possible to better understand what possible processes develop or what are the reaction pathways that emerge during the adsorption technique [167].

It was investigated the time needed to reach the equilibrium in adsorption. The study of kinetics was implemented with the concentration of tetracycline that achieved the best adsorption efficiency results in the previous experiment; 12.5 mg/L. Figure 13 shows the increase in adsorption over the incubation time. For the first 4 h (240 minutes), there is an increase in adsorption as the incubation time increases, after which it stabilises.

By analysing the coefficient of correlation (R^2) from the Figure 13, the most accurate kinetics model to describe the adsorption between the COF TpBD-$(CF_3)_2$ and tetracycline is the pseudo second order (0,995). The other models had values of R^2 closer to 0,8, which are interesting, but not as promising as the the pseudo second order.

On an investigation of adsorption of trimethoprim antibiotic by activated carbon in distilled and natural water, it was observed that increasing the contact time between the antibiotic and the activated carbon potentiated the adsorption, until the equilibrium was achieved [166]. This phenomenon happens because with more contact time, there is a greater chance of filling all the sites for adsorption, until saturation is reached (at equilibrium).

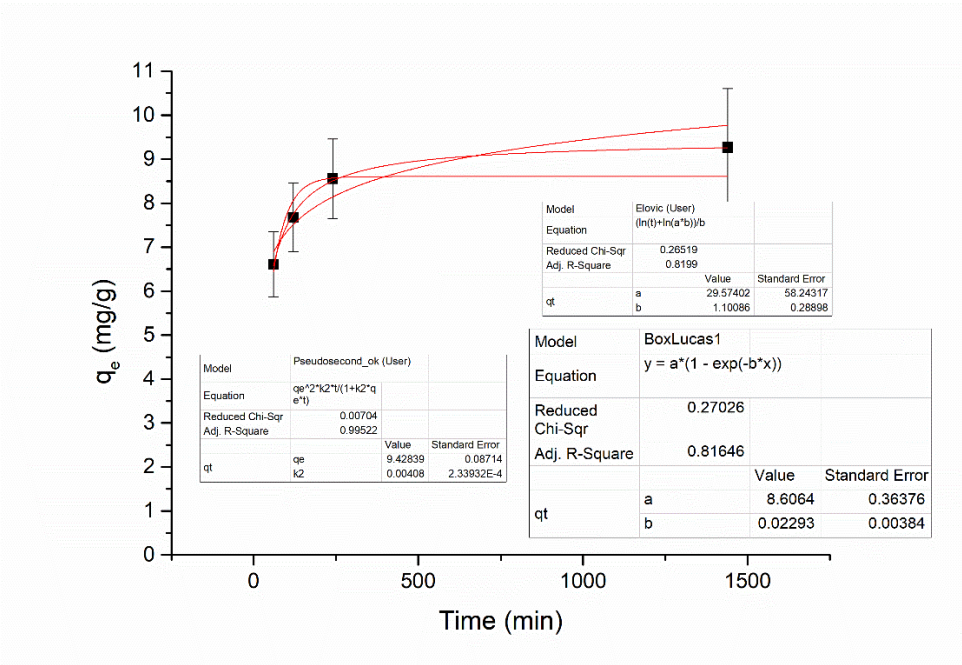

Figure 13 - Kinetics of adsorption capacity of tetracycline with final concentration of 12.5 mg/L by COF TpBD-$(CF_3)_2$. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments. The samples were in the Thermomixer for 5 different time periods, for 30 minutes, 1h, 2h, 4h and 24h (overnight).

4.1.3.2 TpBD-Me_2

As it can be verified on Figure 14, the data shows a rapid adsorption by the COF TpBD-Me_2, which keeps increasing over time until it reaches a clearly defined equilibrium point at 24 hours. When analysing the coefficient of correlation (R^2), the best kinetics model to describe the adsorption between the COF TpBD-Me_2 and tetracycline is the Elovich (R^2 = 0.732), which is not a high value, so it's not the best model to describe this adsorption, which could be because it needs more results to be better fitted, or another type of model would be better to describe the adsorption.. The other models had R^2 values closer to 0.

Similar result was obtained in the kinetics adsorption study of Ibuprofen by COF TpBD-$(CH_3)_2$ at 21 °C, in ultrapure water with a pH between 6 to 7 [1]. On that study only 2 h of adsorption were analysed, but

it was shown that the capacity of adsorption will be bigger if the time of contact between the pharmaceutical and the COF is longer [1].

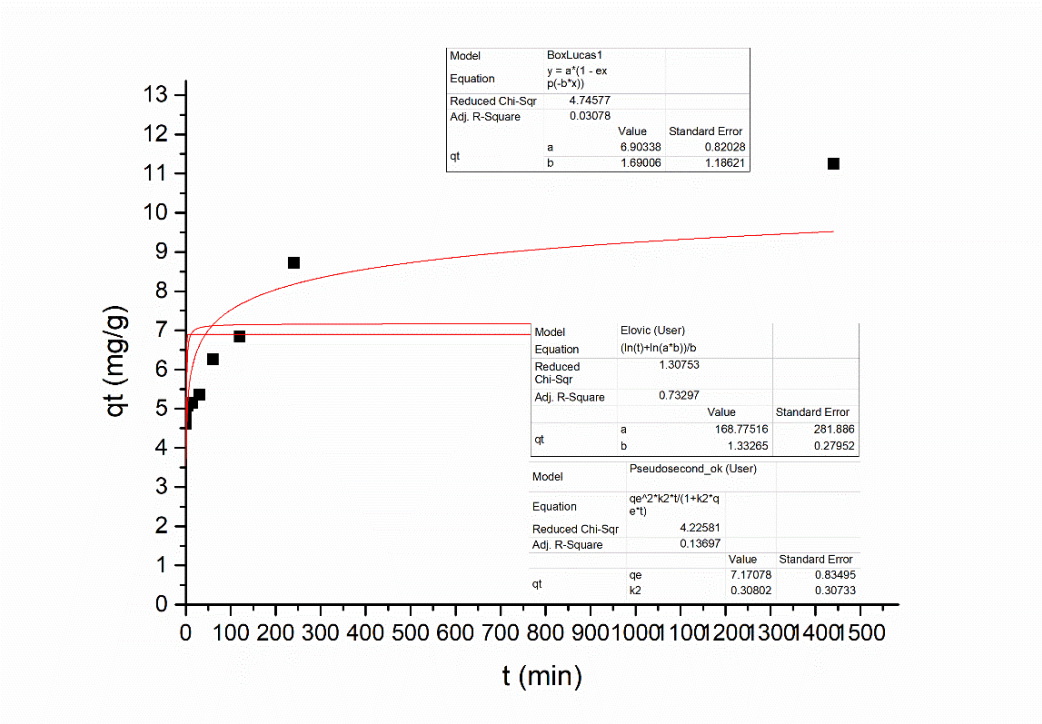

Figure 14 - Kinetics of adsorption capacity of tetracycline with final concentration of 12.5 mg/L with COF TpBD-Me$_2$. The samples were in the Thermomixer for 9 points of time, for 30 seconds, 1, 5, 15, 30 minutes, 1h, 2h, 4h and 24h (overnight).

4.1.3.3 TPB-DMTP

A kinetics study was conducted to better understand the time needed to reach the adsorption equilibrium of Tetracycline by COF TPB-DMTP. The results, presented in in Figure 15, show that, in this test, adsorption stabilizes at 30 minutes, which proved to be a very fast adsorption and it is in concordance with past investigations [152].

It is described in the literature that within the first 10 minutes of adsorption of sulfamerazine from an aqueous solution, the COF TPB-DMTP was capable of almost achieving 71 % of the adsorption capacity [152]. A significant factor that potentiates COFs rapid adsorption is their very high pore surface area and a larger pore size than TpBD-Me$_2$ or –(CF$_3$)$_2$ [152].

From the correlation coefficient, none of the applied kinetics models could describe the adsorption between the COF TPB-DMTP and tetracycline. All those models have negative values.

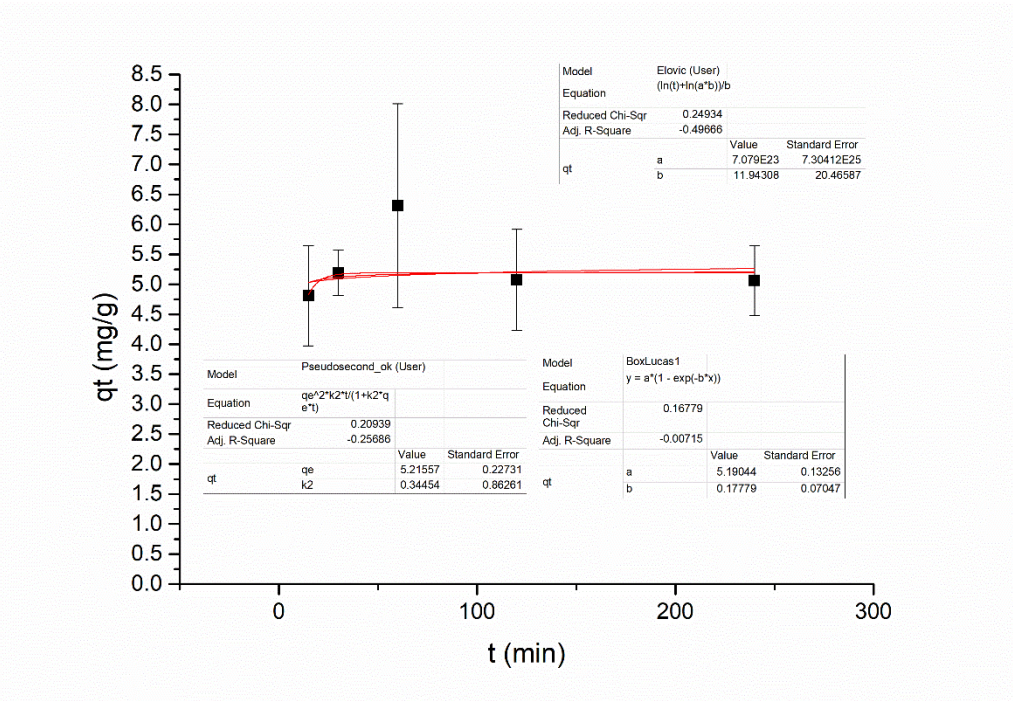

Figure 15 - Kinetics of adsorption capacity of tetracycline with final concentration of 12.5 mg/L with COF TPB-DMTP. The samples were in the Thermomixer for 5 points of time, for 15, 30 minutes, 1h, 2h, and 4h. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.1.7 Influence of pH on tetracycline adsorption by COF TpBD-Me$_2$

As it can be seen in Figure 16, tetracycline was more efficiently adsorbed when prepared in the pH 6 buffer (> 90%). On the other hand, when present with pH 2 and pH 9 buffers, only 43% and 34% adsorption efficiency was achieved, respectively. The lowest values obtained compared to the previous studies, can be explained by the ionizable functional groups from tetracycline that suffer protonation or deprotonation reactions in different pH environments [168]. When tetracycline is at pH < 3.3, it produces cation species such as H_2TC^+; when the pH is between 3.3 and 7.7, the dominant species are zwitterionic, which is the neutral state of the compound with H_2TC^0; however, when the pH is > 7.7 it forms anion species of tetracycline, for example HTC^-/TC^{2-} (Figure 17) [168,169].

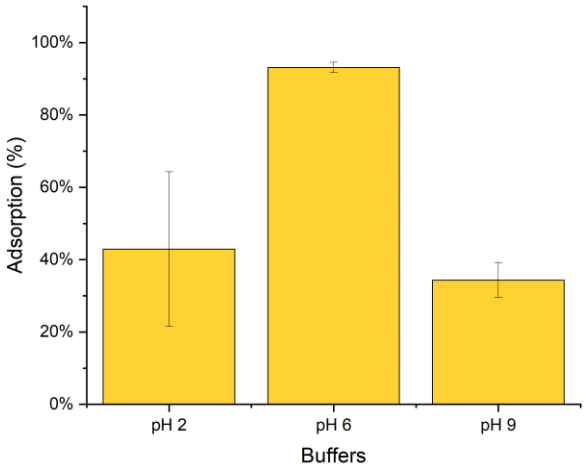

Figure 16 - Adsorption efficiency of tetracycline with final concentration of 12.5 mg/L with COF TpBD-Me₂. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

The most efficient adsorption occurred at pH 6, which indicated that COF TpBD-Me$_2$ has a stronger affinity to tetracycline when it is at its neutral state [168,169]. Another article on the adsorption of tetracycline with biochar showed a higher adsorption capacity when the solution had a pH in the range of 3.5 to 8 [169]. It also explains why, in buffers at pH 2 and 9 lower adsorption results occurred, i.e., the COF TpBD-Me$_2$ is not attracted to the anion and cation states of tetracycline. However, when in ultrapure water, tetracycline is dominantly in the zwitterionic form, which is probably why adsorption results were good in water and are expected to be good if applied in natural waters (pH 5.5-8.5). This result is very interesting, as it validates the application of these COFs to a real application for monitoring this pharmaceutical.

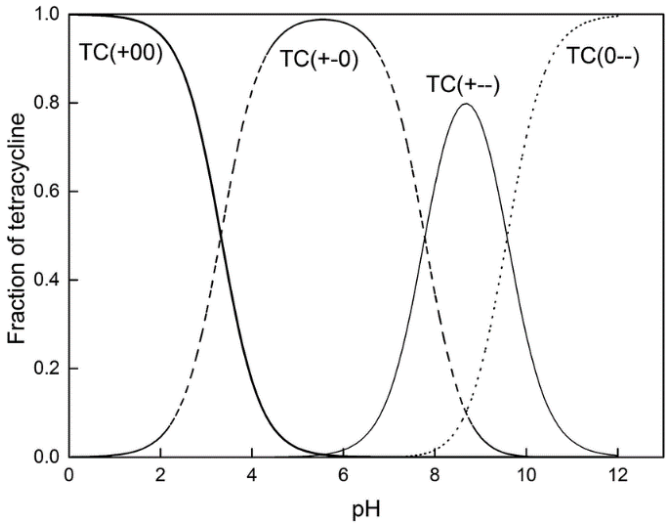

Figure 17 - Tetracycline species distribution on the pH scale. Reproduced from Wang et al, [169].

4.1.8 Desorption test of tetracycline by COF TpBD-Me$_2$

To investigate how to efficiently desorb tetracycline from TpBD-Me$_2$ and better understand their interaction, the desorption tests were conducted with isopropanol, pH 2 and pH 9 buffers. These buffers were applied based on the knowledge from previous studies [10,80]. Figure 18 shows that isopropanol presented the highest desorption efficiency (circa 95 %). Tetracycline is a hydrophilic molecule, and is easily dissolved in isopropanol, an organic polar solvent [170]. Neither pH 2, nor pH 9 buffers, were able to efficiently desorb this pharmaceutical compound (52 % and 55 %, respectively). This data indicated that promoting anionic or cationic forms of the molecule is not an efficient way to desorb it, despite of the fact that adsorption was very low at those pHs, which would point for a poor interaction of the COF with its charged forms.

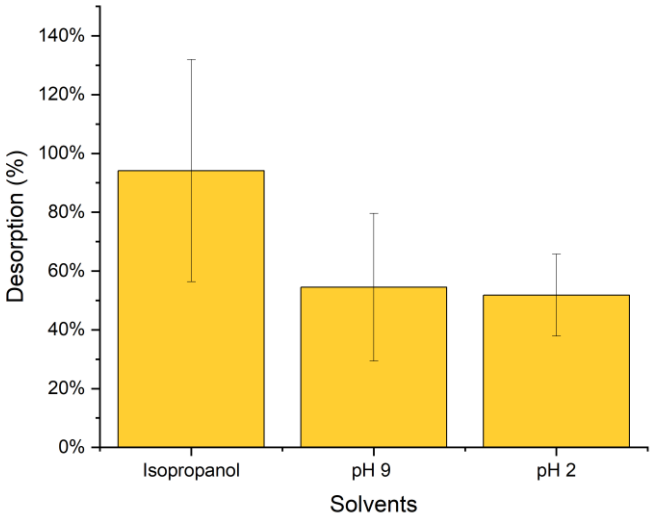

Figure 18 - Desorption efficiency of tetracycline with final concentration of 12.5 mg/L with COF TpBD-Me$_2$. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.1.9 Kinetics desorption test of tetracycline by COF TpBD-Me$_2$ with isopropanol

Once isopropanol was confirmed as a good solvent for tetracycline desorption, kinetics studies were performed to determine the time needed to obtain the maximum desorption. The data in Figure 19 shows that a high desorption efficiency is already obtained after only one minute of incubation with isopropanol

(67%). Maximum desorption was obtained after 24 hours of incubation, however, after 2 hours the desorption was almost the same, so applying this time saves a lot of time in the real application.

A previous work tested the desorption kinetics of okadaic acid from the COF TpBD-Me_2 and a similar result was obtained, after 1 minute it had a desorption of 60%. However, desorption was tested only for 250 minutes, and the equilibrium was achieved after 60 minutes with a desorption efficiency of 80% [1].

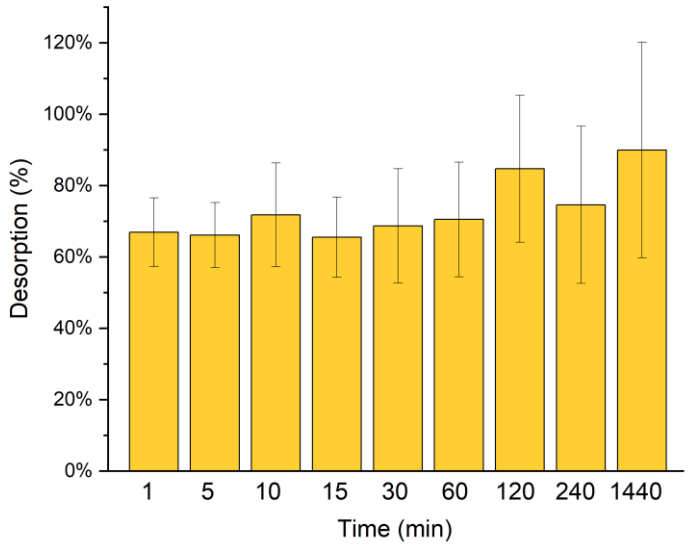

Figure 19 - Kinetics adsorption efficiency of tetracycline with a final concentration of 12, 5 mg/L by COF TpBD-Me2. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.1.10 Desorption test of tetracycline by COF TpBD-Me_2 with isopropanol

The potential of the TpBD-Me_2 COF to be used as adsorbent, for pharmaceuticals monitoring, relies on the possibility of recycling and reusing the material. In this regard, consecutive adsorption/desorption tests were conducted with isopropanol. As it can be seen on Figure 20, great desorption values were obtained in the first cycle, with 80% desorption efficiency. In the following desorption cycles, the results kept increasing, the last two cycles even surpassing the 100% mark. This outcome happened probably because isopropanol removed residual tetracycline from the preceding desorption cycle. For instance, in the first cycle it was only able to desorb 80% of the pharmaceutical compound, which means that 20% of tetracycline remained adsorbed on the COF. Hence, in the second cycle of desorption, isopropanol might desorb a higher percentage of antibiotic than the quantity it was used to adsorb in that cycle, and so on.

On a previous article, it was evaluated the adsorption of sulfamerazine from an aqueous solution by covalent organic frameworks and recyclability was studied [152]. The organic solvents used for the three desorption cycles of sulfamerazine by COF consisted of THF, ethanol, and methanol and were very successful on the regeneration of the COFs [152]. The data obtained from this experiment revealed there was an increase of concentration of the pharmaceutical compound with each cycle, which indicates that desorption was not completely efficient and some residual sulfamerazine was still present on the subsequent cycle, which is in concordance with the results from the present study (Figure 20) [152].

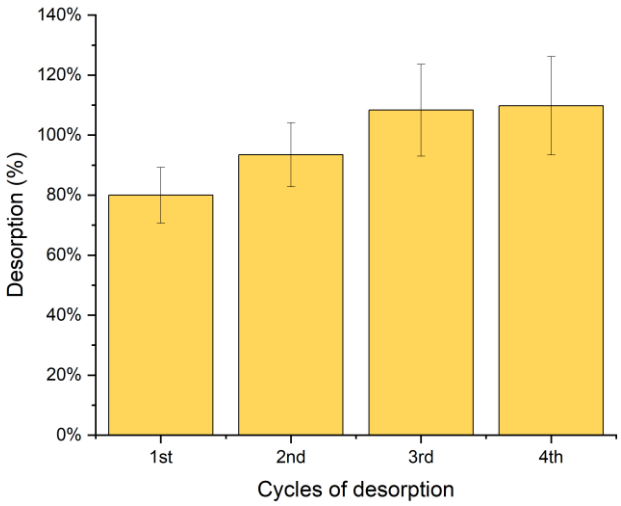

Figure 20 - Desorption efficiency of tetracycline with final concentration of 12.5 mg/L with COF TpBD-Me₂. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.1.11 Recyclability test of COF TpBD-Me₂ for tetracycline adsorption

The COFs were consecutively used for four cycles of adsorption/desorption to understand their potential to be reutilized for adsorbing 12.5 mg/L of tetracycline by COF TpBD-Me₂. The results can be seen in Figure 21, which indicate a decrease in the adsorption values, however small. A lower adsorption efficiency is expected with the increase in the number of cycles, as, according to the desorption studies, tetracycline is not entirely removed from the COF. From the first to the fourth cycle, there is only a 20% decrease in the adsorption efficiency at a high concentration (12.5 mg/L) which will always be much higher than the concentration expected in natural waters [11,24]. However, these results demonstrate that COF TpBD-Me₂ can be recycled and reutilized.

The same type of results was found in previous investigations where the recycling of three COFs TpBD-$(CF_3)_2$, TpBD-$(NO_2)_2$, and TpBD-$(NH_2)_2$ in three consecutive cycles of adsorption/desorption of the biotoxin microcystin was shown, using ultrapure water at 19 °C, and a pH between 6 to 7 for adsorption, and a methanol/ammonium hydroxide mixture for desorption [10]. However, the chemical relationship between the solvent to desorb, the adsorbate, and the adsorbent is critical because it determines the desorption efficiency and the possible efficiency of being reutilized for new adsorption. Only limited combinations of solvents and pHs were tested in this study due to time restrictions. Further investigations should be performed to improve the desorption efficiencies.

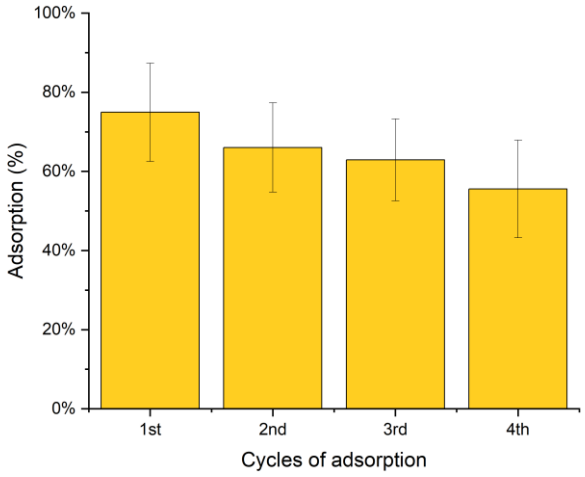

Figure 21- Adsorption recycling test of COF TpBD-Me_2 for tetracycline with a final concentration of 12.5 mg/L. Graphics with error bars correspond to the standard deviation of the mean from three independent experiments.

4.2 Diclofenac

4.2.1 Adsorption test of diclofenac with different COFs

Two types of COFs with diverse functionalities were tested for their capacity of adsorption of diclofenac: TpBD-$(CF_3)_2$ and TPB-DMTP COF. The results obtained on the Figure 22 revealed that COF TpBD-$(CF_3)_2$ reached a value of 8,47 mg/g of capacity of adsorption, and the COF TPB-DMTP showed to have a slightly higher capacity of adsorption, with a value of 8,87 mg/g. Both COFs showed potential to efficiently adsorb diclofenac, however, due to their constitution, COF TpBD-$(CF_3)_2$ has already been reported to adsorb diclofenac highly efficiently, but not compared with TPB-DMTP [1].

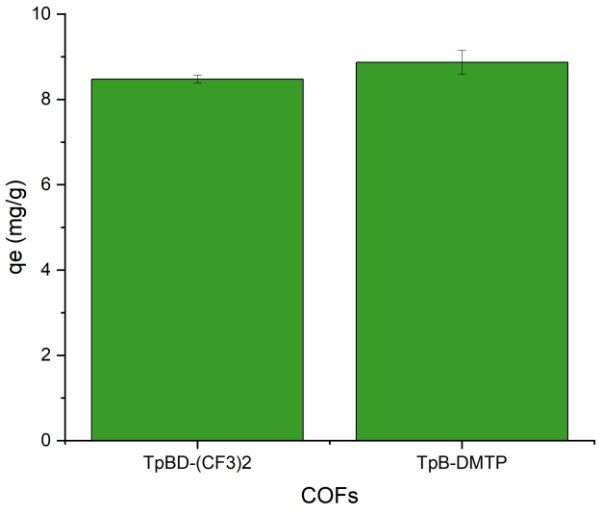

Figure 22 - Adsorption capacity of 10 mg/L of diclofenac by two types of COFs. Graphic with error bars correspond to the standard deviation of the mean from between samples of the same experiment.

4.2.2 Kinetics of adsorption tests of diclofenac by different COFs

4.2.2.1 TpBD-$(CF_3)_2$

Figure 23 shows the data from the kinetics study indicating an initial increase of adsorption with time. The adsorption of diclofenac reaches the equilibrium at 24 hours of incubation. The effect of time was studied on a similar work where authors concluded that the adsorbed amount of diclofenac by activated carbon and Eucarbon increases with the increase of contact time [171]. The period of this test was of 300 minutes (5 hours) and the equilibrium was achieved at 120 and 150 minutes for activated carbon and Eucarbon, respectively [171]. The adsorption of diclofenac proved to be faster for activated carbon in comparison with Eucarbon, which is explained by the attractive forces that were stronger for activated carbon than Eucarbon [171].

As can be observed in Figure 23, the Elovich kinetic model is the one that fits better the adsorption of diclofenac by COF TpBD-$(CF_3)_2$ with an R^2 value of 0.806. This model was developed for gases but has been applied to molecules in liquid and usually describes adsorption into heterogeneous surfaces.

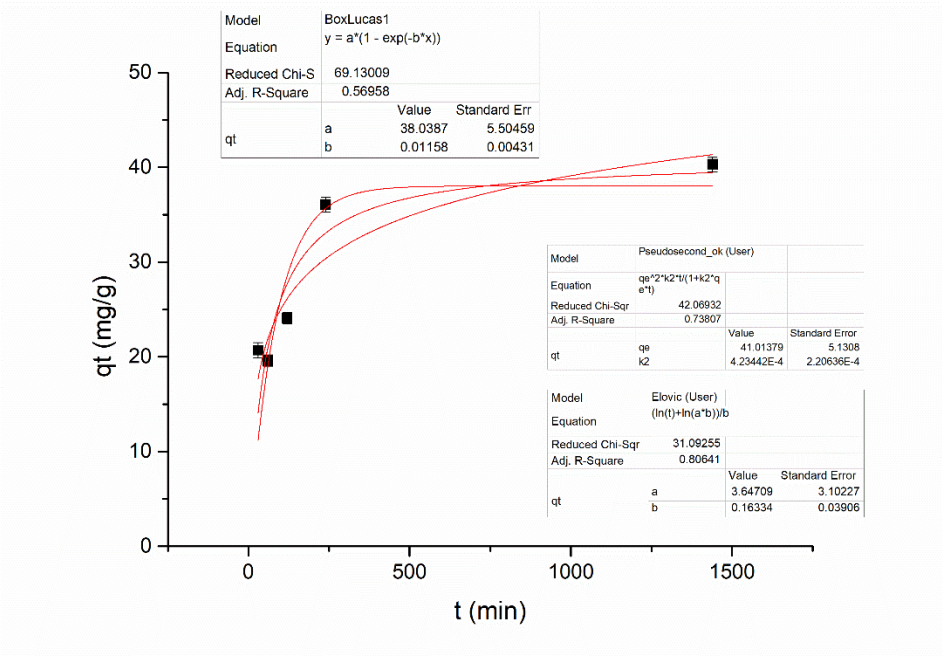

Figure 23 - Kinetics adsorption test of diclofenac with a final concentration of 10 mg/L with COF TpBD-$(CF_3)_2$. The samples were in the Thermomixer for 5 points of time, for 30 minutes, 1h, 2h, 4h and 24h (overnight).

4.2.2.2 TpBD-Me_2

The adsorption equilibrium of diclofenac was obtained at 15 minutes of incubation with TpBD-Me_2 (Figure 24), which means that the adsorption is much faster than with TpBD-$(CF_3)_2$. It was not possible to fit the data into any of the most common models for kinetics due to all R^2 values being negative. The reason for these rates is probably the high variability of the data in the first time points due to the extremely fast adsorption.

The study of adsorption of diclofenac sodium by sycamore ball activated carbon from an aqueous solution is in concordance with rapid adsorption of diclofenac [172]. The kinetics experiment used different initial concentrations of 10, 20, 30, 40, and 50 mg/L of diclofenac, and the results proved that within the first 10 minutes, the adsorption efficiency percentage were already at 86.87, 85.00, 67.51, 58.28, and 48.26 %, respectively [172]. These values show how diclofenac molecule being lipophilic induces a faster adsorption.

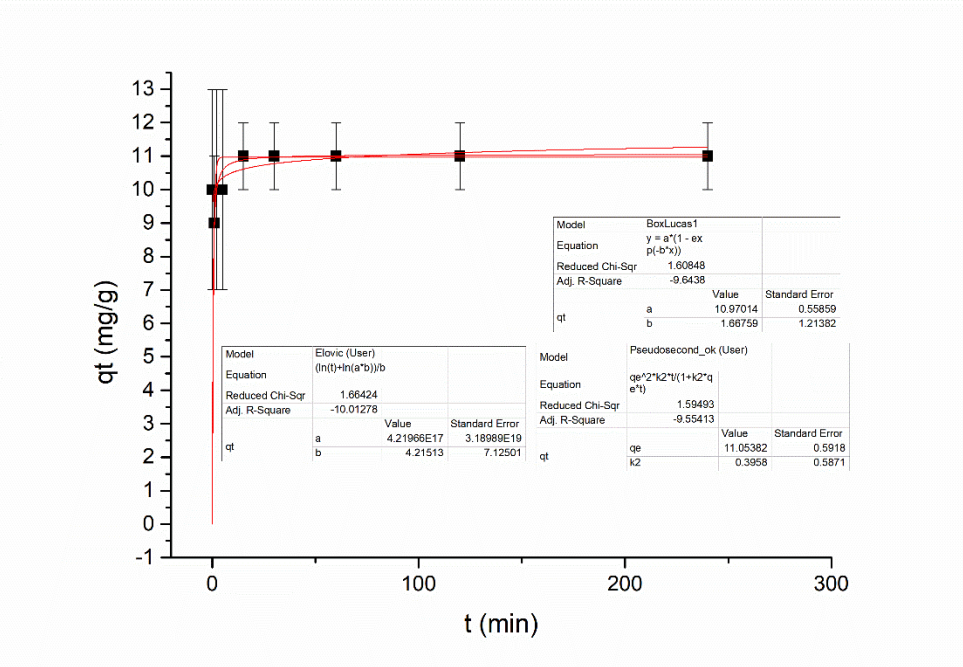

Figure 24 - Kinetics adsorption capacity of diclofenac with a final concentration of 12.5 mg/L with COF TpBD-Me$_2$. Graphic with error bars correspond to the standard deviation of the mean from between samples of the same experiment. The samples were in the Thermomixer for 9 points of time, for 30 seconds, 1 minute, 2 minutes, 5 minutes, 15 minutes,30 minutes, 1h, 2h and 4h.

4.2.2.3 TPB-DMTP

The adsorption of diclofenac with TPB-DMTP occurs at least as fast as with TpBD-Me$_2$, as can be observed in the Figure 25. The equilibrium was achieved at 15 minutes, proving it is not necessary for COF to remain in contact with diclofenac for four hours to achieve equilibrium. The rapid adsorption with COF TPB-DMTP at the beginning of contact with the pharmaceutical compound happens due to an extensive surface with vacant adsorption sites and the great concentration gradient [152].

By analysing the Figure 25, the kinetics model that better fits the adsorption of diclofenac by COF TPB-DMTP is the pseudo-second-order model (R^2=0.885), which was described to be the model that better fit the sorption of divalent metals, although it also describes well the adsorption of organic molecules.

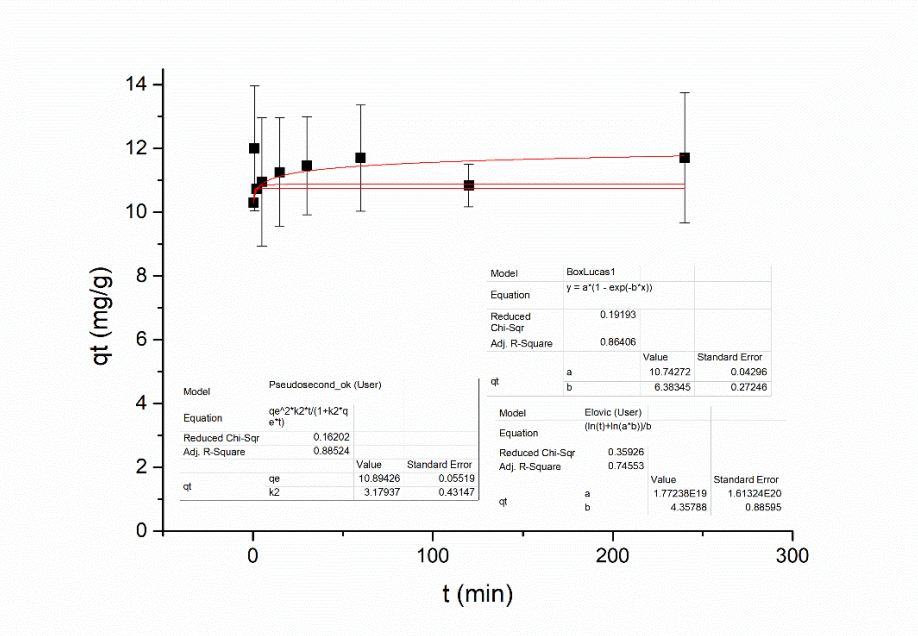

Figure 25 - Kinetics adsorption capacity of diclofenac with a final concentration of 12.5 mg/L with COF TPB-DMTP. Graphic with error bars correspond to the standard deviation of the mean from between samples of the same experiment. The samples were in the Thermomixer for 9 points of time, for 30 seconds, 1 minute, 2 minutes, 5 minutes, 15 minutes,30 minutes, 1h, 2h and 4h.

4.2.3 Isotherms of diclofenac adsorption by different COFs

4.2.3.1 TpBd-Me_2

Different concentrations of diclofenac (100 mg/L; 75 mg/L; 50 mg/L and 25 mg/L) were incubated with a TpBD-Me_2 dispersion at 1mg/mL to better understand the mechanism of adsorption. It is clearly observed in Figure 26 that there is an increase in the adsorption capacity as diclofenac concentration values increase while maintaining the same concentration of COF. Although, it was not possible to obtain any good fitting model with the most common isotherm models (Figure 26) and more experiments with intermediate and lower concentrations, should be performed because it seems to fit a subclass 3 isotherm. The short plateau (Figure 26) indicates that at lower concentrations there is affinity between diclofenac and COF TPB-DMTP, which is followed by a stage of high adsorption when the concentration is higher. This fact could mean that the diclofenac adsorbed in the COF has intermolecular interactions with the diclofenac in solution, leading to the formation of multilayers [173].

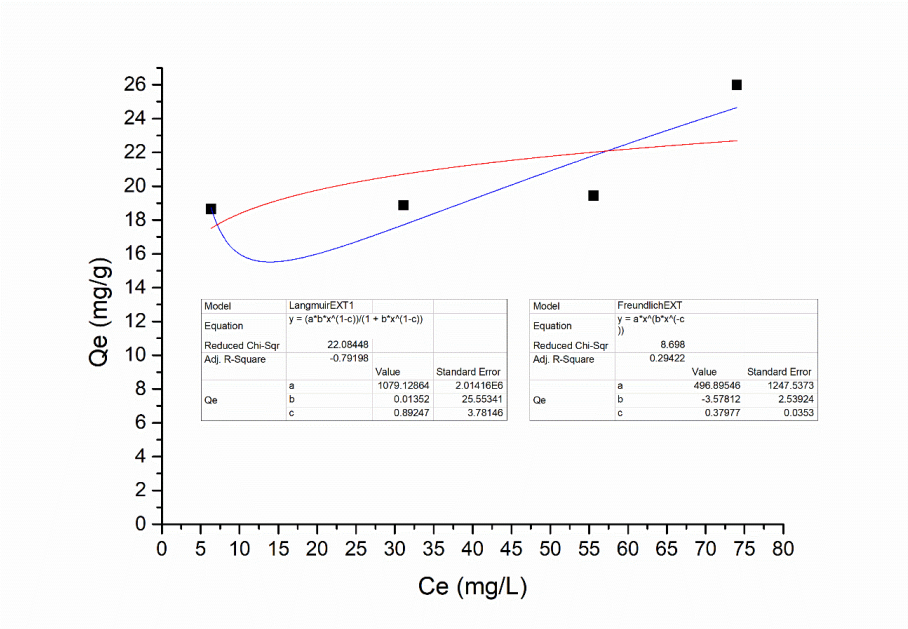

Figure 26 - Adsorption isotherm of diclofenac into COF TpBD-Me$_2$ with final concentration of 25, 50, 75 and 100 mg/L adapted to the model of Langmuir and Freundlich.

4.2.3.2 TPB-DMTP

The same isotherm study was performed with diclofenac and TPB-DMTP. Again, it was not possible to fit the isotherm to any of the tested models, most probably due to the variability and the scarcity of enough concentration points. However, it seems to fit the subclass mx, which explains how a fall in slope after a first stage of exponential increase in adsorption. The most likely explanation for this phenomenon is how the diclofenac molecules interact with each other at high concentrated solutions, where it seems they have more affinity for each other than for the adsorbent (Figure 27). Nonetheless, concentrations as high as the ones tested here will never be found in natural waters and the adsorption rate is very high, so, this adsorbent is still very interesting to be tested for monitoring diclofenac in the environment. Therefore, while testing natural waters, it is not expected this kind of interference.

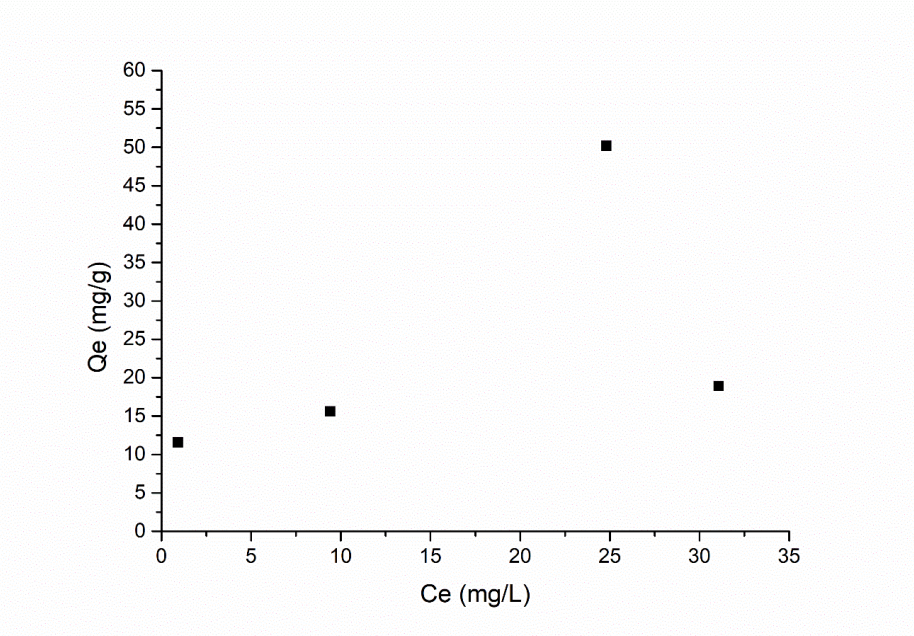

Figure 27 - Adsorption capacity of diclofenac with final concentration of 12.5, 25, 50 and 75 mg/L by TPB-DMTP.

4.2.4 Effect of pH in diclofenac adsorption by COFs

Until now, both TpBd-Me_2 and TPB-DMTP showed a good diclofenac adsorption efficiency with rapid kinetics. Characteristics that are very attractive for a possible application as adsorbents for passive samples to monitor this compound in natural waters. These COFs were then incubated with diclofenac in solutions in different pHs buffers to investigate the effect of the molecules and COFs protonation states and charges in the adsorption. The main interest is to check their behaviour at the pH ranges of natural waters.

4.2.4.1 TpBD-Me_2

As both buffers have a pH under 5, it induces the precipitation of Diclofenac and there is interference caused by the higher concentration of the hydrogen ion, and it might rise the adsorbance values (Figure 28) [174]. On the case of the results with the pH 6 buffer it is a different scenario, it has a good adsorption efficiency, 61% (Figure 28), but not the best. This is explained due to COF TpBD-Me_2 not having the strongest linkage with diclofenac anion species, it is more attracted to Diclofenac neutral species. The same was reported in an article of the study of the adsorption of diclofenac sodium from neutral aqueous solution by low-cost lignite activated cokes [98]. The results from this investigation showed that the adsorption capacity values were very good on pH 7 [98].

There is a clear trend, with the lower the pH the higher the adsorption observed. At low pH, diclofenac is protonated, without any charge and in its most hydrophobic form (Figure 29). Diclofenac seems to have more affinity for TpBD-Me_2 when it is protonated, as previously described for ibuprofen and TpBD-$(CF_3)_2$, indicating that the most dominant forces in the adsorption are hydrophobic interactions [54].

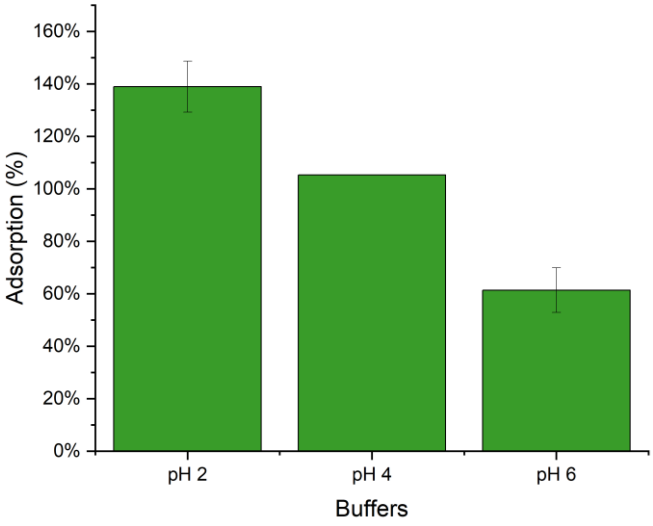

Figure 28 - Adsorption efficiency of diclofenac with final concentration of 12.5 mg/L with COF TpBD-Me_2. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

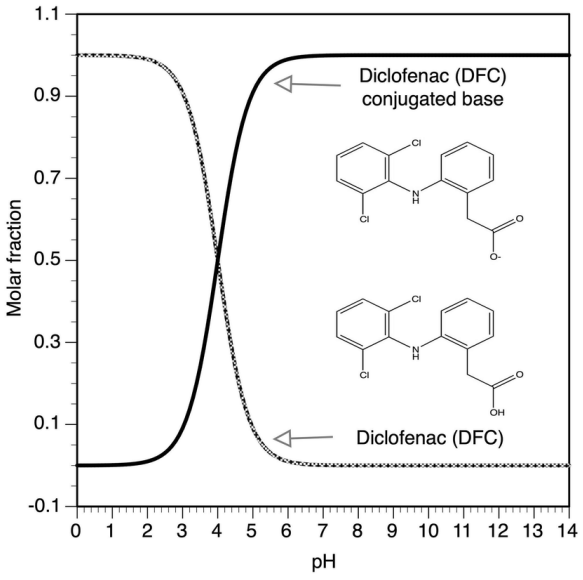

Figure 29 - Diclofenac species distribution on the pH scale, reproduced from Lara-Pérez et al, [175].

4.2.4.2 TPB-DMTP

As it can be seen in the Figure 30, diclofenac was more efficiently adsorbed at pH 6. Although, at pH 9 the adsorption was lower, which could be related with repulsion forces. These results can be explained because the COF TPB-DMTP has a higher adsorption efficacy when is present on natural environments with pH between 6 to 7 [60]. When the COF TPB-DMTP is present on alkaline or acidic solutions it tends to have lower adsorption results [152]. Moreover, based on previous studies, it was concluded that hydrophobic relationships between the ibuprofen molecule when is protonated (which is very similar to diclofenac), and the TpBD-$(CF_3)_2$ have more potential to have greater adsorption efficiency [54]

With the results analysed so far, it seems that the most promising COF for monitoring and adsorption of diclofenac in natural waters would be TPB-DMTP.

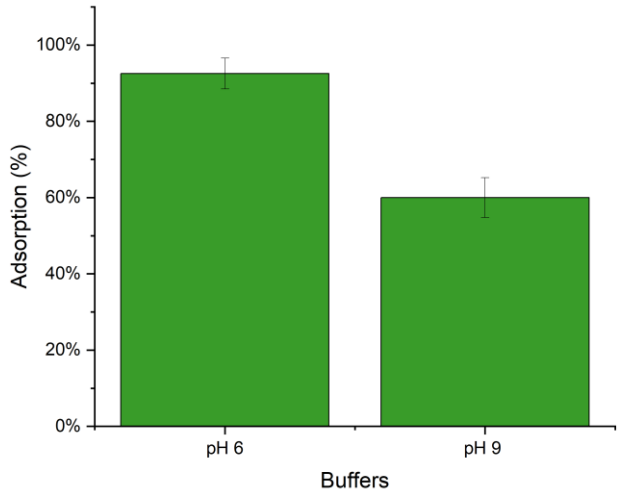

Figure 30 - Adsorption efficiency of diclofenac with final concentration of 12.5 mg/L with COF TPB-DMTP. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.2.5 Desorption test of Diclofenac from TPB-DMTP

The utilisation of COFs for pharmaceuticals monitoring in natural water requires to efficiently recover the adsorbed target compounds from the COF. To better understand what the most effective solvent to desorb diclofenac from the TPB-DMTP COF it was used isopropanol and a pH 9 buffer. Isopropanol is a polar solvent that was previously used to efficiently desorb other compounds from COFs, and an aqueous solution at pH 9 was tested as diclofenac is expected to be deprotonated and present a negative charge,

which seems to make the molecule less attractive for the COF. The results from the experiment can be observed on Figure 31, which demonstrate that isopropanol presented the best efficiency in desorbing diclofenac. Diclofenac is a lipophilic molecule, therefore is more soluble in organic solvent and alcohols as isopropanol. The efficiency levels above 100% obtained with isopropanol can be explained by an interference of some TPB-DMTP remaining in the supernatant or the leaching of some building blocks that were not sufficiently washed out after the synthesis. Nonetheless, this topic must be further investigated to determine what the best option for these conditions is and to possibly recycle the COFs TPB-DMTP. Isopropanol, however, was not able to remove diclofenac from TpBD-$(CF_3)_2$ in a recently reported work [1].

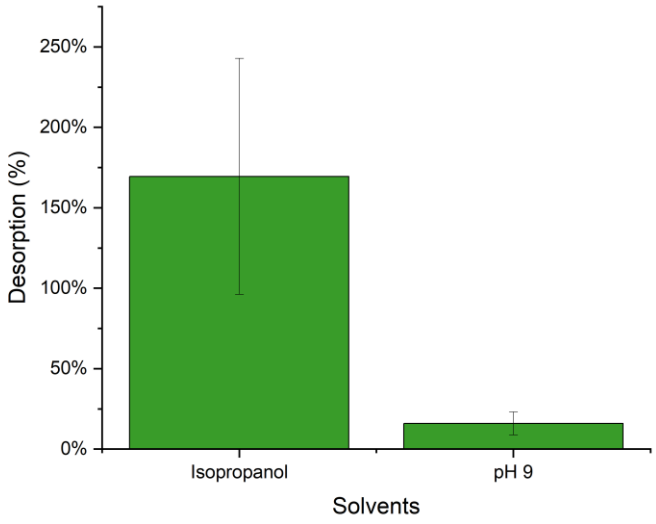

Figure 31 - Desorption efficiency of diclofenac with final concentration of 12.5 mg/L with TPB-DMTP. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

4.3 Sulfapyridine

4.3.1 Application of a TPB-DMTP composite for pharmaceuticals adsorption from natural waters

COF TPB-DMTP showed very promising results for the adsorption of different pharmaceuticals in the laboratory studies. However, as synthesized, this material is a powder, and it would be very difficult to apply and retain in cartridges of passive samplers for *in situ* adsorption. Taking this into account, a

composite material based on the direct growth of TPB-DMTP on the surface of polystyrene particles was carried out.

This material was tested for the adsorption of sulfapyridine, as sulfonamides are one of the most problematic antibiotics responsible for the appearance of antimicrobial resistances, and poorly eliminated in the conventional wastewater treatments [176]. TPB-DMTP had been recently demonstrated in our lab to efficiently adsorb these compounds.

Two concentrations of composite were tested: 1 and 2 mg/g. Previous studies used 1 mg/g of the TPB-DMTP COF alone, showing good results.

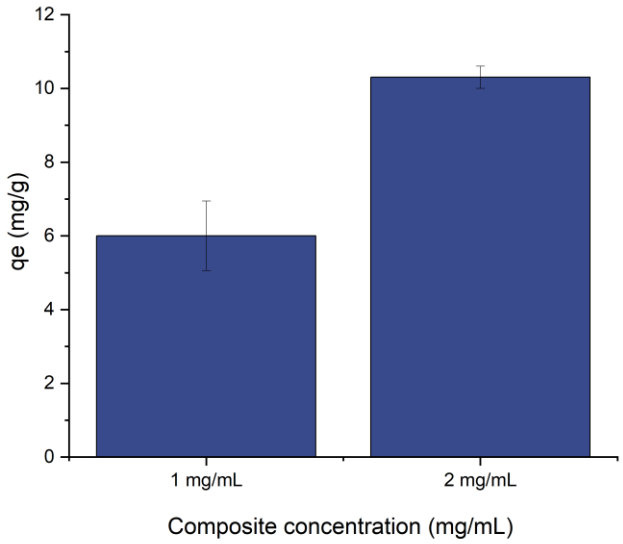

Figure 32 - Adsorption capacity of 15 mg/L of sulphapyridine by two concentrations of composite. Graphic with error bars correspond to the standard deviation of the mean from between samples of the same experiment.

As expected, the amount of sulfapyridine adsorbed with 2 mg/mL of composite was higher and comparable with the results previously obtained with 1 mg/mL. This indicated that the COF efficiency when supported in the particles is higher, as the percentage of mass of COF is expected to be much less than half of the total mass of the composite. This follows onto the hypothesis that there's a chemical sorption between sulphapyridine and the COF surface [177].

4.3.2 Desorption of a TPB-DMTP composite

To investigate whether sulfapyridine can be efficiently extracted from the composite, incubations of the composite with isopropanol were carried after adsorption. It was obtained good desorption values (up to 87%), however, a high variability was observed (Figure 33), most probably due to the causes referred in the diclofenac desorption experiments (interference of some COF remaining in the supernatant or building block leaching).

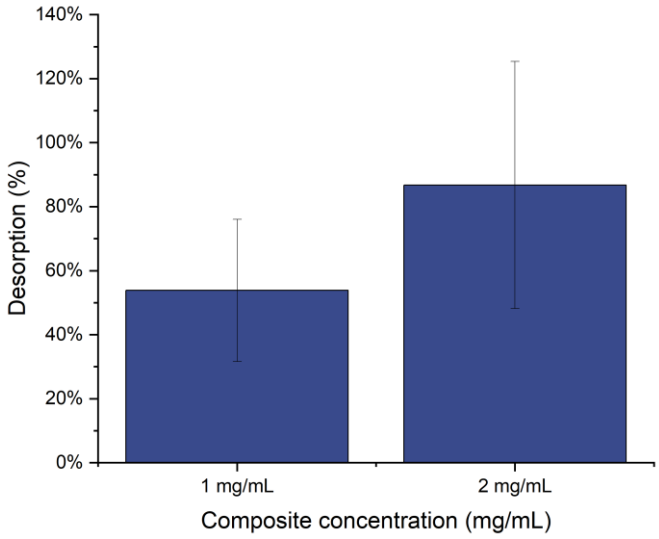

Figure 33 - Desorption efficient of diclofenac with final concentration of 12.5 mg/L with COF TPB-DMTP. Graphic with error bars correspond to the standard deviation of the mean from three independent experiments.

This composite is being applied in an ongoing study for the adsorption of pharmaceuticals in natural waters and the first investigations were performed at the final part of the experimental part of this thesis.

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

The results obtained in this work demonstrate that the application of specific selective COFs for the adsorption and monitoring of pharmaceutical contaminants in water is a promising area. The key to optimize this process lies in chemical knowledge of the adsorbate and the COF compositions, as well as the relationship between them. Therefore, it is necessary to analyse different concentrations of adsorbate, investigate the kinetics, the effect of different pH environments, to better understand how the molecules interact with these parameters and how this impacts the adsorption.

For the adsorption of tetracycline, COF TpBD-Me_2 was found to be the most convenient and highly efficient adsorbent for tetracycline, due to being a more eco-friendly alternative to TpBD-$(CF_3)_2$. The interactions between tetracycline and COF TpBD-Me_2 could possibly be described as electrostatic and/or π-π.. When tested at different pHs, the adsorption of tetracycline by TpBD-Me_2 was more efficient at pH 6 when the pharmaceutical compound was at the dominant zwitterionic form. These characteristics made it the most attractive adsorbent for tetracycline because of its potential for application in natural waters. The desorption of TpBD-Me_2 was efficiently done with isopropanol and the tests of adsorption/desorption proved that this COF can be recycled and reutilized. However, it was only studied a restricted combination of pHs and solvents with TpBD-Me_2, and the optimization of desorption efficiency should be further investigated in future works.

For the adsorption of diclofenac, COF TPB-DMTP showed the highest adsorption capacity between the analysed COFs. This COF stands out for its structure, being highly stable in water, and having a huge surface area and large pores, which makes it ideal for capturing pharmaceutical pollutants. The adsorption of diclofenac was very fast, attaining equilibrium in less than 15 min and was very efficient at pH 6, which is very appealing when considering the application on natural waters (pH 5.5-8.5). With the information obtained, it is possible to conclude that the most promising adsorbent for the adsorption and monitoring in natural waters is COF TPB-DMTP and the possibility of successfully recycling and reusing it. However, due to the results from the desorption studies, further investigation is needed on which are the best solvents to recover pharmaceutical compounds from the pores of the COF and prepare for another adsorption test. So, it will be possible to successfully recycle and reuse the COF TPB-DMTP on adsorption/desorption experiments.

For the adsorption with composite NPS@TPB-DMTP, sulfapyridine was efficiently adsorbed with an adsorption rate comparable with the bulk COF (data not shown, Brantuas TFG) when the concentration of composite was 2 mg/mL. Giving the fact that the mass of COF in the composite is much lower than 50% of the total mass, the concentration of TPB-DMTP was lower than 1mg/mL and, consequently, the adsorption efficiency of the composite is better than the bulk COF.. Although, it is necessary to develop more studies with this composite, but with a more even surface of COFs TPB-DMTP and analyse if it will react differently in the same conditions. For now, based on the initial results, it seems promising.

To summarize, COFs proved to be competently tailored with the desired qualities for adsorption of specific pharmaceutical contaminants. The ability to adapt their structures promotes various possibilities to be explored for the monitoring applications in diverse natural water matrices.

This work had a great impact on me, I was able to learn, expand my knowledge, and explore COFs as an adsorbent material to monitor the presence of pharmaceutical compounds in the water.

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