National University of Science and Technology
POLYTECHNIC of BUCHAREST
Faculty of Biotechnical Systems Engineering
Biotechnical Systems Engineering Doctoral School

DOCTORAL THESIS

TREATMENT OF WATERS CONTAINING PHARMACEUTICAL COMPOUNDS

(summary)

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TREATMENT OF WATERS CONTAINING PHARMACEUTICAL COMPOUNDS

CONTENT

FOREWORD

The doctoral thesis entitled **"Treatment of waters containing pharmaceutical compounds"** includes research and experimental studies whose main objective was the adsorption of pharmaceutical products from wastewater using 4 types of adsorbent materials: magnetite (F_3O_4), zeolites, carbon nanotubes and activated carbon, in order to define a water treatment process containing xenobiotic pollutants from the classes of nonsteroidal anti-inflammatory drugs and fluoroquinolone antibiotics.

The doctoral thesis is structured in 6 chapters.

In chapter 1 of the doctoral thesis entitled **"The objectives of the doctoral thesis and the importance of the thesis"** the objectives proposed and obtained as a result of the experimental studies and the importance of the theme chosen to be developed in this doctoral thesis are presented.

Chapter 2, entitled **"Literature study on the treatment of waters containing pharmaceutical compounds"** contains data from specialist research on the main sources of pollution with pharmaceutical compounds, their concentration level in the environment, the types of nanomaterials and adsorbent materials, their advantages and not finally, environmental risk assessment according to the concentrations of anti-inflammatory and antibiotic studied.

Chapter 3, entitled **"Methods for the detection and quantification of anti-inflammatories"** and antibiotics contains data from the literature regarding the techniques for determining medicinal products in wastewater. Among the methods mentioned in the studies in the literature, HPLC-type techniques are used most often, because they allow the rapid and high-sensitivity determination of a large number of drugs present in complex sample types such as wastewater.

Chapter 4, entitled **"Experimental research on the development and validation of some methods for the identification and quantification of some pharmaceutical compounds from the class of NSAIDs (non-steroidal anti-inflammatory drugs) and from the class of antibiotics FQs (fluoroquinolones)"** presents the methods developed and implemented for the detection and quantification of some pharmaceutical compounds from the class of NSAIDs (non-steroidal anti-inflammatory drugs) and from the class of FQs (fluoroquinolones) antibiotics. The developed methods were validated and the performance parameters of each method were calculated.

Chapter 5, entitled **"Experimental research on the treatment of waters containing pharmaceutical pollutants based on adsorption on 4 types of adsorbent materials (zeolites, activated carbon, carbon nanotubes, magnetite)**" presents the experimental research on the treatment of waters containing pharmaceutical pollutants using as a technique of treatment, adsorption on 4 types of adsorbent materials (zeolites, activated carbon, carbon nanotubes, magnetite). The pollutant adsorption process on the four types of adsorbent materials was highlighted using the Langmuir and Freundlich adsorption isotherms.

Chapter 6 has in its composition the sub-chapters, final conclusions, original contributions and Future Perspectives.

The final part of the thesis includes the list of published articles, participation in conferences and the list of bibliographic references.

CHAPTER 1. THE OBJECTIVES OF THE DOCTORAL THESIS AND THE IMPORTANCE OF THE TOPIC

1.1. The objectives of the doctoral thesis

Current research results clearly demonstrate that current water treatment technologies do not sufficiently remove pharmaceuticals and/or their metabolites from wastewater.

Therefore, pharmaceuticals enter surface water, groundwater and soil.

To justify the doctoral thesis, the following objectives were achieved:

I) Development and implementation of two chromatographic methods (HPLC) for the detection and quantification of some compounds from:

a) anti-inflammatory class: acetaminophen (paracetamol), diclofenac, ibuprofen and ketoprofen

b) antibiotic class (ciprofloxacin and norfloxacin)

II) Development and implementation of a spectrophotometric method (UV-VIS) for the identification and quantification of acetaminophen

III) Adsorption studies of some pollutants (compounds from the class of anti-inflammatories and antibiotics) in the presence of adsorbent materials (F_3O_4, active carbon, zeolites and carbon nanotubes)

IV) Mathematical models applied to describe the adsorption process: Langmuir, Freundlich, SIPS, Redlich Peterson, etc.

The main objective of the doctoral thesis is water treatment through the adsorption process using 4 types of adsorbent materials.

For the identification and quantification of the six compounds: paracetamol, diclofenac, ketoprofen and ibuprofen (non-steroidal anti-inflammatory drugs - NSAIDs), as well as norfloxacin and ciprofloxacin, 3 methods of analysis were developed and implemented at the laboratory level:

a) a chromatographic method (HPLC-DAD) for the anti-inflammatory class;
b) a chromatographic method (HPLC-FLD) for the class of antibiotics;
c) a spectrophotometric method (UV-VIS) for acetaminophen.

The pharmaceutical compounds containing paracetamol, diclofenac, ketoprofen and ibuprofen were quantified by the total organic carbon (TOC) determination method.

1.1. The importance of theme

- At national level, there are no limits on the concentration of these drugs in drinking water, surface water or waste water.
- Article 8c of the Priority Substances Directive provides for the obligation of the European Commission to propose a strategic approach regarding water pollution with pharmaceutical substances, in the context of a circular economy (2008/105/CE5, as amended by Directive 2013/39 /EU6)

- The objective of the European Commission in Brussels, 11.03.2019 COM(2019) 128, in the context of a circular economy, is to build a sustainable Europe by 2030, regarding the achievement of the objective of obtaining clean drinking water.

CHAPTER 2. Literature study on the treatment of wastewater containing pharmaceutical products

2.1. Introduction

The treatment of waters containing pharmaceutical compounds is an important concern in protecting the environment and human health. Pharmaceutical compounds can enter surface water and groundwater from various sources, such as municipal wastewater, residues of expired or improperly disposed drugs, and human or animal excreta containing incompletely metabolized drugs. Some of the methods and technologies used for the treatment of waters containing pharmaceutical compounds could be:

Biological treatment: Process in which bacteria and other microorganisms are used to degrade pharmaceutical compounds. Biological treatment processes, such as biodegradation, can be effective in removing some of the pharmaceutical compounds from waters.

Ozonation: Ozone is a strong oxidizer and can be used to degrade pharmaceutical compounds in water. Ozonation involves passing water through contact with ozone, which results in the oxidation and breakdown of pharmaceutical compounds into less toxic substances.

Adsorption: Adsorbent materials such as activated carbon and ion exchange resins can be used to adsorb pharmaceutical compounds from water. These materials retain pharmaceutical compounds in their structure.

Advanced filtration: Technologies such as advanced microfiltration and ultrafiltration membranes can remove some of the pharmaceutical compounds from water.

Advanced oxidation processes: Technologies such as advanced oxidation with peroxides or UV can be used to deactivate or degrade pharmaceutical compounds in water.

Catalytic adsorption processes: These involve the use of catalysts to accelerate the degradation reactions of pharmaceutical compounds in water.

Technologies based on nanomaterials: Nanomaterials such as iron or carbon nanoparticles can be used to degrade pharmaceutical compounds through specific chemical reactions.

Treatment with specialized microorganisms: Microorganisms, such as denitrifying bacteria, can be modified or used in specific treatment processes to degrade pharmaceutical compounds.

Drug contamination of drinking water can be caused indirectly by effluents from sewage treatment plants, which are the main carriers of pharmaceutical products and their metabolites from receiving water sources, such as rivers, lakes and underground aquifers, which are used to obtain drinking water [9]. Pharmaceutical products are present at trace levels in drinking water; even in this case, it is required to treat wastewater polluted with drugs and their degradation products in sewage treatment plants.

2.2. Types of adsorbent materials used in water depollution

2.2.1. Zeolites

Nano-structured materials containing zeolites are complex matrices that are used in adsorption studies due to their high adsorption capacity, which is the main reason for increasing applicability in the pharmaceutical field. Zeolitic materials are mesostructured and have high specific surface area (>200m^2/g) and appropriate pore size to adsorb compounds with high molecular masses [1].

2.2.2. Carbon Nanotubes (CNTs)

The advantages of non-conventional materials (carbon nanotubes) used in water depollution are: large specific surface area, high adsorption capacity, easy reuse. In aqueous media, CNTs form loose aggregates, due to the hydrophobicity of their graphitic surface.

Aggregates of CNTs contain spaces that are high adsorption energy sites for bulky organic molecules due to their larger packing pores and more accessible adsorption sites. Carbon nanotubes (CNTs) adsorb polar organic compounds due to: hydrophobic effect, π-π interactions, electrostatic interactions, hydrogen bonding and covalent bonding [5-7].

Having a surface rich in π electrons, the surface of CNTs can allow π-π interactions with organic molecules that have C=C bonds or benzene rings and polar aromatic compounds in their composition. Choosing the best methods and materials for wastewater treatment is a complex challenge, as a number of factors must be taken into account: such as quality standards to be met, efficiency and cost [9]. For the preparation of nanomaterials for wastewater treatment technologies, the following criteria must be taken into account:

(1) flexibility and ultimate efficiency of treatment;
(2) reuse of treatment agents;
(3) environmental security;
(4) low cost.

2.2.3. Activated carbon

Activated carbon is a microporous adsorbent material with a very large adsorption surface. It can be obtained from any organic material with a high carbon content: coal, wood, peat, coconut shells. Granular activated carbon is most commonly produced by grinding the raw material, adding a suitable binder to provide the desired hardness and shape. Activated carbon is used for the treatment of liquids, and the one with larger granules is used for the treatment of air, gases or as a catalyst for certain oxidation-reduction reactions [1-5]. Activated carbon is a widely used adsorbent for the retention of pollutants present in wastewater. The main characteristic of activated carbon is the particularly large specific surface, due to the presence of pores, which can reach several hundred m2/gram. Another important and controllable characteristic of activated carbons is their affinity for various types of pollutants; through the production process, the surface of the activated carbons contains weakly polar groups (carbonyl, carboxyl) that allow the effective adsorption of non-polar or polar species, mainly organic.

2.2.4. Fe_3O_4 (Magnetite)

Magnetite was prepared in the laboratory by the PhD supervisor and is the subject of his PhD thesis. The adsorbent material was made available to the PhD student for testing it in the adsorption studies of the four compounds from synthetic wastewater samples and real wastewater samples.

2.3. The advantages of adsorbent materials (nanomaterials) used in water treatment

Nanomaterials consist of tens or hundreds of atoms or molecules and can have different sizes and morphologies (amorphous, crystalline, spherical). Examples of nanomaterials used as adsorbent materials: SiO_2, TiO_2, Al_2O_3, Fe_3O_4, zeolites (ZSM-5), carbon nanotubes, activated carbon, polymers, etc. [2-3].

Advantages: a) Very good chemical stability
b) High specific surface area
c) High adsorption capacity
d) The possibility of regeneration of adsorbent materials
e) High selectivity
f) Fast kinetics
g) Specific affinity to different pollutants

2.4. The main generating sources of pharmaceutical residues in the aquatic environment

Drug contamination of drinking water can be indirectly caused by wastewater treatment plant effluents, which are the main carriers of pharmaceuticals and their metabolites, from receiving water sources such as: rivers, lakes and underground aquifers, which are used to obtain drinking water [9]. Pharmaceutical products are present at trace levels in drinking water; even in this case, it is required to treat wastewater polluted with drugs and their degradation products in sewage treatment plants.

Factors influencing the efficiency of wastewater treatment containing drugs depend on the structure of the drug, the temperature of the treatment process, the degree of hydrophobicity and the retention time of the drug in the environment [9-14].

Below are some data from the specialized literature regarding drug concentration and maximum limits found in the environment according to Table 3 and WHO [15].

The main generating sources of pharmaceutical residues in the aquatic environment

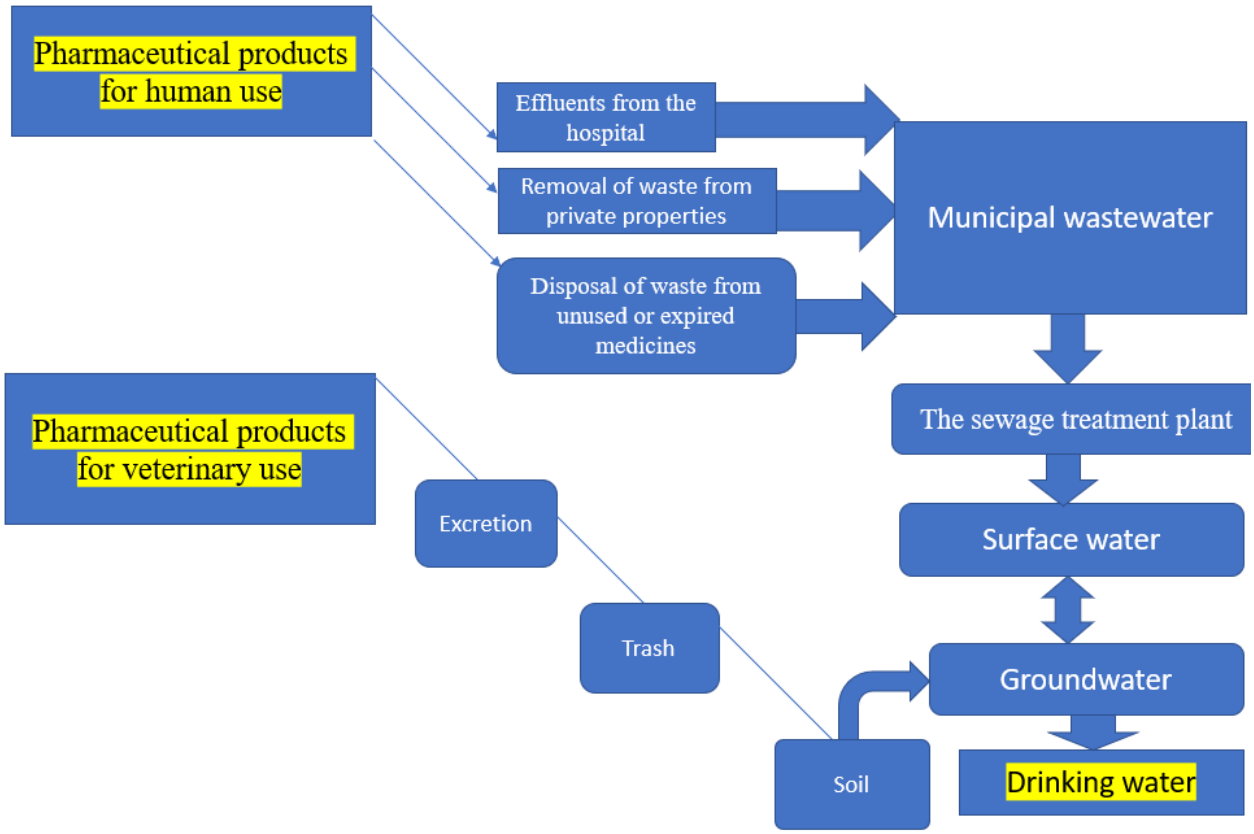


Figure 1. Generating sources of pharmaceutical residues

Table 3. Concentrations of diclofenac, ibuprofen and acetaminophen in environmental samples

Compound	Effluent from water (max.conc.,ng/L)	River waters (max. conc., ng/L)	Reference
Diclofenac	2349	568	[16]
	598	<LOQ	[17]
Ibuprofen	27,256	5044	[16]
	4239	2370	[17]
Acetaminophen	<20	nd	[17]
	nd	555	[18]

"nd" undetectable; **"LOQ"** = limit of quantification of the method

Table 4. Concentrations of diclofenac and ibuprofen in surface waters

Compound	Austria max.conc.(ng/L)	Finlanda max.conc.(ng/L)	Franța max.conc.(ng/L)	Germania max.conc.(ng/L)	Reference
Diclofenac	64	40	41	1200	[19]
Ibuprofen	nd	65	120	530	[19]

"nd" undetectable; **"LOQ"** = limit of quantification of the method

As can be seen from Tables 3 - 4, the concentration of pollutants is expressed in ng/L. At the national level, there are no limits on the concentration of the four drugs in drinking water or wastewater.

Description of pollutants and adsorbent materials, as well as stages of preparation of experiments

Table 5. Presentation of the chemical properties of the main pollutants studied

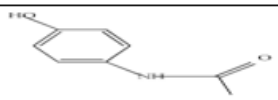
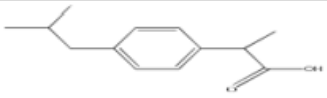
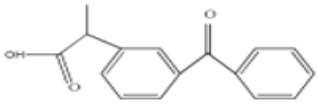
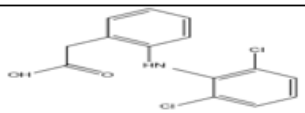
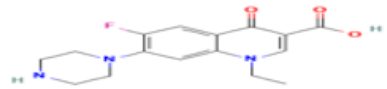
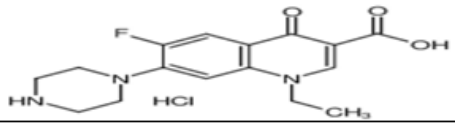
Pollutants	Chemical formula	Molecular formula	Molecular mass (g/mol)	Log Kow
Acetaminophen (ACF)		$C_8H_9NO_2$	151,06	0,46
Ibuprofen (IBU)		$C_{13}H_{18}O_2$	206,13	3,97
Ketoprofen (KTF)		$C_{16}H_{14}O_3$	254,09	3,12
Diclofenac (DCF)		$C_{14}H_{11}Cl_2NO_2$	295,02	4,51
Norfofloxacin (NRF)		$C_{16}H_{18}FN_3O_3$	319,33	0,46
Ciprofloxacin (CIP)		$C_{17}H_{18}FN_3O_3$	331,40	0,28

Table 6. Presentation of the main adsorbent materials used in this study

Adsorbent material	Specific surface area (m^2/g)	Total pore volume (cm^3/g)	Average micropore radius (Å)
Active coal	604	12,7	870
Zeolite (ZSM-5)	500	11,5	750
Carbon Nanotubes (CNTs)	410	8,6	520
Fe_3O_4	275	5,5	390

CHAPTER 3. METHODS OF DETERMINATION AND QUANTIFICATION OF MEDICINES AND APPLIED MATHEMATICAL MODELS

3.1.Determination methods

Analytical methods for the simultaneous quantification of paracetamol (acetaminophen), diclofenac, ibuprofen and ketoprofen are essential given their versatile nature and importance for pain treatment. Data in the literature refer to several analytical methods for the determination of paracetamol, diclofenac, ibuprofen and ketoprofen either separately or in combination with other drugs in the solid pharmaceutical dosage by spectrophotometry [1], liquid chromatography coupled to mass spectrometry [1] high performance liquid chromatography (HPLC) [1], capillary electrophoresis [1], voltammetry [1] and thin layer chromatography (TLC) [1].

3.2. Mathematical models applied to adsorption processes. Adsorption isotherms

Four analytes were studied: acetaminophen, diclofenac, ketoprofen and ibuprofen. The experimental results were processed using various mathematical models, presented in table 5. For the quantification of these pharmaceutical compounds, HPLC methods developed and implemented at the laboratory level were used. The experiments were carried out at room temperature of approximately 20±20C. Adsorption parameters and mathematical models were determined by linear and non-linear regression and then presented with their help.

Table 7. Mathematical models of adsorption isotherms

Model	Mathematical equation	Remarks
Henry	$q = K_H \cdot c$	The linear adsorption model
Langmuir	$q = Q\frac{K_L \cdot c}{1 + K_L \cdot c}$	Isotherms with two parameters
Freundlich	$q = K_F \cdot c^{1/n}$	
Sips (Modified Langmuir)	$q = Q\frac{(K_S \cdot c)^m}{1 + (K_S \cdot c)^m}$	Isotherms with three parameters
Redlich –Peterson	$q_e = \frac{K_{RP} \cdot C_e}{1 + a_{RP} \cdot C_e^{\beta}}$	
Temkin-Pzyhev	$\theta = (\frac{RT}{b}) \cdot \ln(K_T C_e)$	

Among them, the Langmuir isotherm model and the Freundlich isotherm model have the widest applicability, considered to be the classical models of adsorption isotherms [3].

The choice of the most appropriate model, which can best describe the experimentally obtained adsorption isotherms, was made, in this study, with the help of linear regression, for all the cases studied [3].

CHAPTER 4. ORIGINAL CONTRIBUTIONS

4.1. Development of an HPLC method for the identification and quantification of anti-inflammatories (acetaminophen, diclofenac, ibuprofen, ketoprofen) present in wastewater samples

The aim of this study was the simultaneous determination of four anti-inflammatory drugs namely acetaminophen, diclofenac, ibuprofen and ketoprofen from wastewater samples by a simple and rapid method using the High Performance Liquid Chromatography (HPLC) technique with DAD (Diode Array) detection Detector).

After the development and implementation of the HPLC method for the four compounds of interest, the adsorption studies of these compounds on adsorbent materials were also carried out.

The study aimed at both the quantification of the four compounds through the developed HPLC method, and the degree of their removal from wastewater, with the help of adsorbent materials (zeolites and activated carbon).

At the same time, the pharmaceutical products of the four analytes present in the wastewater were monitored by the total organic carbon (TOC) quantification method, expressed in mg C/L.

Chromatographic equipment used:

The experiments to establish the optimal conditions for chromatographic separation and detection were carried out on an Agilent 1200 HPLC system consisting of:

- solvent container and membrane degasser;
- the quaternary pump with isocratic and gradient elution capable of supplying a mobile phase with up to 4 components, with variable flow rate;
- autosampler with a capacity of 100 positions and variable injection volume (0.1-100 μl);
- thermostat for maintaining constant temperature in the chromatographic column;
- the Eclipse C18 chromatographic column with the length of 15 cm, the inner diameter of 4.6 mm and the diameter of the stationary phase particles of 5 μm;
- UV-VIS detector (DAD) with variable wavelength and ability to simultaneously record up to 8 different wavelengths;
- Agilent ChemStation software for data acquisition, processing, reporting.

Due to the fact that the simultaneous determination of several compounds is aimed at, an attempt was made to establish the chromatographic separation conditions using gradient elution. Different mixtures of the mobile phase of phosphate buffer pH 3.3 and acetonitrile were tested for elution, using mixtures in different proportions of phosphate buffer pH 3.3 and acetonitrile (Table 5). The composition of the optimal mobile phase determined experimentally is 20 mM phosphate buffer in ultrapure water with a pH value of pH 3.3 (solvent A) and acetonitrile (solvent B) with gradient elution. The detection of the compounds of interest was carried out at the optimal wavelengths identified by the absorption maximum from the UV-VIS spectra at 248 nm for acetaminophen, 255 nm for ketoprofen, and the non-steroidal anti-inflammatory drugs diclofenac and ibuprofen had absorption maxima at 280 nm and 220 nm respectively .

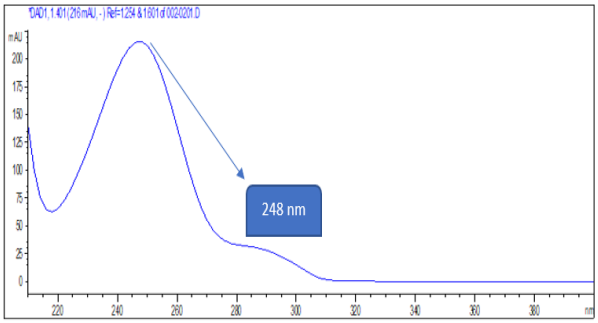


Figure 1. UV-Vis spectrum for Acetaminophen (ACF) (210-700 nm) (RT=2.1 minutes)

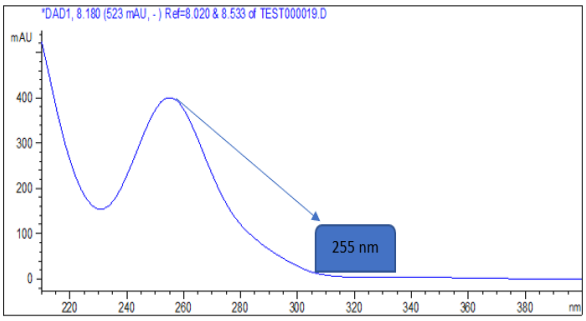


Figure 2. UV-Vis spectrum for Ketoprofen (KTF) (210-700 nm) (RT=6.3 minutes)

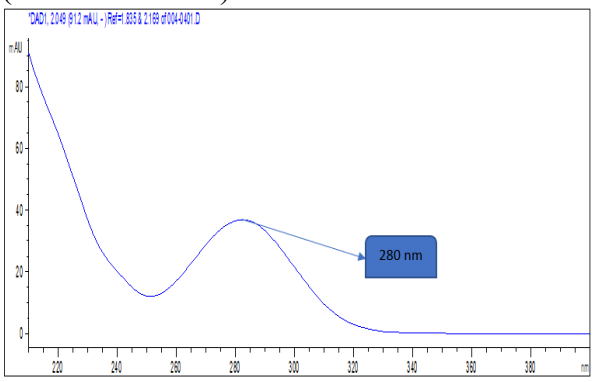


Figure 3. UV-Vis spectrum for Diclofenac (DCF) (210-700 nm) (RT=8.3 minutes)

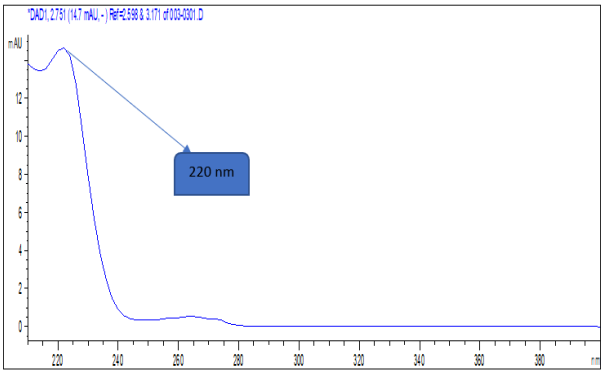


Figure 4. UV-Vis spectrum for Ibuprofen (IBF) (210-700 nm) (RT=9.0 minutes)

4.1.1. Optimization of the detection method to increase the sensitivity of the determination of the four analytes

Having a hydrophilic character due to the presence of -OH groups in the molecule of the analytes of interest (log Kow = 0.46, for ACF; log Kow = 3.12, for KTF; log Kow = 4.51, for DCF and log Kow = 3.97 for ibuprofen) at development of the method, it was decided to use an Eclipse C18 chromatographic column.

A column temperature range between 20 and 40°C was tested for a better separation of the four analytes, and for the sensitivity of the method, an injection volume range between 2 – 100 µL was tested. The temperature of 20°C and the injection volume of 10 µL were found to be the optimal values for the separation with the highest resolution, the best efficiency and the shortest time, while achieving the maximum sensitivity of the method.

For the gradient elution, different compositions of the buffer solution and acetonitrile were tested using different ratios between aqueous and organic solvent shown in table 5. The optimal composition was 80% aqueous solvent (KH_2PO_4 20 mM pH 3.3) and 20% solvent organic (Acetonitrile), which led to obtaining narrow peaks with high chromatographic efficiency. The

optimized conditions of the liquid-chromatographic parameters allowed the separation of the 4 analytes in a chromatographic run-time of only 10 minutes.

Taking into account the very low concentrations in which the 4 anti-inflammatories are found in wastewater (at the level of μg/L), an HPLC method was developed to allow their simultaneous detection at a concentration level between 0.5 – 20 μg/L.

To optimize these parameters, a mixture solution of acetaminophen, ketoprofen, ibuprofen and diclofenac was used with a concentration of 1 mg/L, 5 mg/L, 10 mg/L and 20 mg/L, respectively. With the modification of the parameters, their effect on the peak area and the signal/noise ratio (S/N) was followed. Following these experiments, the chromatographic parameters that generated the best sensitivity (maximum peak area and respectively maximum signal/noise ratio) were chosen for the studied compounds.

After the LC detection optimization procedure, the parameters that generated maximum sensitivity (minimum peak width, maximum efficiency, maximum signal-to-noise ratio) were chosen for all compounds analyzed in conjunction with a minimum duration of chromatographic separation. Following parameter optimization, method limits of quantification for (LOQ) were determined for the four analytes. These correspond to an S/N ratio of about 10.

The optimal HPLC separation parameters of nonsteroidal anti-inflammatory drugs established experimentally are the following:

- Eclipse C18 chromatographic column with a length of 15 cm, an inner diameter of 4.6 mm and a stationary phase particle diameter of 5 μm
- Column temperature: 20°C
- Injection volume: 10 μL
- Mobile phase: Phosphate buffer 20 mM in ultrapure water (brought to pH=3.3): Acetonitrile
- Mobile phase flow rate: 1 mL/min
- Elution: gradient
- UV detection: λ = 248 nm for Acetaminophen, 255 nm for Ketoprofen, and for Diclofenac and Ibuprofen the absorption maxima were at 280 nm and 220 nm, respectively.
- Duration of separation: 10 minutes

4.1.2. Performance parameters of the analytical method developed in the laboratory

Considering the provisions regarding the internal validation of the analytical method, the following performance parameters were taken into account: selectivity/specificity, working range, linearity, accuracy (recovery), precision, limit of detection and limit of quantification.

Linearity

The working range is specific to each analyte of interest and is established to confirm that the developed analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing the analyte of interest within or at the extremes of the specified range.

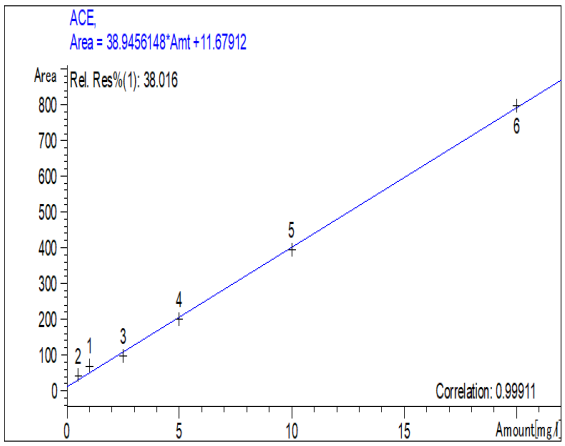


Figure 5. Calibration curve for Acetaminophen

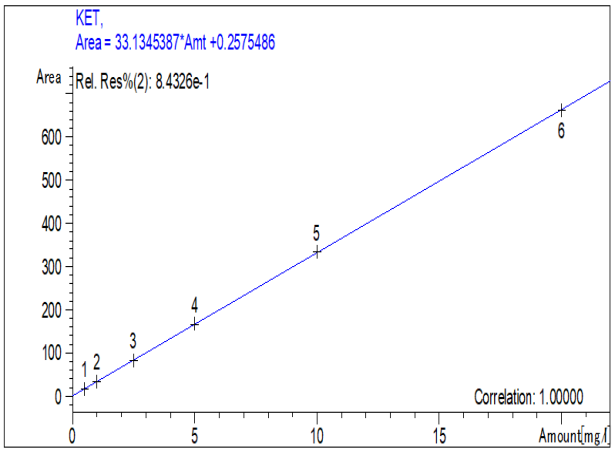


Figure 6. Calibration curve for Ketoprofen

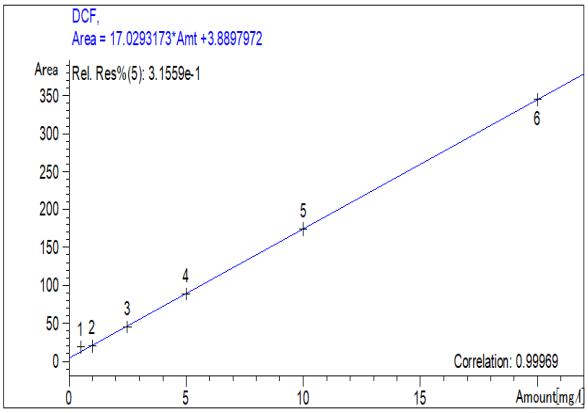


Figure 7. Calibration curve for Diclofenac

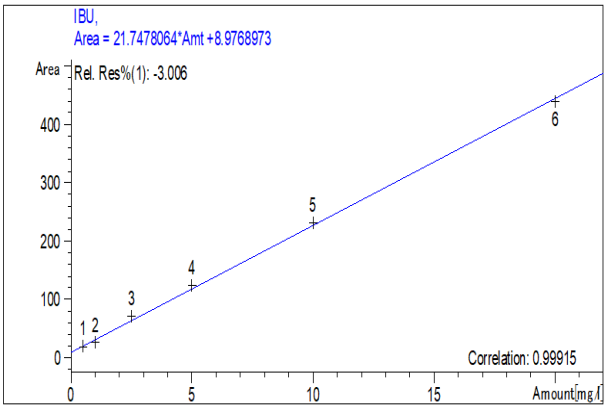


Figure 8. Calibration curve for Ibuprofen

The linearity of a quantitative analytical method represents its ability to obtain results proportional to the concentration of the analyte in the samples. From the regression lines presented in figures 5-8, a good correlation can be observed between the concentration values of the compounds and the information values expressed in area units ($R^2 > 0.999$).

After setting the program for chromatographic separation and UV detection, the 6 working solutions for calibration were injected, in the order of increasing their concentration. In the chromatograms of the calibration solutions, the retention time and the area values of the chromatographic peaks corresponding to the studied compounds were recorded, as well as the chromatographic profile of the base line, shown in figures 5-8.

Using the Agilent ChemStation software of the equipment used, the calibration functions for each compound were drawn, passing on the ordinate the concentration values, in µg/L, and on the abscissa the average values of the areas of the chromatographic peaks, expressed in area units, corresponding to the injections performed for each calibration solution.

The parameters obtained after drawing the calibration curves (working area, regression equation, correlation coefficient) are given in table 8.

Table 8. Linear regression parameters obtained for the concentration range tested for linearity

Compound	Concentration range	The regression equation	R^2
Acetaminophen	0,5–20 μg/L	y = 38,94x + 11,68	0,9991
Ketoprofen	0,5–20 μg/L	y = 33,13x + 0,26	1,0000
Diclofenac	0,5–20 μg/L	y = 17,03x + 3,89	0,9997
Ibuprofen	0,5–20 μg/L	y = 21,75x + 8,98	0,9991

From the analysis of the data presented in the table and the charts of the drawn calibration curves, the following aspects emerge:

- the equation of the linear regression function corresponding to the calibration curve for each analyte, has a linear dependence of the area values of the chromatographic peaks on its concentration;
- the concentration range on which the response of the detector is proportional to the concentration of the compounds is between 0.5-20 μg/L for the four analytes, with values of the correlation coefficients (R^2) higher than 0.999.

Selectivitatea/Specificitatea

Selectivitatea (specificitatea) metodei a fost evaluată prin injecția unui etalon cu cei patru analiți, a unei probe de apă uzată care nu conține analiții de interes și a unei probe de apă ultrapură (blank). Având în vedere ca la lungimile de undă de interes nu au apărut peak-uri interferente la timpii de retenție corespunzători celor 4 analiți se poate considera că metoda este selectivă.

PRECISION

The precision of an analytical method expresses the fit or degree of agreement between a series of determinations obtained from several samples originating from the same homogeneous sample under conditions of specificity. It is expressed as a standard deviation (s) or as a percentage relative standard deviation (RSD%)

Precision can be evaluated at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses analytical variability under the same working conditions, over a short period of time. It is obtained when the test is carried out in a single laboratory, by a single operator, using a single type of equipment and the same method over a short period of time.

Intermediate precision represents the long-term variability of the measurement process when identical samples are analyzed by the same method, by the same laboratory, by different operators, over a longer period of time and is determined by comparing the results of a method for a single laboratory for a certain number of days.

The repeatability of the analysis was assessed by repeating the sample preparation and analysis procedure on 6 subsamples from the same sample. Intermediate precision was assessed by applying the sample preparation procedure to a single sample divided into 12 subsamples but prepared on different days by multiple analysts.

The precision of the entire analytical procedure, expressed as percentage relative standard deviation (RSD%), was determined by repeated analysis of effluent samples from several wastewater treatment plants.

Table 9. Accuracy data obtained on repeated samples

Analyte	Concentration µg/L	Repeatability (RSD %) (n=6)	Reproducibility (RSD %) (n=12)
Acetaminophen	5	0,15	0,29
Ketoprofen	5	0,11	0,28
Ibuprofen	5	0,29	0,40
Diclofenac	5	0,17	0,23

The results obtained for the precision of the method are presented in table 9. The precision of the method varied in the case of repeated measurements below 10%.

Accuracy and retrieval efficiency

To determine the recovery yield (recovery of the analytes of interest) the wastewater samples were enriched with a known concentration of the four studied compounds of about 10µg/L.

The analytes of interest were quantified by the HPLC method and then the recovery (recovery) yield was calculated based on the formula:

$$Recovery\ yield\ (\%) = \frac{The\ concentration\ obtained}{The\ concentration\ added} * 100 \quad (1)$$

Recovery refers to the ratio of the experimentally determined concentration using calibration curve interpolation of each analyte to the concentration added to the aqueous matrix.

Table 8. Recovery yield results

Analyte	The concentration added (µg/L)	The concentration obtained (µg/L)	Recovery yeld (%)
Acetaminophen	10	9.599	95.99
Diclofenac	10	8.145	81.45
Ketoprofen	10	8.392	83.92
Ibuprofen	10	9.022	90.22

The accuracy of the method was demonstrated, the recovery yields being between 81-96% (table 8).

Limit of detection and quantification

The instrumental limits of detection (LOD) and quantification (LOQ) were determined by injecting solutions having increasingly lower analyte concentrations until the experimentally determined signal-to-noise ratio was equal to 3 (LOD) and 10 (LOQ), respectively). The values of the limits of detection and quantification thus determined are shown in table 10.

Table 10. Limits of detection and quantification of the studied analytes

Analyte	LOD (µg/L)	LOQ (µg/L)
Acetaminophen	0,07	0,21
Ketoprofen	0,16	0,46
Ibuprofen	0,05	0,14
Diclofenac	0,20	0,60

Limit of detection (LOD); Limit of quantification (LOQ)

4.1.3. Anti-inflammatory concentrations quantified in wastewater samples

The laboratory experiments on the separation of drugs from the class of analgesics from aqueous matrices were carried out with the aim of establishing the optimal filtration method, following the recovery yields of these compounds and the elimination of potential interferences. StrataTM-X 33µm Polymeric Reversed Phase filters with a pore size of 0.33µm were used to filter the samples. This optimized method of sample preparation has the following advantages: the consumption of environmentally toxic organic solvents is eliminated, the use of large-capacity laboratory glassware is avoided, and the working time is reduced.

Wastewater samples were taken in 1000 ml brown glass containers filled in such a way that the amount of air present in the container was as small as possible. They were transported in refrigerated boxes and were pre-treated within 24 hours. The samples were first analyzed as such, without the addition of standard to observe the possible presence of any target compound in the water sample.

For the determination of the four compounds studied by the HPLC method, 6 samples of wastewater were subjected to analysis. The samples were filtered through StrataTM-X 33µm Polymeric Reversed Phase filters with a pore size of 0.33µm. An injection volume of 20 µL was used (Table 11).

Table 11. Concentrations determined in wastewater samples using the HPLC method

Samples	ACF conc. (µg/L)	KET conc. (µg/L)	IBF conc. (µg/L)	DCF conc. (µg/L)
P1	1,15	1,36	1,55	2,90
P2	1,51	1,15	1,56	1,63
P3	1,26	1,21	2,59	3,20
P4	1,09	1,26	1,63	1,41
P5	<LOQ	<LOQ	<LOQ	<LOQ
P6	<LOQ	<LOQ	<LOQ	<LOQ

4.2. Development of a spectrophotometric method for the quantification of acetaminophen present in wastewater

For the quantification of acetaminophen in wastewater, a spectrophotometric method (UV-VIS) was developed and implemented at the laboratory level.

Acetaminophen calibration curve

For the calibration curve, 5 working solutions were prepared from the 10mg/L acetaminophen stock solution. A calibration curve was plotted: absorbance as a function of concentration over a working range of 0.1-1mg/L.

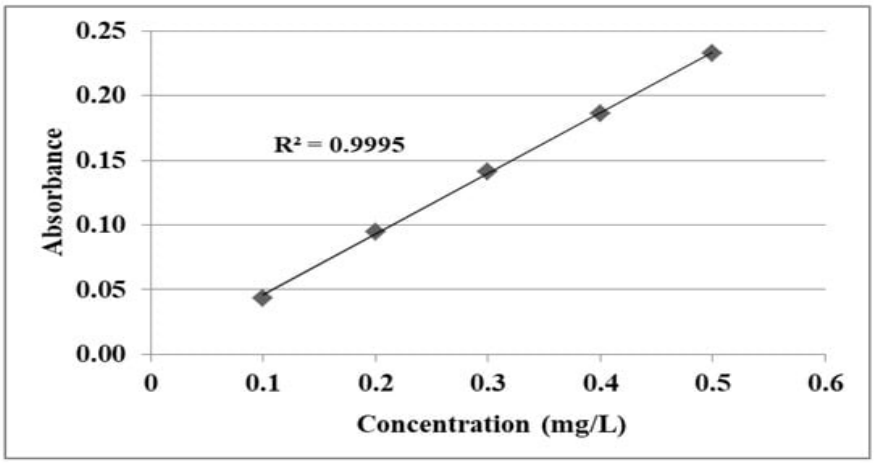


Figure 9. Acetaminophen calibration curve at λ-302nm

4.2.1 Performance parameters of the method for the determination of acetaminophen by a UV-VIS method

Performance parameters of the test method:

►the working range is between 0.1 - 1 mg/l paracetamol

In order to highlight the results obtained in this study, the performance parameters of the method were studied: precision, accuracy, limit of quantification, limit of detection, extended uncertainty.

Internal validation of the applied analytical method, carried out in order to evaluate the performance parameters of the method: LOD detection limit, LOQ quantification limit, repeatability, intermediate precision, extended uncertainty. The method developed in this study was validated according to the ICH guideline. (Badea et al. 2012, Cruceru et al. 2011, ICH Q2(R1) 2015). Ten determinations were made for repeatability and precision, limit of quantification, limit of detection, and extended uncertainty for acetaminophen in the range of 0.10-0.50 mg/L and using two dissolved media.

Table 12. Internal validation of the analysis method

Parameters	Experiments
LOQ and LOD	5 independent blank solutions fortified with 0.05mg/L
Repeatability	10 independent standard solutions of concentration 0.25mg/L
Intermediar precision	12 independent standard solutions of 0.35mg/L

The expanded uncertainty of the analytical results was calculated using the following formula [5]:

$$U_{extended} = k * U_{combined} \quad (2)$$

$$U_{extended} = k * \sqrt{U_c^2 + U_v^2 + U_{rep}^2 + \left(\frac{1}{U_{mas}}\right)^2 + \left(\frac{1}{U_{rec}}\right)^2} \quad (3)$$

where k is a coverage factor; with the value 2 for a 95% confidence level;
Ucombinata – combined standard uncertainty; Uc – concentration uncertainty (instrument calibration, flasks, pipettes, standard reference material); Uv – 50 ml volumetric flask (calibration, temperature); Urep – repeatability uncertainty (mass, volume, concentration, extraction recovery); U(mass) – weight uncertainty (balance calibration, linearity);
Urec – extraction recovery uncertainty.

Linearity

The linearity of the method was checked on five working solutions prepared for each dissolved medium (for 0.1 M HCl and for 0.01 M KOH). The concentrations of the working solutions were between 0.1 - 0.5 mg/L for acetaminophen in both dissolved media. The linearity parameters showed that the intercept values are small and the correlation coefficient is close to one for all studied compounds (Table 13), which suggests that Lambert Beer's law is verified.

Table 13. Linear regression parameters obtained for the concentration range tested for linearity

Experimental conditions	Concentration range (mg/L)	The regression equation	R^2	λ(nm)
Acetaminophen dissolved in 0.1M HCl	0,1-0,5	y=0.4591x+0.0004	0,9995	302
Acetaminophen dissolved in 0.01M KOH	0,1-0,5	y=0.4595x+0.0003	0,9992	297

Table 14. Performance parameters for paracetamol dissolved in 0.1M HCl medium

No.	Acetaminophen		
	Performance parameters	**M.U.**	**Obtained results**
1	Precision	mg/L	0,03
2	Accuracy	mg/L	0,02
3	Detection limit (LOD)	mg/L	0,04
4	Quantification limit (LOQ)	mg/L	0,12
5	Extended uncertainty	%	17,5

Table 15. Performance parameters for paracetamol dissolved in 0.01M KOH medium

No.	Acetaminophen		
	Performance parameters	**M.U.**	**Obtained results**
1	Precision	mg/L	0,06
2	Accuracy	mg/L	0,04
3	Detection limit (LOD)	mg/L	0,06
4	Quantification limit (LOQ)	mg/L	0,18
5	Extended uncertainty	%	19,5

4.3. Development and validation of an HPLC-FLD method for the identification and quantification of fluroquinolones (norfloxacin and ciprofloxacin) present in wastewater samples

Development of a method for pretreatment of water samples using separation, treatment and concentration techniques for the isolation of compounds of interest with high yields of their recovery from water samples.

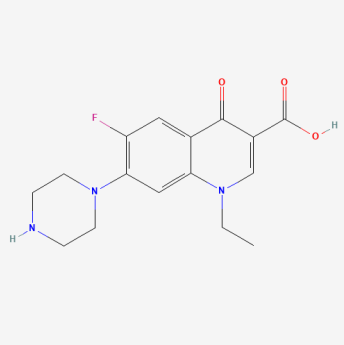
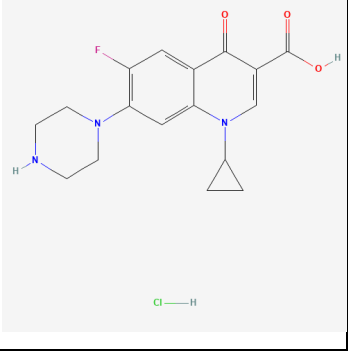
- Establishing the chromatographic conditions for specific detection and separation using columns with optimal selectivity, resolution and efficiency;
- Validation of the method developed for the detection and quantification of the 2 antibiotics;
- The aim of this method was the simultaneous determination of 2 fluoroquinolones, namely norfloxacin and ciprofloxacin from environmental samples and synthetic samples through a simple and rapid method using the High Performance Liquid Chromatography (HPLC) technique with FLD - G1312A fluorescence (Fluorescence Detector) .

Chromatographic equipment used

The experiments to establish the optimal conditions for chromatographic separation and detection were carried out on an Agilent 1200 HPLC system consisting of:

- solvent container and membrane degasser;
- quaternary pump with isocratic and gradient elution capable of supplying the system with a mobile phase with up to 4 components, with variable flow rate;
- autosampler with a capacity of 100 positions and variable injection volume (0.1-100 µl);
- thermostat for maintaining a constant temperature in the chromatographic column;
- chromatographic column Zorbax Eclipse Plus C18 with the length of 15 cm, the inner diameter of 4.6 mm and the diameter of the stationary phase particles of 5 µm;
- detector (FLD) with FLD wavelength λex 280nm and λem 440nm;
- Agilent ChemStation software for data acquisition, processing and reporting.

Table 16. Physico-chemical properties of the analyzed compounds

No.	Analytes Color/Shape	Molecular formula	Molecular mass	Log Kow*	pKa* (25°)	Structural formula
1	**Norfloxacin** White to pale yellow crystalline powder	$C_{16}H_{18}FN_3O_3$	319.33	0.46	pKa_1 = 6.34; pKa_2 = 8.75	
2	**Ciprofloxacin** Weak to light yellow crystalline powder	$C_{17}H_{18}FN_3O_3$	331.4	0.28	pKa_1 = 6.09; pKa_2= 8.74	

4.3.1. Optimization and validation of the method developed in the laboratory

Optimization of the detection method to increase the sensitivity of the determination of the two analytes (norfloxacin and ciprofloxacin)

A column temperature range between 20 oC and 40 oC was tested for a better separation of the 2 analytes, and for the sensitivity of the method an injection volume range between 1 – 20 µL was tested. The temperature of 30oC and the injection volume of 20 µL were found to be the optimal values for the separation with the highest resolution, the best efficiency and the shortest time, while achieving the maximum sensitivity of the method.

Different percentage compositions of the aqueous solvent, H_3PO_4 25 mM pH 3, and the organic solvent, acetonitrile, were tested for gradient and isocratic elutions. The optimal composition was 88% aqueous solvent (H_3PO_4 25 mM pH 3) and 12% organic solvent (Acetonitrile), which led to obtaining narrow peaks with high chromatographic efficiency. The optimized conditions of the liquid-chromatographic parameters allowed the separation of the 2 analytes in a chromatographic run-time of only 7 minutes. Taking into account the low

concentrations at which the 2 fluoroquinolones are found in environmental samples (at the µg/L level), an HPLC method was developed to allow their simultaneous detection at a concentration level between 5 – 100 µg/L.

To optimize these parameters, a mixed solution of norfloxacin and ciprofloxacin with a concentration of 5 µg/L was used. Along with the modification of the parameters, their effect on the peak area and the signal/noise ratio (S/N) was followed. Following these experiments, the chromatographic parameters that generated the best sensitivity (maximum peak area and respectively maximum signal-to-noise ratio) were chosen for the studied compounds. Following parameter optimization, method limits of quantification for (LOQ) were determined for the two analytes. These correspond to an S/N ratio of about 10.

The optimal HPLC separation parameters of fluoroquinolones established experimentally were as follows:

- Chromatographic column Zorbax Eclipse Plus C18 (15 x 3 mm, 5 µm);
- Column temperature: 30°C;
- Injection volume: 20 µL;
- Mobile phase: H3PO4 25mM (brought to pH=3): ACN; isocratic: 88/12, v/v;
- Mobile phase flow rate: 1 mL/min;
- Elution: isocratic;
- FLD detection: λex (excitation wavelength), 280nm and λem (emission wavelength), 440nm;
- Separation time: 7 minutes.

Figure 24 shows the chromatogram obtained from the analysis of a mixed solution of NOR and CIP with a concentration of 100 µg/L, and the elution order of the compounds on the Eclipse C18 chromatographic column according to the retention time (TR) is as follows:
TR norfloxacin < TR ciprofloxacin.

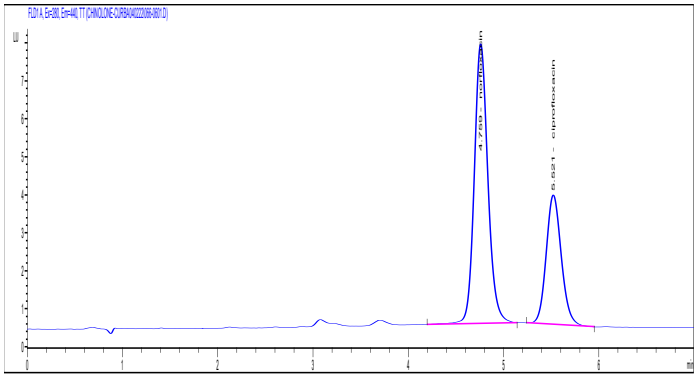


Figure 24. Chromatogram of a mixed solution of NOR and CIP with a concentration of 100 µg/L

Preparation of calibration solutions and plotting of calibration curves

After establishing the optimal separation and detection program, it is necessary to draw the calibration curve based on the values of the peak areas of the 2 analytes in order to validate the HPLC method of quantitative determination of the established analytes.

a) Preparation of basic standard solutions

Preparation of calibration solutions: Individual stock standard solutions were prepared in methanol. The concentrations of these solutions are shown in Table 17.

Table 17. Concentrations of individual standard stock solutions

Weighed quantity (g)	Volume of volumetric flask (ml)	Concentration of stock solution (mg/L)
0.025 g	50	500
0.025 g	50	500

All stock solutions and their dilutions were protected from light and stored in the dark at 4 °C.

b) Preparation of the mixed working standard solution

In a 10ml volumetric flask, add 2 ml of the stock solution of Norfloxacin and 2 ml of the stock solution of Ciprofloxacin and bring it up to the mark with mobile phase: H3PO4 25mM (brought to pH=3): ACN, 88/12, resulting concentrations 100 µg/L for NOR and 100 µg/L CIP.

c) Preparation of working standard solutions and plotting the calibration curve

To plot the calibration curve, 5 standard solutions were prepared in the mobile phase (H_3PO_4 25mM, pH=3: ACN, 88/12). From the mixed working standard solution, 7.5 ml, 5 ml, 2.5 ml, 1 ml, 0.5 ml were used in 10 ml volumetric flasks, which were then brought up to the mark in the mobile phase

The concentrations of the solutions that were used to plot the calibration curve are provided in table 18.

Table 18. Concentrations of working standard solutions

Fluoroquinolones	Norfloxacin	Ciprofloxacin
Conc. Solution, µg/L	5	5
Conc. Solution, µg/L	10	10
Conc. Solution, µg/L	25	25
Conc. Solution, µg/L	50	50
Conc. Solution, µg/L	75	75
Conc. Solution, µg/L	100	100

4.3.2. Performance parameters of the analytical method developed in the laboratory

Considering the provisions regarding the internal validation of the analytical method, the following performance parameters were taken into account: selectivity/specificity, working range, linearity, accuracy (recovery), precision, limit of detection and limit of quantification.

Linearity

The working range is specific to each analyte of interest and is established to confirm that the developed analytical procedure provides an acceptable degree of linearity, accuracy and

precision when applied to samples containing the analyte of interest within or at the extremes of the specified range.

The linearity of a quantitative analytical method represents its ability to obtain results proportional to the concentration of the analyte in the samples. After setting the program for chromatographic separation and FLD detection, the 6 working solutions for calibration were injected, in order of increasing concentration, figure 25. From the regression lines shown in figures 26-27, a good correlation can be observed between the values of the concentrations of the compounds and the values of information expressed in area units ($R^2 > 0.99$).

Using the Agilent ChemStation software of the equipment used, the calibration functions for each compound were drawn, passing on the ordinate the concentration values, in µg/L, and on the abscissa the average values of the areas of the chromatographic peaks, expressed in area units, corresponding to the injections performed for each calibration solution.

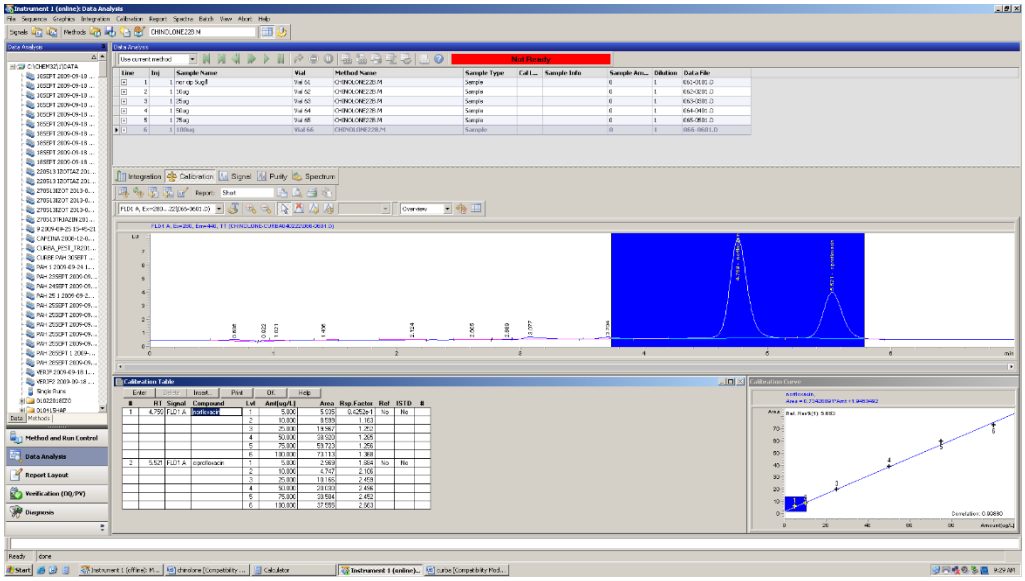


Figure 25. Working solutions for calibration with concentrations of 5 µg/L, 10 µg/L, 25 µg/L, 50 µg/L, 75 µg/L, 100 µg/L for norfloxacin and ciprofloxacin

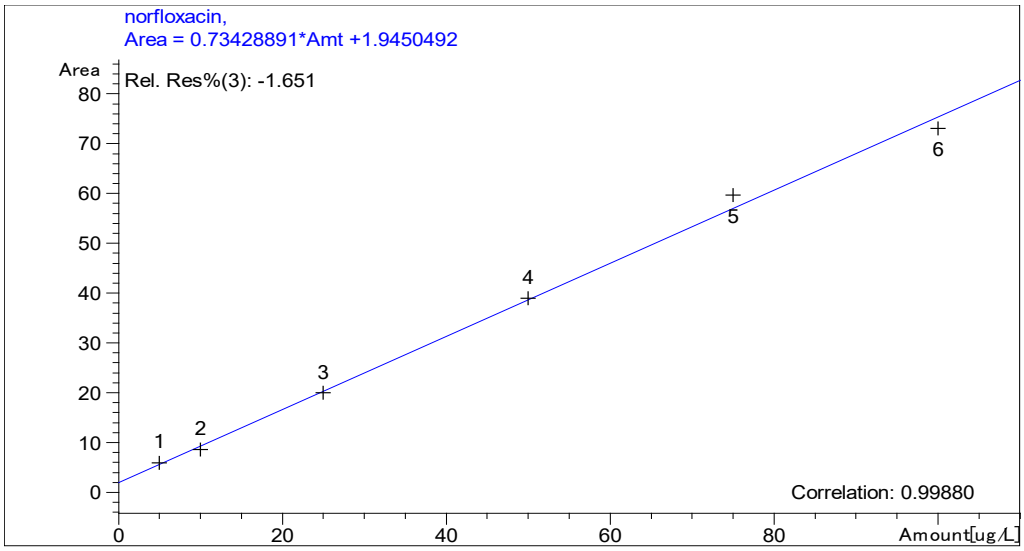


Figure 26. Norfloxacin calibration curve

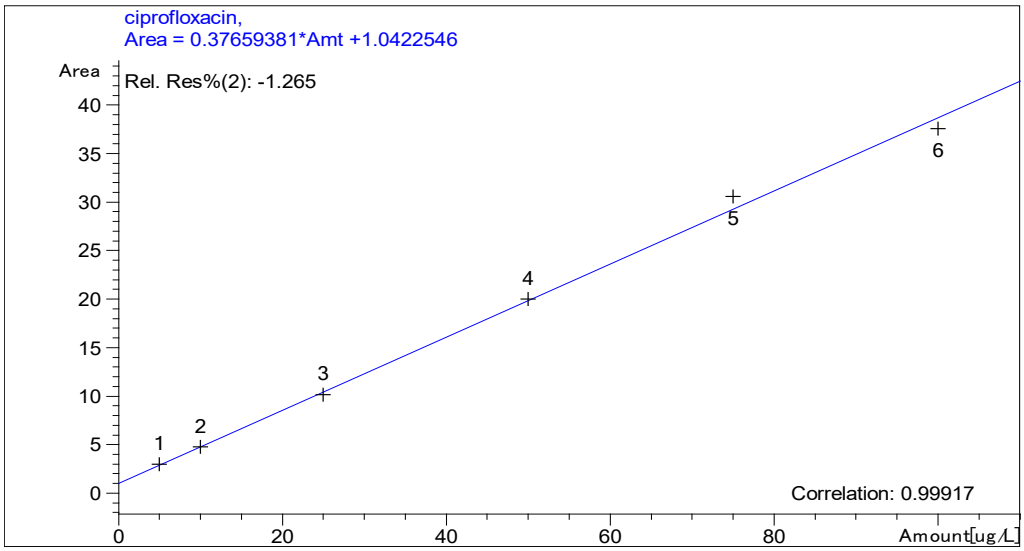


Figure 27. Calibration curve for Ciprofloxacin

The parameters obtained after drawing the calibration curves (working range, regression equation, correlation coefficient) are presented in Table 19.

Table 19. Linear regression parameters obtained for the concentration range tested for linearity

Analytes	Concentration range	The regression equation	R^2
Norfloxacin	5–100 µg/L	y = 0.73x + 1.94	0,9988
Ciprofloxacin	5–100 µg/L	y = 0.37x + 1.04	0.9991

From the analysis of the data presented in the table and the charts of the drawn calibration curves, the following aspects emerge:

- the equation of the linear regression function corresponding to the calibration curve for each analyte, has a linear dependence of the area values of the chromatographic peaks on its concentration;

- the concentration range in which the response of the detector is proportional to the concentration of the compounds is between 5-100 μg/L for the 2 analytes, with values of the correlation coefficients (R^2) higher than 0.99.

Selectivity/Specificity

The selectivity (specificity) of the method was evaluated by injecting a standard with the 2 analytes, a wastewater sample containing no analytes of interest and an ultrapure water sample (blank). Considering that no interfering peaks appeared at the wavelengths of interest at the retention times corresponding to the 2 analytes, it can be considered that the method is selective.

Precision

The precision of an analytical method expresses the agreement or degree of agreement between a series of determinations obtained from several samples from the same homogeneous sample under conditions of specificity. It is expressed as a standard deviation (s) or as a percentage relative standard deviation (RSD%).

Precision can be evaluated at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the analytical variability under the same working conditions over a short period of time. It is obtained when the test is carried out in a single laboratory, by a single operator, using a single type of equipment and the same method over a short period of time.

Intermediate precision represents the long-term variability of the measurement process when identical samples are analyzed by the same method, the same laboratory, by different operators, over a longer period of time and is determined by comparing the results of a method for a single laboratory for a certain number of days.

Assay repeatability was assessed by repeating the sample preparation procedure and analysis on 6 subsamples from the same sample. Intermediate precision was assessed by applying the sample preparation procedure to a single sample divided into 12 subsamples, but prepared on different days by multiple analysts.

The accuracy of the entire analytical procedure, expressed as percentage relative standard deviation (RSD), was determined by repeated analysis of surface water samples (table 26 and figure 28) and effluent samples of some treatment plants (table 20 and figure 29).

Table 20. Accuracy data obtained on repeated surface water samples

Analyte	Concentration (μg/L)	Repeatability (RSD %)	Reproducibility (RSD %)
Norfloxacin	25	4.17	6.87
Ciprofloxacin	25	3.14	7.03

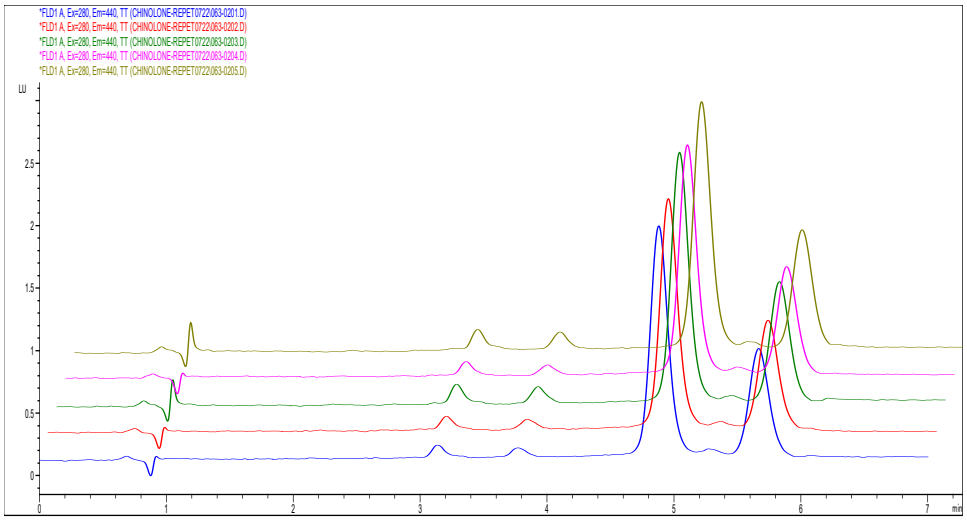


Figure 28. Repeatability obtained for surface water samples at the concentration of 25 µg/L

Table 21. Accuracy data obtained on repeated wastewater samples

Analyte	Concentration (µg/L)	Repeatability (RSD %)	Reproducibility (RSD %)
Norfloxacin	75	7.78	11.20
Ciprofloxacin	75	6.71	13.20

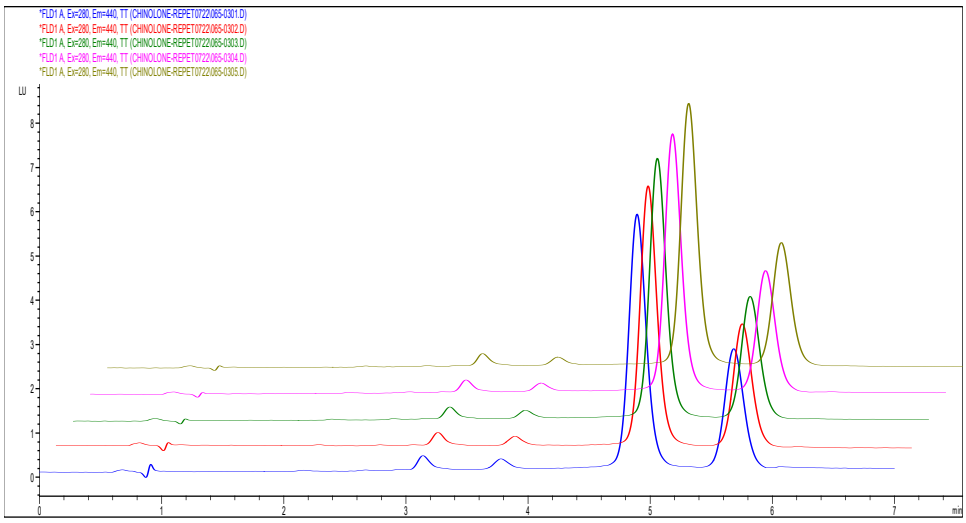


Figure 29. Repeatability obtained for wastewater samples at the concentration of 75 µg/L

Accuracy and recovery yield

The recovery yield for the 2 analytes was calculated following the application of the extraction and concentration procedure of the ultrapure water samples devoid of the analytes of interest in order to establish the optimal recovery method following the recovery yields of these compounds and the elimination of potential interferences. Dionex SPE AutoTrace 280 Extractor (Thermo Scientific), Strata XAW Anion Polymeric Cartridges 33 µm and size 500mg/6ml from

Phenomenex, and Strata X 33 µm Reverse Phase Polymeric Cartridges were used for the extraction and concentration of analytes of interest from aqueous matrices of 500mg/6ml from Phenomenex.

The calculation of the recovery yield was performed, which refers to the ratio between the experimentally determined concentration using interpolation on the calibration curve of each analyte and the theoretical concentration that was added to the aqueous matrix. The data obtained for the recovery yield are presented in table 28**.**

Table 22. Calculated recovery yields using Strata X Polymeric Cartridges Reverse Phase and Strata X-AW Cartridges

Analyte	Strata X Polymer cartridges Reverse phase		Recovery yield (%)	Strata XAW cartridges		Recovery yield (%)
	Added conc. (µg/L)	Obtained conc. (µg/L)		Added conc. (µg/L)	Obtained conc. (µg/L)	
Norfloxacin	50	25.88	51.7	50	48.59	87.2
Ciprofloxacin	50	23.75	47.5	50	47.14	90.3

For analytes of interest tested using Strata X Reverse Phase Polymer Cartridges recovery yields of 51.7% for norfloxacin and 47.5% for ciprofloxacin were obtained compared to the recovery values obtained on controlled ultrapure water samples contaminated with the 2 analytes which were 87.2% for norfloxacin and 90.3% for ciprofloxacin using Cartridges XAW Polymeric Anion Exchange Layer. Due to the almost double recovery yield values obtained using Strata XAW Anion Cartridges, the latter were chosen for further experiments on the analytical performance of the developed method and for the calculation of environmental samples in the present study.

The accuracy of the method was demonstrated, with recovery yields of 87.2% for norfloxacin and 90.3% for ciprofloxacin in Table 22 and Figure 30.

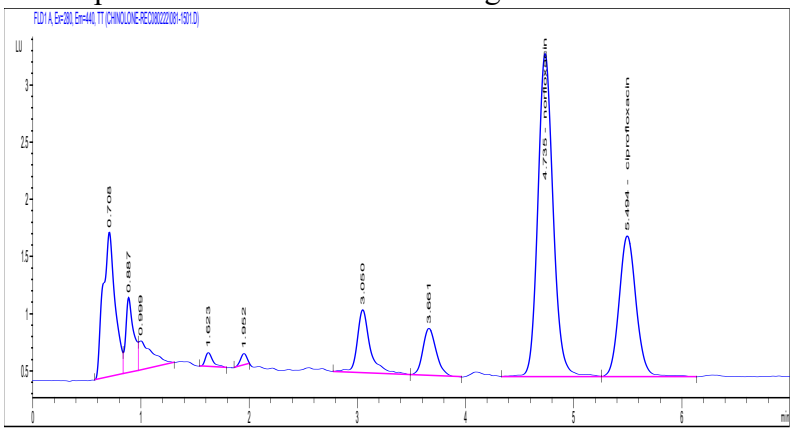


Figure 30. Elution of the 2 pharmaceutical compounds after using the cartridges Strata XAW Polymeric Anion Cartridges

Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) were determined by injecting solutions with increasingly lower concentrations of analytes until the experimentally determined signal-to-noise ratio was equal to 3 (LOD) and 10 (LOQ), respectively). The values of the limits of detection and quantification thus determined are shown in Table 23.

Table 23. Limits of detection and quantification of the studied analytes

Analyte	LOD Wastewater (ng/L)	LOD Surface water (ng/L)	LOD Wastewater (ng/L)	LOD Surface water (ng/L)
Norfloxacin	1.4	0.73	4.3	2.2
Ciprofloxacin	1.9	0.93	5.6	2.8

4.3.3. Application of the developed method for the determination of fluoroquinolones (norfloxacin and ciprofloxacin) in wastewater samples

Surface water samples and wastewater samples were analyzed for the determination of norfloxacin and ciprofloxacin by the previously validated HPLC-FLD method. Waste water and surface water samples were taken in 1000 ml brown glass containers filled so that the amount of air present in the container was as small as possible. They were transported in refrigerated boxes and analyzed within 24 hours. 500 ml of surface water and 250 ml of waste water were analyzed from the samples taken. The wastewater samples were prefiltered through 0.45 μm pore size membranes to remove solids, and all samples were pH adjusted to 2 with 6N HCl. To chelate/block metal ions Ca, Mg that can interact with antibiotic compounds, 0.5g Na_2EDTA was added to each sample. Strata XAW Polymeric Anion Exchange cartridge (6ml/500mg, Phenomenex) was conditioned with 10ml MeOH, then 10ml pH=2 ultrapure water (adjusted with HCl) was added. The cartridge was washed with 10 ml of ultrapure water at pH 2 to eliminate the effect of the sample matrix (humic acids). To dry the adsorbent phase, air was passed through the cartridge for 15 minutes. The elution of the analytes from the cartridge was carried out with 10 ml of methanol, after which the obtained extract was evaporated to dryness with nitrogen in a water bath at 45°C. The obtained residue was resuspended with 1 ml mobile phase (12% acetonitrile and 88% H_3PO_4 25mM, pH=3). The extract was injected into the HPLC-FLD system using the developed and validated method.

Using the validated HPLC method, the concentrations of the 2 analytes of interest were determined from the 10 wastewater samples and 10 surface water samples shown in tables 24 and 25.

Table 24. Concentrations determined in wastewater samples

Samples	NOR conc. (ng/L)	CIP conc. (ng/L)
P1	62.2	131.4
P2	54.3	142.1

P3	67.5	56.7
P4	73.2	203.8
P5	71.8	65.5
P6	90.5	284.5
P7	78.1	130.4
P8	98.9	109.2
P9	87.8	289.8
P10	62.4	31.8

Table 25. Concentrations determined in surface water samples

Samples	NOR conc. (ng/L)	CIP conc. (ng/L)
P11	<LOQ	<LOQ
P12	7.6	<LOQ
P13	<LOQ	<LOQ
P14	<LOQ	<LOQ
P15	<LOQ	6.8
P16	<LOQ	7.5
P17	<LOQ	<LOQ
P18	<LOQ	<LOQ
P19	<LOQ	2.9
P20	<LOQ	<LOQ

CONCLUSIONS

In chapter 4, an HPLC-DAD method was developed and validated for the determination of acetaminophen (ACF), ketoprofen (KTF), diclofenac (DCF) and ibuprofen (IBU) from wastewater samples by the HPLC method and having a separation time of only ten minutes.

All liquid-chromatographic conditions (nature and composition of the mobile phase, injection volume, detection wavelength, column temperature, etc.) were optimized for the rapid separation of the four analytes with high sensitivity in order to determine these anti-inflammatories at levels of concentrations of the order of "µg/L" from complex wastewater matrices.

The average recovery yield of the HPLC-DAD method was 95.99% for ACF, 83.92% for KTF, 81.45% for DCF, and 90.22 for IBU. Thus, it can be seen that the accuracy of the method was within a maximum deviation of 10%.

Regarding the precision of the direct injection method, RSD values between (0.11% - 0.29%) for repeatability and between (0.23% - 0.40%) for intermediate precision were obtained. The limits of quantification of the method were 0.20 µg/L for acetaminophen, 0.46 µg/L for ketoprofen, 0.60 µg/L for diclofenac, and 0.14 µg/L for ibuprofen, respectively. The expanded uncertainty of the analysis method is 12%.

The developed method was successfully applied for the determination of anti-inflammatories in 6 wastewater samples. From the chromatographic analysis of the wastewater samples taken from the entrance to the treatment plants of the towns, the following presence of analytes of interest is observed: acetaminophen > ibuprofen > ketoprofen > diclofenac.

The performance parameters of the method for the determination of acetaminophen using a UV-VIS method were determined for 2 different media, namely: 0.1 M HCl and 0.01 M KOH.

The LOD and LOQ in the case of 0.1M HCL were: 0.04 and 0.12 respectively, and in the case of the 0.01M KOH dissolution medium, the detection limit was 0.06 and the quantification limit was 0.18.

Also within this chapter, an HPLC-DAD method was developed and validated for the determination of norfloxacin and ciprofloxacin from wastewater and surface water samples by an HPLC-FLD method with a separation time of only 7 minutes.

All liquid-chromatographic conditions (nature and composition of the mobile phase, injection volume, detection wavelength, column temperature, etc.) were optimized for the rapid separation of the 2 analytes with high sensitivity in order to determine these anti-inflammatories at levels of concentrations on the order of parts per billion (μg/L) in complex wastewater and surface water matrices. The recovery yields of the HPLC-FLD method were 87.2% for norfloxacin and 90.3% for ciprofloxacin.

Regarding the precision of the developed and validated HPLC method, RSD values of 4.17% for norfloxacin and 3.14% for ciprofloxacin were obtained for repeatability and RSD of 6.87% for norfloxacin and 7.03 % for ciprofloxacin, intermediate precision performed on repeated surface water samples. The wastewater precision data obtained for norfloxacin were: RSD % repeatability 7.78 and RSD% intermediate precision 11.20, and in the case of ciprofloxacin were: RSD % repeatability 6.71 and RSD% intermediate precision, 13.20%.

The developed method was successfully applied for the determination of fluoroquinolones in 10 wastewater samples and 10 surface water samples (tables 30 and 31).

CHAPTER 5. Experimental research on the treatment of waters containing pharmaceutical pollutants based on adsorption on 4 types of adsorbent materials (zeolites, activated carbon, carbon nanotubes, magnetite)

5.1.1. Studiul de adsorbție a celor patru AINS (acetaminofenului , ketoprofenului , diclofenac și ibuprofen) pe materiale adsorbante de tipul zeoliților

Zeolites purchased from Sigma Aldrich were used as adsorbent materials in this study. The zeolites showed a specific surface area of 500m2/g, total pore volume 11.5 cm2/g and average micropore radius 750 Å.

Adsorption studies were carried out in Erlenmeyer flasks (100 ml) using a speed controlled stirrer (stirring speed of 125 rpm) for 2 hours. The amounts of adsorbent material (zeolite) were 100 mg and 200 mg, and the sample volume was 50 ml of synthetic solution of acetaminophen, ketoprofen, ibuprofen and diclofenac, of different concentrations. Samples were collected and filtered at time To and after 2 hours from the beginning of the experiments. All adsorption studies

were performed at room temperature of 20±20C. Adsorption studies were carried out on synthetic solutions with known concentrations (1mg/L, 5 mg/L and 10 mg/L).

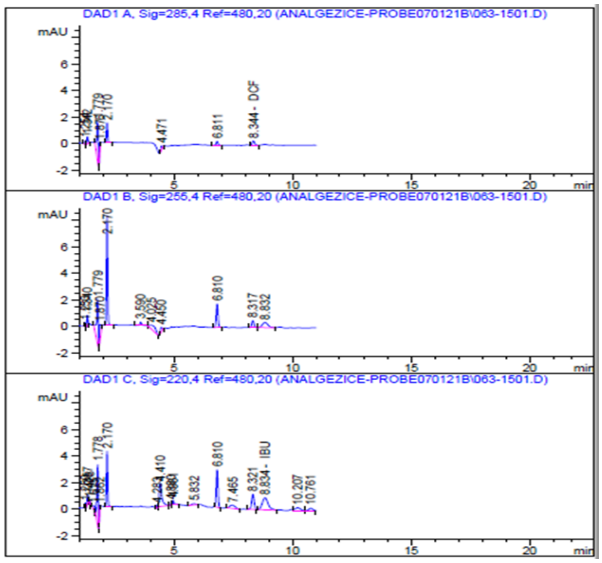


Figure 31. Chromatograms of diclofenac, ketoprofen and ibuprofen at the concentration of 1 mg/L at pH 4

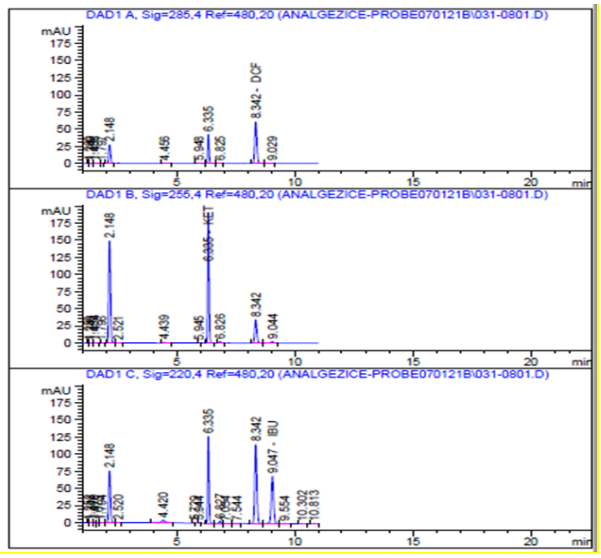


Figure 32. Chromatograms of diclofenac, ketoprofen and ibuprofen at the concentration of 10 mg/L and at pH 4

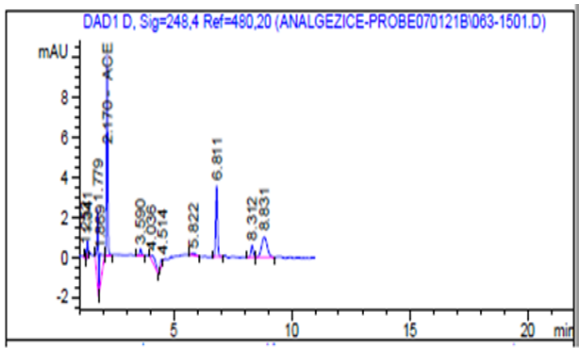


Figure 33. Chromatogram of acetaminophen at a concentration of 1mg/L at pH 4

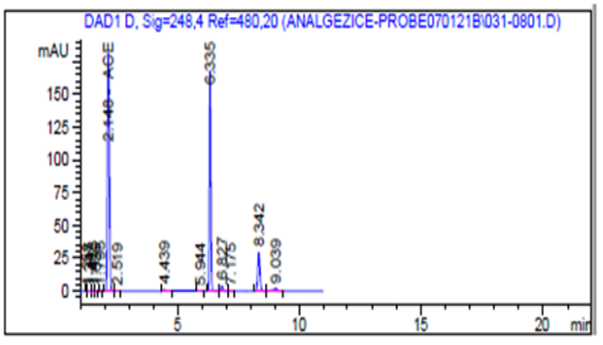


Figure 34. Chromatogram of acetaminophen at the concentration of 10 mg/L

As can be seen from Figures 31 and 33, the separation and quantification of the four compounds was also achieved at a very low concentration of only 1 mg/L.

At the concentration of 10 mg/L studied analyte, 200 mg zeolite was used.

As can be seen from figures 32 and 34 at higher concentrations (10mg/l) the four studied analytes can be quantified better (the peaks are sharper and the separation is better).

The parameters that were followed in the adsorption study:

a) the concentration of the analytes of interest (1, 5, 10 mg/L)

b) pH of the samples (2,4, 6, 8 unit.pH)

c) the amount of adsorbent material

d) contact time

The adsorption capacity of the adsorbent materials was calculated with the formula:

$$C\ ads = \frac{(Ci - Ce) * V}{m} \qquad (4)$$

Where:

C ads- the adsorption capacity of the analyte on the adsorbent material (mg/g);

V - the volume of the analyte solution used (L);

Ci - initial analyte concentration (mg/L);

Ce - concentration at equilibrium (mg/L);

m - the amount of adsorbent material used (mg).

Adsorption of acetaminophen, diclofenac, ketoprofen and ibuprofen was carried out on zeolite-type adsorbent materials using two different amounts of material: 100mg zeolite and 200mg zeolite.

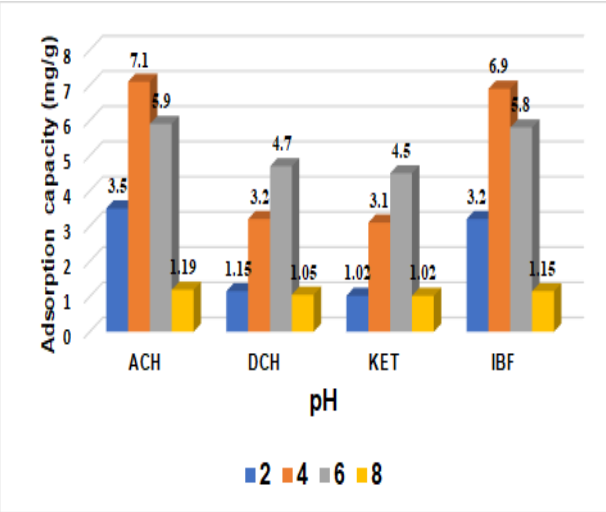


Figure 35. Adsorption capacity for the four studied compounds, using 100mg zeolite at the 4 different pH values

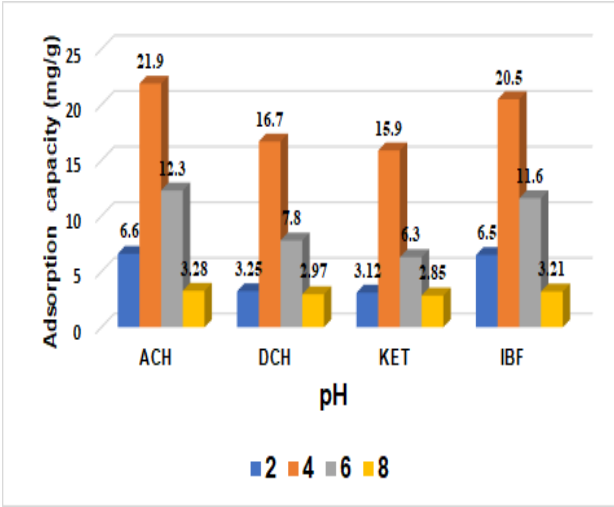


Figure 36. Adsorption capacity for the four studied compounds using 200mg zeolite at the 4 different pH values

The amount of adsorbent material (zeolite) significantly influenced the adsorption process of the four compounds as can be seen from Figures 35 and 36. The highest adsorption capacity for all tested compounds was obtained at pH 4.

The order of increase in adsorption capacity at a value of 4 unit. The pH is as follows: acetaminophen (7.1mg/g)>ibuprofen(6.9mg/g)>diclofenac(4.7mg/g)>ketoprofen (4.5mg/g) for a quantity of zeolite of 100mg, stirring time 2 hours and with a stirring speed on the Shaker of 125 rpm.

Increasing the amount of adsorbent material from 100 mg to 200 mg led to an increase in the adsorption capacity for all four studied compounds as follows: acetaminophen (21.9mg/g)>ibuprofen(20.5mg/g)>diclofenac>ketoprofen (15.9mg/g) (figure 37).

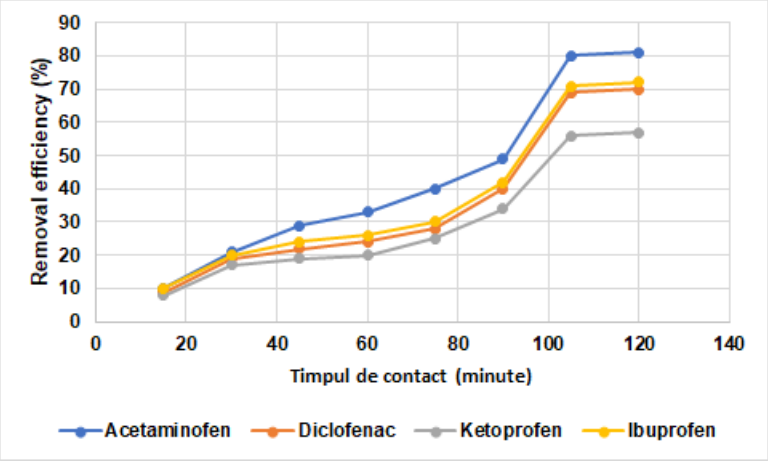


Figure 37. Pollutant removal efficiency as a function of contact time in the presence of 200mg zeolite

Figure 37 shows the removal efficiency of acetaminophen, diclofenac, ketoprofen and ibuprofen as a function of contact time (120 minutes). The removal efficiency of pollutants by the adsorbent material is as follows: (81%) acetaminophen, (72%) ibuprofen, (70%) diclofenac and (57%) ketoprofen, for a concentration of 1mg/L of pollutant. The maximum saturation point of the zeolite was reached after about 100 minutes.

Adsorption isotherms

The linear profile of the Langmuir isotherm was obtained by plotting the ratio Ce/Qe versus Ce in figure 38.

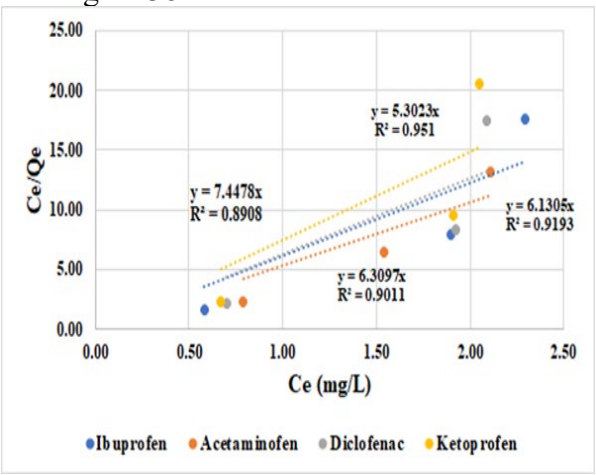


Figure 38. The linearized form of the Langmuir isotherm

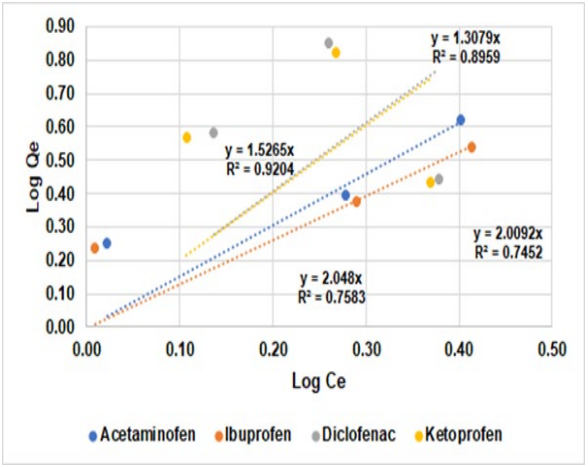


Figure 39. The linearized form of the Freundlich isotherm

The linear profile of the Freundlich isotherm was obtained by plotting of the logarithm of logQe versus logCe in figure 39.

Comparing the correlation coefficients (R^2) shows that the Langmuir isotherm (R^2 = 0.8908 to 0.9510) is more suitable to describe the adsorption process compared to the Freundlich isotherm (from R^2 = 0.7452 to 0, 9204) for the adsorption of the four compounds on zeolites (ZSM-5).

Table 26. Langmuir and Freundlich parameters

Compounds	Langmuir parameters			Freundlich parameters		
	Q_{max}(mg/g)	K_L (L/mg)	R^2	K_F (L/g)	1/n	R^2
Acetaminophen	0,12	0,63	0,9510	0,91	0,21	0,9204
Ibuprofen	0,10	0,67	0,9193	0,70	0,22	0,8958
Diclofenac	0,09	0,77	0,9011	0,57	0,77	0,7583
Ketoprofen	0,07	0,78	0,8908	0,33	0,69	0,7452

Table 27. Equilibrium constant RL for the Langmuir isotherm

Initial concentration (mg/l)	Ibuprofen	Acetaminophen	Diclofenac	Ketoprofen
1	0,5984	0,6151	0,5637	0,5618
5	0,2296	0,2422	0,2053	0,2041
10	0,1297	0,1378	0,1144	0,1136

The Langmuir constant RL is in the range 0–1, indicating that the adsorption of the compounds is favorable as can be seen in Table 27. The adsorption of the four compounds on ZSM-5 is favorable for values of RL (Langmuir constant) of $0.1 < 1/n < 1.0$.

Based on the results obtained in table 26 and figures 38 and 39, it is evident that the Langmuir model (correlation coefficient, from R2 = 0.8908 to R2 = 0.9510) better describes the adsorption processes for the compounds studied on ZSM- 5 compared to the Freundlich model.

The equilibrium concentration was obtained after 120 minutes at pH=6 for all studied compounds. When the concentration of the studied compounds increased from 1mg/L to 10 mg/L, the removal efficiency of the analytes decreased.

The removal efficiency of ZSM-5 was as follows: (81%) acetaminophen, (72%) ibuprofen, (70%) diclofenac and (57%) ketoprofen. The Langmuir isotherm characterizes the experimental data very well.

5.1.2. Quantification of degradation products of anti-inflammatories (acetaminophen, ketoprofen, diclofenac and ibuprofen) present in wastewater using the TOC method

The degradation products of the four analytes of interest and the active substances are found in the wastewater samples as total organic carbon. With the help of the TOC method, it was possible to quantify the total organic carbon (mg C/L) present in the wastewater and synthetic water samples.

To demonstrate the selectivity and accuracy of the analytical method, the TOC value was determined on certified reference materials (CRMs) with known drug concentrations.

The TOC analysis was performed with a total organic carbon analyzer, TOC/TN-LCPN (Shimadzu) controlled by the "TOC-Control V Software" program. The method consisted in the combustion of the samples at 7200 C in an oxygen-rich environment and in the presence of a platinum catalyst. Carbon dioxide generated in the oxidation reaction in oxygen medium was recorded using an infrared gas analyzer. Four certified reference materials (CRMs) with known concentrations of acetaminophen, ketoprofen, ibuprofen and diclofenac were used to verify the TOC method.

Table 34 shows the TOC values obtained for the MRC of acetaminophen present in water, at four studied pHs and after 2h of contact between the zeolite and the synthetic wastewater solution. By applying the uncertainty of the analysis method, good results were obtained at pH values of 4 and 6 unit.pH.

The samples collected at 2h for the quantification of the compounds of interest by the HPLC method, were also introduced on the TOC analyzer for the determination of total organic carbon.

The degradation products of the four analytes of interest were studied at four different concentrations (1, 5, 10, 20 mg/L) and at four pH (2, 4, 6, 8 unit.pH). In the figures below, the graphs of the four compounds at concentrations of 1, 5, 10, 20 mg/L are representative.

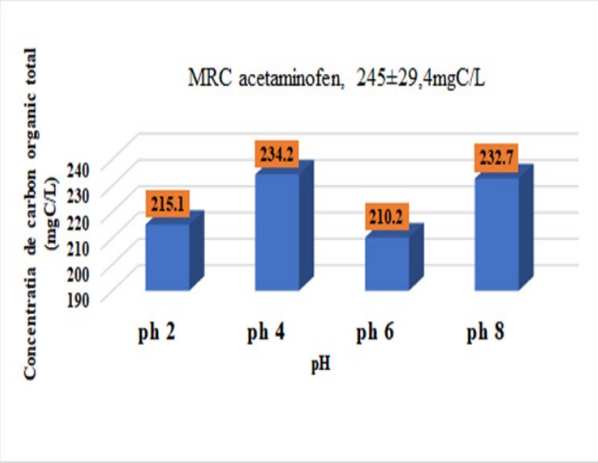


Figure 40. Total organic carbon concentration obtained from the 1mg/L acetaminophen sample and with a certified MRC

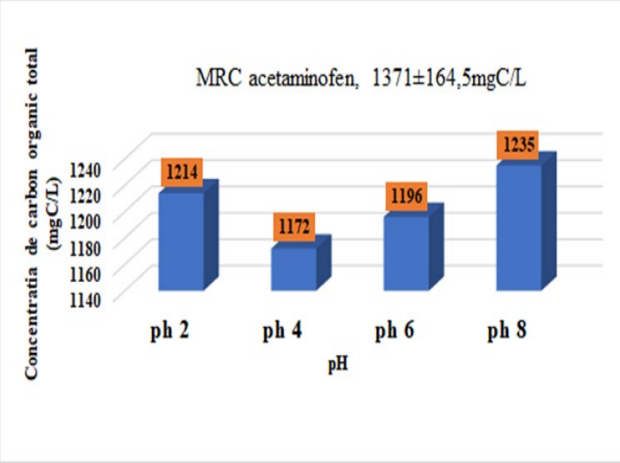


Figure 41. Total organic carbon concentration obtained from the 5mg/L acetaminophen sample and with a certified MRC

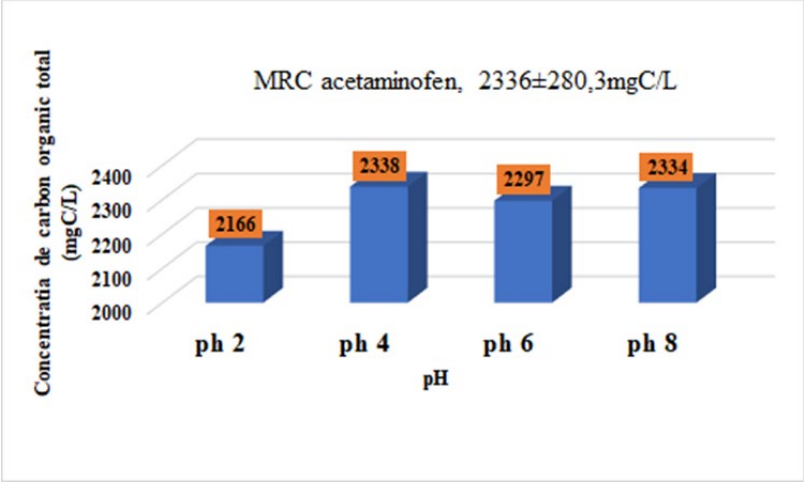


Figure 42. Total organic carbon concentration obtained from the acetaminophen sample of 10mg/L and with a certified MRC

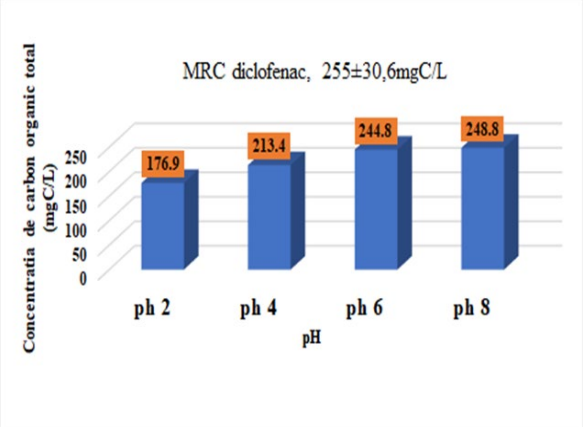


Figure 43. Total organic carbon concentration obtained from the 1 mg/L diclofenac sample and with a certified MRC

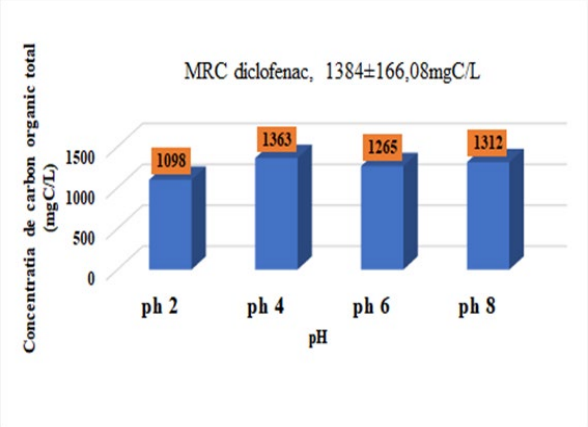


Figure 44. Total organic carbon concentration obtained from the 5 mg/L diclofenac sample and with a certified MRC

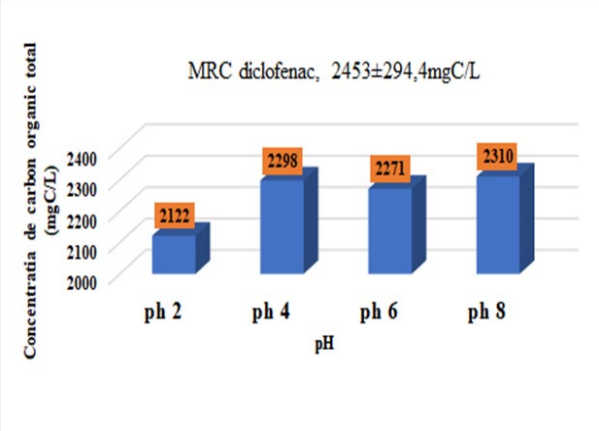


Figure 45. Total organic carbon concentration obtained from the 10 mg/L diclofenac sample and with a certified MRC

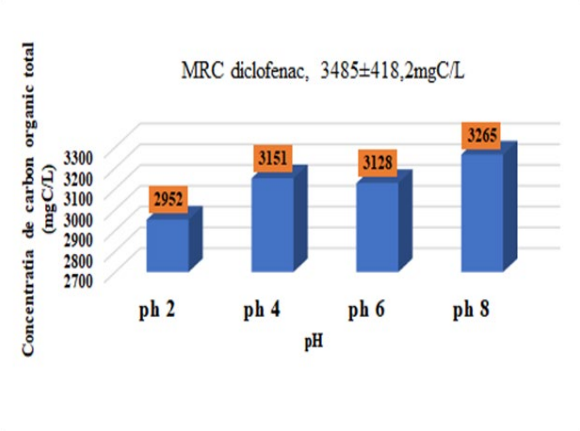


Figure 46. Total organic carbon concentration obtained from the 20 mg/L diclofenac sample and with a certified MRC

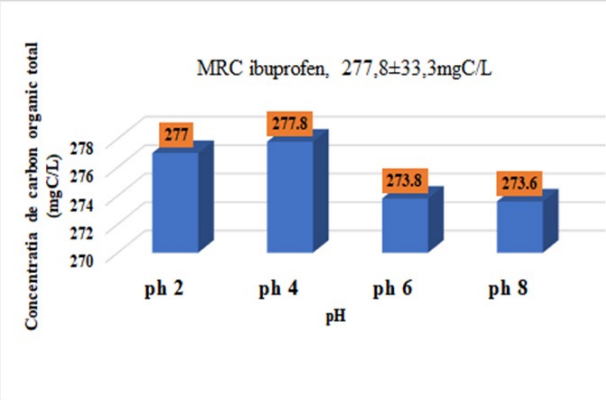


Figure 47. Total organic carbon concentration obtained from the 1 mg/L ibuprofen sample and with a certified MRC

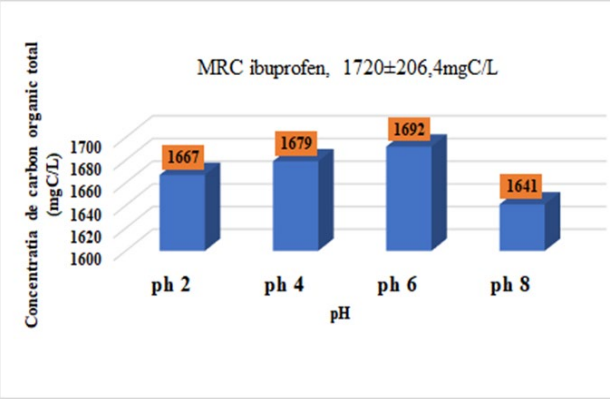


Figure 47. Total organic carbon concentration obtained from the 5 mg/L ibuprofen sample and with a certified MRC

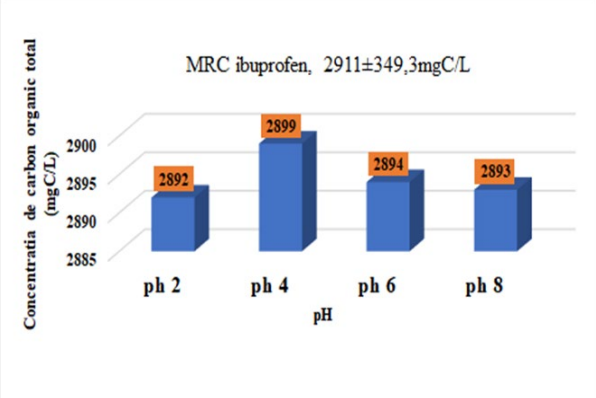


Figure 48. Total organic carbon concentration obtained from the 10 mg/L ibuprofen sample and with a certified MRC

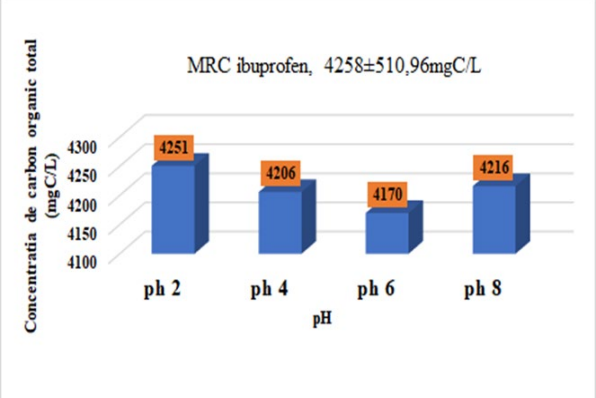


Figure 49. Total organic carbon concentration obtained from the 20 mg/L ibuprofen sample and with a certified MRC

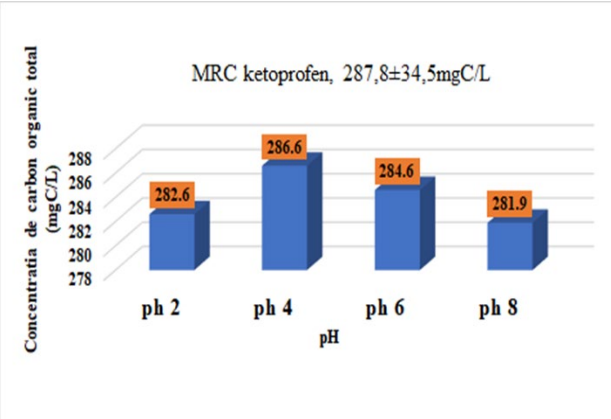


Figure 50. Total organic carbon concentration obtained from the 1 mg/L ketoprofen sample and with a certified MRC

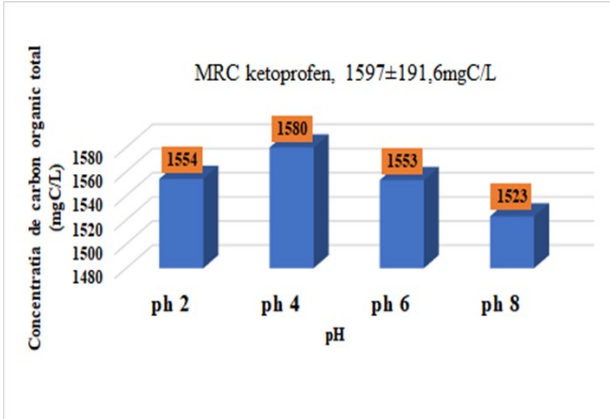


Figure 51. Total organic carbon concentration obtained from the 5 mg/L ketoprofen sample and with a certified MRC

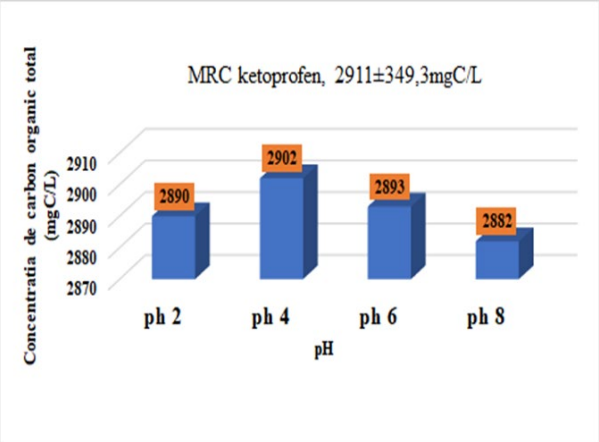


Figure 52. Total organic carbon concentration obtained from the 10 mg/L ketoprofen sample and with a certified MRC

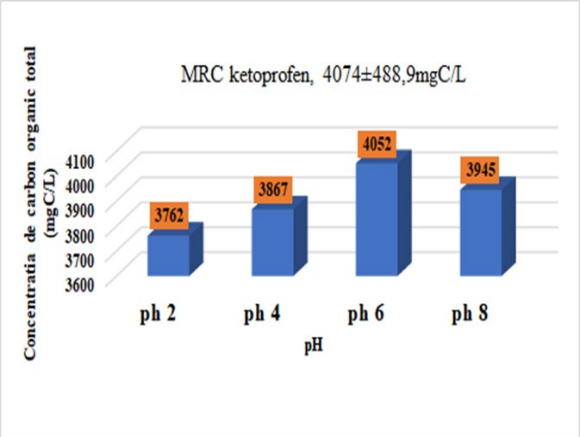


Figure 53. Total organic carbon concentration obtained from the 20 mg/L ketoprofen sample and with a certified MRC

No significant variations were observed in the determination of total organic carbon (TOC) from synthetic water samples prepared from MRCs with certified values. The results obtained were within the uncertainty of the analysis method (TOC=12%) at pH values of 4 and 6 unit. pH (figures 40-53).

To ensure the validity of the obtained results, they were compared with a reference material with certified values, for each studied compound. The obtained results fell within the uncertainty of the analysis method of 12%.

5.1.3. CONCLUSIONS

In subsections 5.1.1. and 5.1.2. the adsorption capacity of the four compounds (acetaminophen, ketoprofen, diclofenac and ibuprofen) on zeolite-type adsorbent materials was studied.

The highest adsorption capacity (mg/g) was recorded for: ibuprofen (21.9 mg/g)>acetaminophen(20.5 mg/g)>diclofenac(16.7 mg/g)>ketoprofen(15, 9 mg/g), when a quantity of 200 mg of zeolite was used.

The removal of the four compounds from synthetic wastewater was achieved by their adsorption on adsorbent materials such as zeolites.

The average recovery yield of the HPLC-DAD method was 95.99% for acetaminophen, 83.92% for ketoprofen, 81.45% for diclofenac, and 90.22% for ibuprofen.

In addition to the analytes of interest, the degradation products of the four anti-inflammatories were also quantified in the form of total organic carbon (TOC). Total organic carbon values varied depending on the compound that was tested and its working concentration (1, 5, 10 and 20 mg/L).

All results obtained for TOC, on certified reference materials (CRMs) were within the uncertainty of the analytical method (uncertainty=10%), at a pH=4 and a concentration of 10 mg/L of the CRM .

The pH value of 4 represents the equivalence point, at which drugs are completely dissolved. The impurities of acetaminophen, ibuprofen, diclofenac and ketoprofen were quantified as total organic carbon (TOC). The two HPLC and TOC methods for quantifying the studied analytes and their impurities are very useful for the treatment of these anti-inflammatories from the waste water matrix.

5.2.1. Adsorption study of the four pharmaceutical compounds (acetaminophen, ketoprofen, diclofenac and ibuprofen) on activated carbon

Compounds from the class of non-steroidal anti-inflammatory drugs (acetaminophen, diclofenac, ibuprofen and ketoprofen) are the compounds most present in wastewater. At the national level, these compounds are used most frequently to treat anti-inflammatory conditions.

This chapter aims to investigate the effect of different experimental parameters on the performance of a "batch" adsorption system for the retention of four pharmaceutical compounds from aqueous solutions on commercial activated carbon. The analysis of the experimental data was carried out by: modeling the adsorption process at equilibrium, using isotherms with two and three parameters and by kinetic modeling, according to the Lagergen, Ho and Morris-Weber models.

Experimental methodology

Adsorption experiments were performed in 100 mL Erlenmeyer flasks with lids. The stock solution was prepared by dissolving acetaminophen, diclofenac, ibuprofen and ketoprofen standards in ultrapure water. Volumes of 50 mL of different concentrations (1-10mg/L) were put in contact with 0.1g - 1g of activated carbon and subjected to agitation on the horizontal shaker at 150 revolutions and at 200 revolutions, for 120 minutes.

To establish the influence of temperature on the adsorption process, tests were performed at two different temperatures (200C and 250C). In order to establish the optimal amount of adsorbent, the mass of activated carbon contacted with the solute of different concentrations in the first phase of the experiments varied between 0.1g and 1g of activated carbon.

For the kinetic studies, the samples were stirred at different time intervals between 5 minutes and 120 minutes, in the horizontal shaker at a speed of 150 rpm and 200 rpm, respectively. After each experiment, the supernatant was filtered and subjected to HPLC analysis at different wavelengths corresponding to the analytes of interest (acetaminophen at 248 nm, diclofenac at 280 nm, ketoprofen at 255 nm and ibuprofen at 220 nm).

And the removal efficiency of the analytes of interest (removal efficiency) was calculated with the following formula:

$$\eta(\%) = \frac{C_i - C_f}{C_i} * 10\mathbf{0} \qquad (5)$$

where: ci,cf – initial concentration and final concentration of the analytes of interest (mg/L).

The parameters that were followed in the adsorption study:

a) the concentration of the analytes of interest (1, 5, 10mg/L)

b) The pH of the samples: 4 and 6 unit.pH

c) amount of adsorbent material: 0.1g, 0.5g and 1g

d) stirring speed: 150rpm and 250rpm

e) contact time between the adsorbent material and the analytes of interest (To up to 120 minutes)

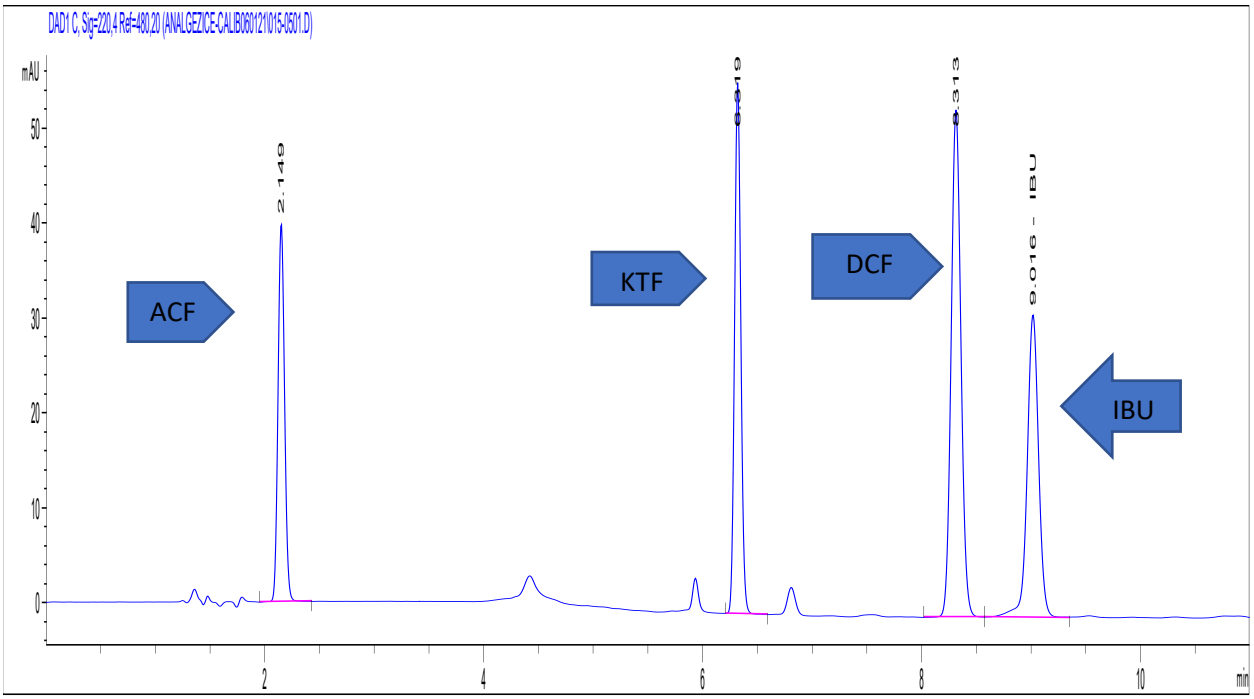


Figure 54. Chromatogram obtained from the analysis of a mixed solution of ACF, KTF, DCF and IBF at the 4 wavelengths corresponding to the adsorption maximum

The order of separation of compounds on the C18 column is as follows: acetaminophen (ACF) > ketoprofen (KTF) > diclofenc (DCF) > ibuprofen (IBU) as shown in figure 34.

Adsorption studies

Adsorption experiments were carried out in 100 mL screw-capped Erlenmeyer flasks. Stock solutions were prepared in methanol and subsequent dilutions were performed using a real wastewater sample.

Volumes of 50 ml of different concentrations (1, 5 and 10 mg/L) of each studied drug were contacted with 0.1, 0.5 and 1g of activated carbon and agitated on a horizontal shaker at 250 rpm.

After each experiment, the supernatant was centrifuged and subjected to HPLC analysis and the chromatogram of the solution was recorded at the wavelengths corresponding to the

analytes of interest (acetaminophen at 248 nm, diclofenac at 280 nm, ketoprofen at 255 nm, and ibuprofen at 220 nm). The mathematical models applied to characterize the adsorption process were Langmuir and Freundlich.

5.2.2. Influence of pH on the removal efficiency of NSAIDs (acetaminophen, ketoprofen, diclofenac and ibuprofen) **on activated carbon**

The experimental parameters that were optimized were: the pH (4 and 6), the contact time (0-120 minutes), the initial concentration of the pollutant (1, 5 and 10mg/L), the amount of adsorbent material (0.1g, 0.5g and 1g) and stirring speed (150 rpm and 250 rpm).

The removal efficiency is directly influenced by the amount of adsorbent material. As the amount of adsorbent material increases, the removal efficiency of the studied analytes from the synthetic wastewater also increases.

5.2.3. Influence of contact time on NSAID removal efficiency on activated carbon

To determine the stirring time required to reach the equilibrium between the species pollutants and activated carbon, the experiments were carried out at a temperature of 20±2°C, in a time interval between 0 and 120 minutes, using 1g activated carbon and 1mg/L of acetaminophen, diclofenac, ketoprofen and ibuprofen. Two different stirring speeds were used at 150 rpm and 250 rpm. The most representative values were obtained for pH=6.

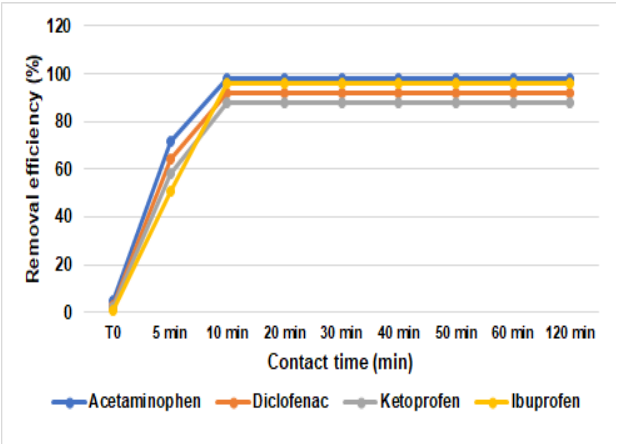


Figure 55. Influence of removal efficiency on contact time for a rotation speed of 250 rpm and 1 mg/L pollutant species

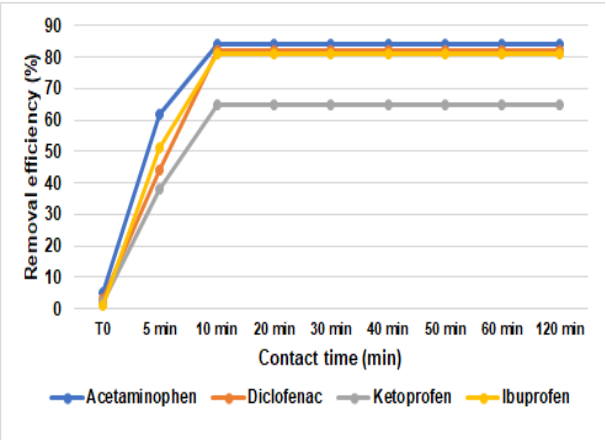


Figure 56. Influence of removal efficiency on contact time for a rotation speed of 150 rpm and 1mg/L pollutant species

The order of removal efficiency (adsorption on activated carbon) was as follows: acetminophen (98%)>diclofenac (92%)>ketoprofen (88%)>ibuprofen (96%), for a stirring speed of 250 rpm, at a pH value of 6 unit.pH (figure 55).

The order of removal efficiency (adsorption on activated carbon) was as follows: acetaminophen (84%)>diclofenac (78%)>ketoprofen (65%)>ibuprofen (81%), for a stirring speed of 150 rpm, at a of the pH of 6 unit.pH (figure 56).

As can be seen from figures 55 and 56, the highest removal efficiency (adsorption on activated carbon) was recorded for a stirring speed of 250 rpm, at a concentration of adsorbent

material of 1g, at an initial concentration of 1mg /L pollutant species, at a pH value of 6 and at a contact time of only 10 minutes.

5.2.4. Influence of the amount of adsorbent material on the removal efficiency of pollutants on activated carbon

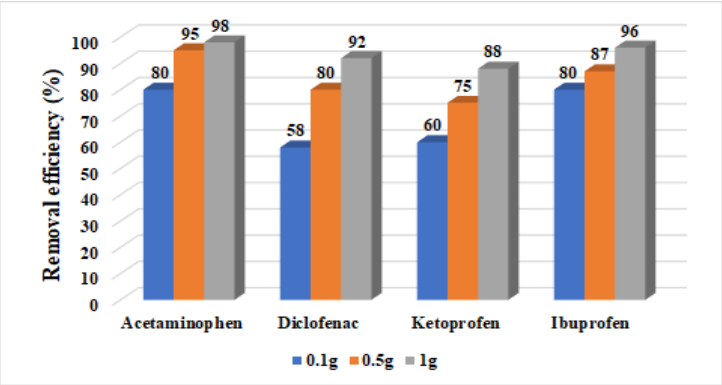


Figure 57. Influence of the amount of adsorbent material on the pollutant removal efficiency at a stirring speed of 250 rpm

The removal efficiency increased considerably when the amount of adsorbent material was increased from 0.1g to 1g of activated carbon. The highest removal efficiencies of 98% for acetaminophen and 92% for diclofenac were achieved at 1mg/L for each studied compound and 1g of adsorbent material. The highest removal efficiencies, 88% for ketoprofen and 96% for ibuprofen, were obtained using 1mg/L of ketoprofen and ibuprofen and 1g of activated carbon (Figure 57).

5.2.5. Evaluation of adsorption processes using mathematical models

Four analytes were studied: acetaminophen, diclofenac, ketoprofen and ibuprofen. The experimental results were processed using the mathematical models: Langmuir and Freundlich. The obtained results are described below.

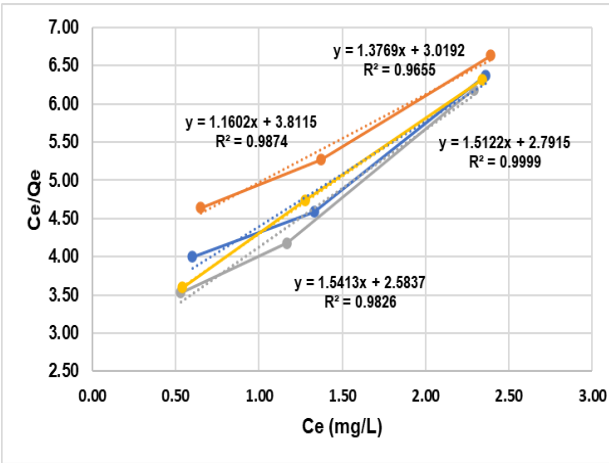


Figure 58. Langmuir isotherm

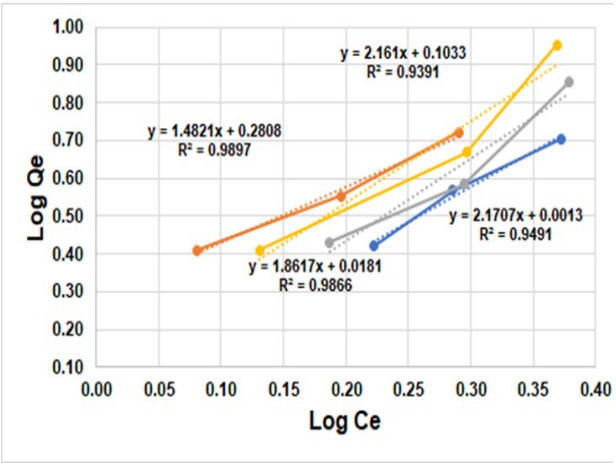


Figure 59. Freundlich isotherm

Since the Langmuir model showed correlation factors (R^2) higher than 0.9999, compared to the Freundlich model, it is claimed to be used for the characterization of the entire adsorption process on the adsorbent material of the activated carbon type (figure 58, figure 28 and table 29).

Table 28. Langmuir and Freundlich parameters for the four studied compounds

	Langmuir parameters			Freundlich parameters		
Analyte	**Q_{max} (mg/g)**	**K_L(L/mg)**	**R^2**	**K_F (L/g)**	**1/n**	**R^2**
Acetaminophen	6,986	1,013	0,9999	2,648	0,501	0,9897
Diclofenac	4,697	0,904	0,9826	1,575	0,349	0,9491
Ketoprofen	3,978	0,856	0,9655	1,324	0,326	0,9391
Ibuprofen	3,127	0,843	0,9874	1,297	0,232	0,9866

The essential feature of Langmuir isotherms can be expressed in terms of a dimensionless separation factor or equilibrium constant (RL), which is described in the following equation:

$$R_L = \frac{1}{(1 + K_L * C_0)} \quad (6)$$

Where:

K_L = is the Langmuir constant

Co = initial concentration (mg/L)

If:

R_L>1, the value indicates that the process is unfavorable;

R_L=1, the value indicates that the process is linear

0<R_L<1, the value indicates that the process is favorable

R_L=0, the value indicates that the process is irreversible

Table 29. Equilibrium constant R_L for the Langmuir isotherm

Pharmaceutical compound	**Concentration 1 mg/L**	**Concentration 5 mg/L**	**Concentration 10 mg/L**
Acetaminophen	0,3569	0,1819	0,1011
Diclofenac	0,3120	0,1512	0,0814
Ketoprofen	0,3318	0,1796	0,0933
Ibuprofen	0,3432	0,1716	0,0997

As can be seen from table 36, the constant R_L is between 0<R_L<1, so we can say that the adsorption process is favorable.

Another important parameter that influenced the adsorption process was the equilibrium concentration. Each studied analyte corresponds to a certain equilibrium concentration, above this concentration nothing is retained on the surface of the adsorbent material. The adsorption process remains at a constant value, nothing is adsorbed or desorbed.

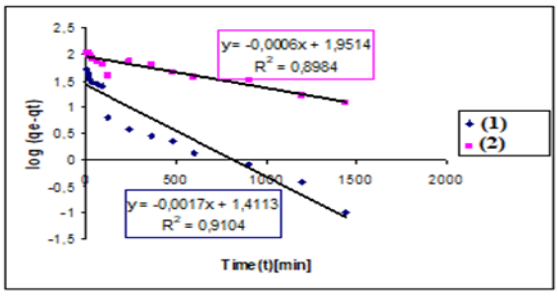
5.2.6. Kinetic modeling of the adsorption process carried out under static and dynamic conditions

In the specialized literature, the kinetics of the adsorption process is described with the help of some mathematical models, which try to approximate as faithfully as possible, the mechanism by which this process takes place. The kinetics of adsorption processes under dynamic conditions

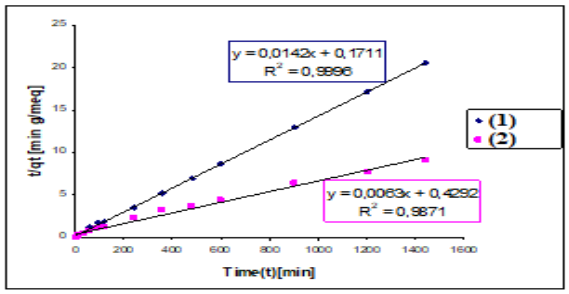
are tested using different mathematical models, which applied to experimental data allow the characterization of the dynamic adsorption system. The most frequently used kinetic models applicable to the adsorption of compounds from aqueous solutions are:

- Lagergren kinetic model (pseudo-first order kinetic model);
- pseudo-second order Ho&Mckay kinetic model
- intra-particle diffusion model (Morris-Weber)

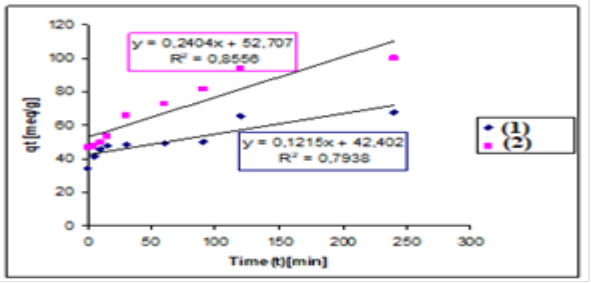
For the kinetic modeling of the adsorption process, only two studied analytes were used: paracetamol and ibuprofen. The results obtained for the two compounds adsorbed on the activated carbon were modeled mathematically with the models: Lagergren, Ho&Mckay, Morris - Weber.



(a) Lagergren



(b) HO&Mckay



(c) Morris-Weber

Figure 60. Linear representation of the Lagergren (a), HO&Mckay (b) models, Morris-Weber(c) for the adsorption of (1) acetaminophen and (2) ibuprofen

For acetaminophen and ibuprofen, the following results were obtained for the Lagergren, oMckay and Morris-Weber models, expressed by the value obtained for the regression factor (R^2):

- 0.9996 ACH > 0.9871 IBU (Ho&Mckay for the two compounds)
- 0.9104ACH > 0.8984 IBU (Lagergren for the two compounds)
- 0.8556 ACH > 0.7938 IBU (Morris-Weber for the two compounds).

Three models were used for the kinetic modeling of the adsorption process of acetaminophen and ibuprofen on activated carbon: the first-order pseudo-kinetic model, the second-order pseudo-kinetic model and the Weber-Morris intra-particle diffusion model.

The results corresponding to the kinetic parameters obtained for the adsorption of the 2 pharmaceutical compounds for the concentration of 1mg/L are presented in table 30 and figure 60.

For the pseudo kinetic models I and II, these were obtained from the slopes and the intercept (ordinate at the origin) of the dependences log($qe - qt$) versus t, respectively t/qt versus t.

Table 30. Kinetic parameters obtained for the adsorption of acetaminophen and ibuprofen, initial concentration of 1mg/L on activated carbon

Analyte	$Q_{exp(mg/g)}$	Lagergren Pseudo first-order kinetics			HO&Mckay Second order pseudo kinetics			Morris-Weber Intraparticle diffusion		
		Q_{calc} (mg/g^{-1}	K_1 (min^{-1})	R^2	Q_{calc} (mg/g^{-1}	K_2 (g/mg min)	R^2	K_{dif} ($mg/g\ min^{1/2}$)	I (mg/g)	R^2
ACF	1,70	1,15	0,0014	0,9104	1,72	0,00001	0,9998	0,444	0,313	0,7938
IBU	0,98	0,53	0,0007	0,8984	0,99	0,0001	0,9671	0,318	0,333	0,8556

ACH= acetaminophen; IBU = ibuprofen

The concordance obtained for the pseudo-kinetic model of ordinal II indicates considerably higher values of the correlation coefficients compared to the model of pseudo-order I (Table 34). The dependence of t/qt versus t led to obtaining a straight line over the entire contact time interval studied for the two compounds, and the values of the adsorption capacities calculated at equilibrium are close to those obtained experimentally.

This allows us to say that the second order pseudo kinetic model describes the most well the behavior of the adsorption systems studied. To identify the diffusion mechanism in the adsorption of the two compounds on activated carbon, the Weber-Morris intraparticle diffusion model was used.

The rate constants are obtained by plotting the linear dependences between qt as a function of t 1/2. If this representation is a straight line passing through the origin, the intraparticle diffusion model controls the adsorption process. However, if the graph shows multilinearity, the adsorption process is influenced by two or more factors.

The first step of the graph is completed within the first 10 min for all concentrations indicating that diffusion occurs initially in the macro and mesopores. Also, the lines obtained indicate a high initial adsorption rate that decreases with increasing contact time.

The choice of the most appropriate kinetic model for the verification of the experimental data was made with the help of linear regression, the corresponding regression coefficients being calculated using the Microsoft Office Excel program.

5.2.7. CONCLUSIONS

Using the HPLC method developed and implemented at laboratory level, the following parameters were optimized for the adsorption tests on activated carbon of the four pharmaceutical compounds: pH (4 and 6), contact time (0-120 minutes), concentration initial pollutant concentration (1, 5 and 10 mg/L), amount of adsorbent material (0.1g, 0.5g and 1g) and stirring speed (150 rpm and 250 rpm).

The highest value of removal efficiency (%) was recorded for: acetaminophen (98%) > ibuprofen (96%) > diclofenac (92%) > ketoprofen (88%) using an amount of adsorbent material of 1g, a concentration of the pollutant species of 1 mg/L and a stirring speed of 250 rpm, at a pH value of 6 unit.pH.

To characterize the adsorption process, two mathematical models Langmuir and Freunlich were used. Based on the results obtained, it can be stated that the Langmuir model (R^2=0.99)

presented higher correlation coefficients (R^2) compared to the Freundlich model (R^2=0.98). The Langmuir model can be used to characterize the entire adsorption process on activated carbon.

The second-order pseudo kinetic model (HO&Mckay) best describes the adsorption behavior of acetaminophen and ibuprofen on activated carbon, as it indicates values of considerably higher correlation coefficients compared to the pseudo I order kinetic model.

5.3.1. Comparative studies of paracetamol adsorption on two adsorbent materials: Fe_3O_4 and ZSM-5

This study presents the comparison of magnetite and zeolite materials for the removal of acetaminophen from wastewater. The effects of various experimental parameters on acetaminophen adsorption were investigated through an adsorption study: adsorbent dosage, contact time, initial acetaminophen concentration, and pH. The procedure for the determination of acetaminophen was validated in-house and is described below.

The performances of the two adsorbent materials Fe3O4 and ZSM-5 were systematically investigated using adsorption kinetics. During the experiments, the initial concentration of acetaminophen in the wastewater was varied from 50 to 280 mg/L, and the maximum Fe3O4 adsorption capacity increased from 25.3 mg/g to 68.9 mg/g. The study of the adsorption capacity of both materials was carried out for three pH values (4, 6, 8) of synthetic wastewater. Langmuir and Freundlich isotherm models were used to characterize the adsorption of acetaminophen on the two adsorbent materials.

The highest water treatment was obtained at pH value 6. The results show that Fe_3O_4 could be used as an effective adsorbent for the removal of acetaminophen from wastewater.

Fe_3O_4 and ZSM-5 were used as adsorbent materials due to their high pollutant removal efficiencies, small particle sizes, and maximum internal surface area. The specific surface area of the adsorbent material is important because removal efficiency generally increases with increasing specific surface area.

The effect of pH is due to the fact that at a different pH value, the adsorbent surface has a negative or positive charge, and the pH value affects the species of metal ions in aqueous solution. Consequently, repulsive or attractive forces may occur between the pollutants and the adsorbent surface.

The adsorption process was influenced by a number of factors such as: pH value, time, temperature, initial concentration of paracetamol and the presence of competing ions. In addition, most acetaminophen-laden industrial wastewater effluents have different pH values depending on the type of industrial activities performed.

The adsorption method is cheap and suitable for wastewater treatment. This technique consists in the ability of an adsorbent material to adsorb pollutants and their degradation products from wastewater on its surface

The concentration of acetaminophen in the solution was measured using UV-Vis spectrophotometer (Specord 200Plus, Analytic Jena). Synthetic solutions of acetaminophen with varying initial concentrations (50 – 280 mg/L) were prepared and analyzed at the maximum wavelength of 302 nm. The absorbance values were plotted against the corresponding acetaminophen concentrations to obtain the calibration curve and the unknown concentrations of the acetaminophen solutions were determined on this calibration curve.

The internal method of validation of the analysis method

The internal validation of the analytical applied method was performed in order to evaluate the performance parameters of the method: limit of detection LOD, limit of quantification LOQ, repeatability, intermediate precision and extended uncertainty. The method developed in this study was validated according to the ICH guideline [12]. Ten determinations were performed for repeatability and precision, limit of quantification, limit of detection and extended uncertainty for acetaminophen in the range of 0.10–0.50 mg/L and using two dissolved media [13]. Linearity studies are shown in Table 31.

Table 31. Results obtained for linearity

Compound	Concentration range (mg/L)	The regression equation	R^2	λ(nm)
Acetaminophen dissolved in 0.1M HCl	0.10 - 0.50	y=0.4591x+0.0004	0,9995	302

Table 32. Internal validation experiments

Parameters	Experiments
LOQ and LOD	5 independent blank solutions fortified with 0.05mg/L
Repeatability	10 independent standard solutions of 0.25mg/L
Intermediate accuracy	12 independent standard solutions of 0.35mg/L

Table 32 shows the performance parameters for acetaminophen using an in-house method obtained with 0.1M HCl as the dissolution medium.

Table 33. Performance parameters of acetaminophen

No.	Paracetamol		
	Performance parameters	M.U.	Results
1	Precision	mg/L	0.03
2	Detection limit (LOD)	mg/L	0.04
3	Quantification limit (LOQ)	mg/L	0.12
4	Extended uncertainty	%	17.5

Adsorption studies

The adsorption study was carried out by adding 0.2 g and 0.5 g of adsorbent in 250 ml stoppered Erlenmeyer containers containing 100 ml of acetaminophen solution of different concentrations (50-280mg/L). Containers were shaken in a mechanical shaker and samples were collected at predetermined time intervals for analysis. The samples were centrifuged for 5 minutes and analyzed UV-VIS spectrophotometrically to determine the concentration of acetaminophen in the wastewater solution.

The adsorption capacity of acetaminophen was calculated with the formula:

$$Q_e = \frac{(C_0 - C_e)}{m} * V \qquad (7)$$

Where: Qe (mg/g) acetaminophen adsorption capacity at equilibrium

Co and **Ct** are the initial and final concentrations of acetaminophen (mg/L),

V is the volume of the solution (L)

m is the amount of adsorbent (g).

The removal efficiency of acetaminophen was calculated with the formula:

$$R(\%) = \frac{(C_0 - C_e)}{C_0} * 100 \quad (8)$$

Where: R(%) = removal efficiency (%)

C_0 = initial concentration of paracetamol (mg/L)

Ce = equilibrium concentration of paracetamol (mg/L)

Adsorption studies involved several parameters such as: pH, contact time, adsorbent dosage and initial acetaminophen concentration.

The adsorption mechanism is an important issue in the design of water/wastewater treatment systems that can be determined using the adsorption isotherm. Therefore, establishing the adsorption capacity of adsorbent materials with different isotherm patterns allows the design of a treatment process and proper optimization of operating conditions. Langmuir and Freundlich adsorption isotherms were used to describe the adsorption data for the adsorption process of acetaminophen on Fe_3O_4 and ZSM-5.

The Langmuir model is widely used to describe the adsorption process in which acetaminophen is chemically adsorbed at a fixed number of adsorption sites. It is based on the assumption that each site will be occupied by a single acetaminophen ion and all adsorption sites are energetically equivalent.

The Freundlich model assumes that the adsorbate concentration on the adsorbent surface increases with the adsorbate concentration and an infinite amount of adsorption can occur.

This model is generally used to describe an adsorption process on heterogeneous surfaces.

The objective of this study was to determine the concentration of acetaminophen in solution (using the UV-VIS method) before adsorption and after the adsorption step.

Influence of pH on the removal efficiency of paracetamol in the presence of Fe_3O_4 and ZSM-5

In this study, the effect of pH on the removal efficiency of paracetamol in the presence of Fe_3O_4 and ZSM-5 was evaluated by using acetaminophen wastewater solutions adjusted from pH 4 to pH 8 (figure 64).

The adsorption process was influenced by a number of factors such as: pH value, time and initial concentration of acetaminophen. The effect of pH was due to the fact that at different pH values, the adsorbent surface has negative or positive charge, and the pH value affects the

speciation of acetaminophen in aqueous solution. Consequently, repulsive or attractive forces may occur between the pollutant and the adsorbent surface. Thus, it is necessary to conduct studies to establish the influence of pH value on acetaminophen adsorption by Fe_3O_4 nanomaterial and ZSM-5.

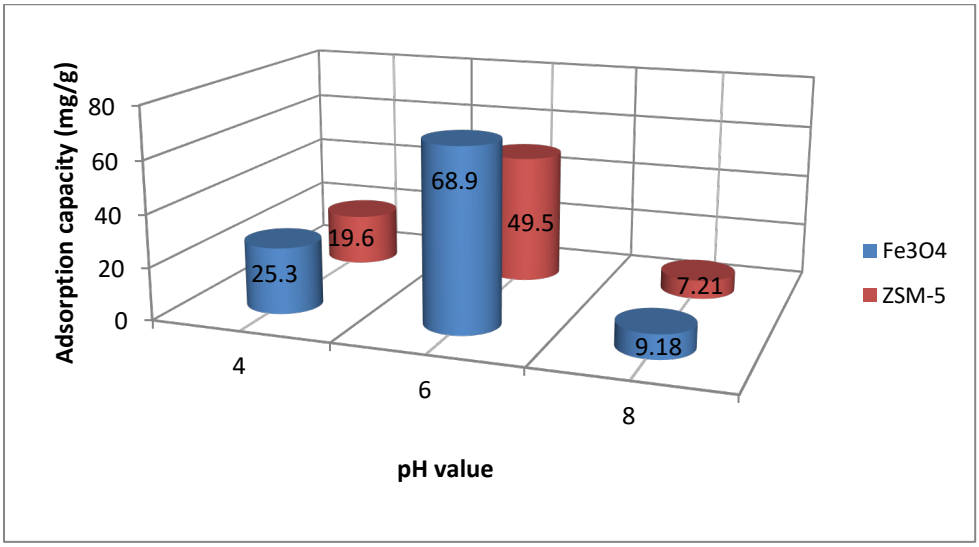


Figure 64. The influence of solution pH on the adsorption capacity of Fe_3O_4 and ZSM-5

At pH 6, the maximum adsorption capacity was obtained both for Fe_3O_4 (68.9mg/g) and for ZSM-5 (49.5mg/g) as can be seen in figure 64.

The influence of the amount of adsorbent on the removal efficiency of paracetamol in the presence of Fe_3O_4 and ZSM-5

Two amounts of adsorbent material were used in the study: 200 mg and 500 mg for both materials (Fe_3O_4 and ZSM-5). Adsorption studies were performed using two initial concentrations of acetaminophen: 50 and 280 mg/L acetaminophen for both materials tested. Figure 65 shows the results obtained using different amounts of adsorbent material and different pH values.

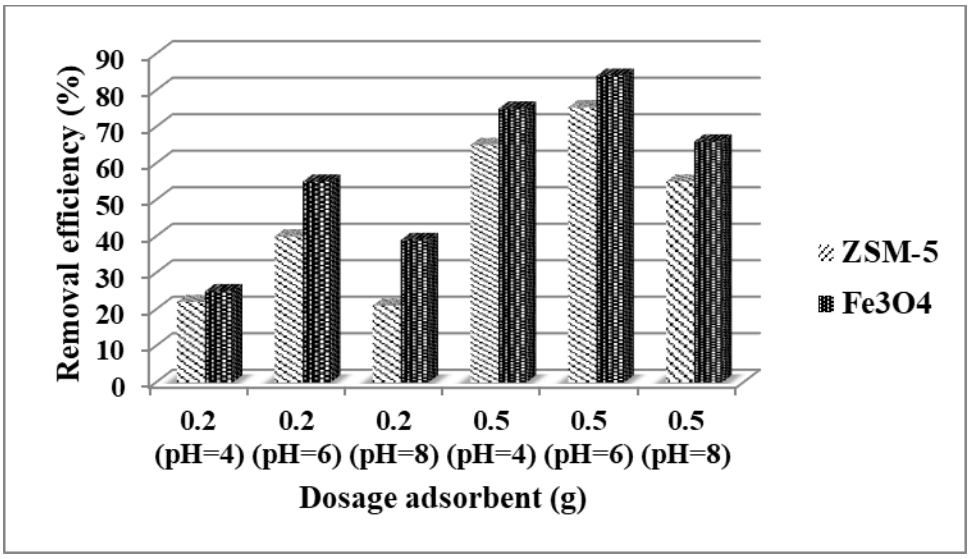


Figure 65. Influence of adsorbent amount and pH on acetaminophen removal efficiency using Fe_3O_4 and ZSM-5

The highest removal efficiency was obtained at pH 6, using 0.5 g as adsorbent material. Fe_3O_4 obtained the highest results for removal efficiency (84% at pH =6) compared to ZSM-5 which showed only 75% removal efficiency (figure 65).

The influence of contact time on the removal efficiency of paracetamol in the presence of Fe_3O_4 and ZSM-5

The results shown in Figure 66 showed that for both materials tested, the optimal contact time was 8 hours and adsorption was rapid in the first 4 hours.

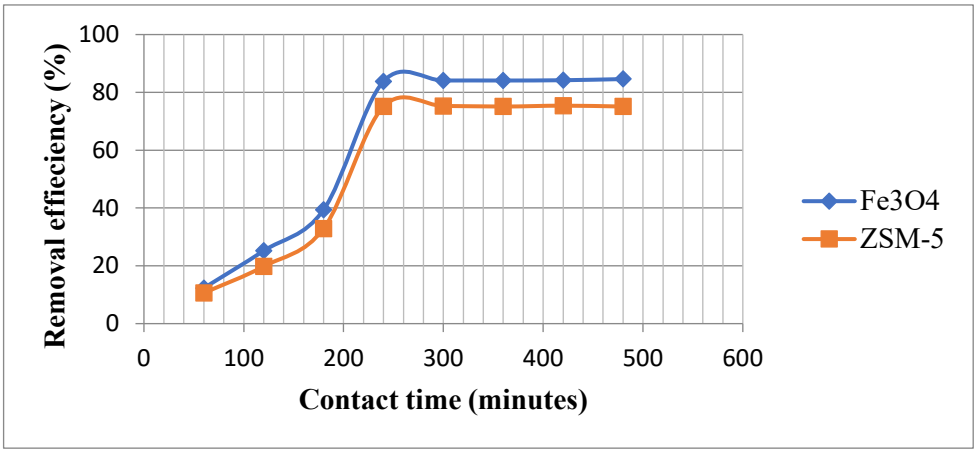


Figure 66. Influence of contact time on acetaminophen removal efficiency using Fe_3O_4 and ZSM-5

After 4 h, a slow increase in adsorption was observed until adsorption equilibrium was reached. In the first stage of rapid adsorption (4h), all the adsorption pores of the adsorbent materials are available and have been occupied by acetaminophen molecules. As the adsorption of acetaminophen continues, more adsorption pores are successively occupied, and consequently, the number of free adsorption pores will decrease and the adsorption will work more slowly. A different behavior of the two adsorbent materials during the adsorption process was observed. The Fe_3O_4 adsorbent material shows a higher removal efficiency (84.6%) compared to the ZSM-5 material (75.4%), as can be seen in figure 66.

The adsorption processes were carried out only on the surface of the adsorbent materials, where some weak van der Walls type bonds were created between acetaminophen molecules and the surface of the materials. The adsorption process involves only physical reactions, not chemical reactions.

Langmuir and Freundlich adsorption isotherms

The Langmuir model is based on the assumption that each pore (adsorption site) will be occupied by a single molecule and then all adsorption sites are energetically equivalent.

The Freundlich model assumes that the adsorbate concentration on the adsorbent surface increases depending on the adsorbent concentration. This model is generally used to describe an adsorption process on heterogeneous surfaces.

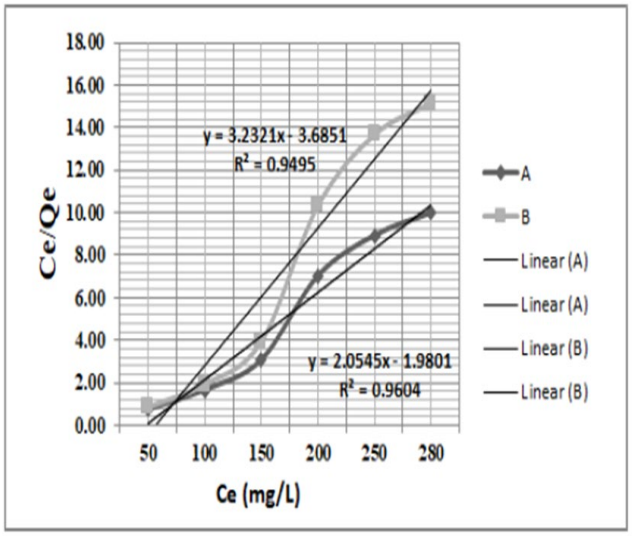


Figure 67. Linearized Langmuir isotherm for acetaminophen adsorption using A (Fe_3O_4) and B (ZSM-5)

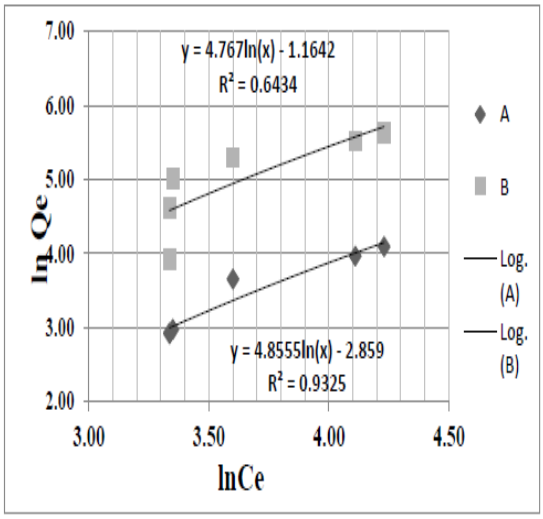


Figure 68. Linearized Freundlich isotherm for acetaminophen adsorption using A (Fe_3O_4) and B (ZSM-5)

Figures 67 - 68. show the linear forms of the equilibrium isotherms. These were used to determine the values of the adsorption parameters.

The experimental data shown in figures 67 and 68 and table 34 showed that the Langmuir isotherm model fits the experimental data very well for all tested adsorbent materials.

Table 34. Langmuir and Freundlich adsorption parameters

Adsorbent material	Langmuir parameters			Freundlich parameters		
	Qmax (mg/g)	K_L (L/mg)	R^2	K_F (L/g)	1/n	R^2
Fe_3O_4	68,9	0,149	0,9604	42,13	0,356	0,9325
ZSM-5	49,5	0,103	0,9495	33,89	0,314	0,6434

Table 38 and figures 67 and 68 highlighted the value of the correlation coefficient of the Langmuir equation, R^2=0.9604 and R^2=0.9495, respectively, which describes very well the adsorption process for both Fe_3O_4 and ZSM-5 tested. The Langmuir constant R_L is in the range (0-1), indicating that the adsorption process of acetaminophen is favorable using these adsorbent materials. The Freundlich isotherm has correlation coefficients of R^2=0.9325 for Fe_3O_4 and R_2=0.6434 for ZSM-5 much lower than the Langmuir isotherm, as can be seen in Figures 67 and 68.

Based on the correlation coefficients, it can be stated that the Langmuir isotherm characterizes the adsorption process very well, compared to the Freundlich isotherm.

It can be seen from figure 67 that the adsorption of acetaminophen decreases with increasing pH of the synthetic wastewater solution, and the maximum adsorption of acetaminophen is obtained at a pH of 6. At a lower pH of the solution, the acetaminophen molecules are not protonated in other molecules, so it leads to a higher adsorption capacity.

On the other hand, at higher pH values there is a competitive adsorption between acetaminophen and (OH^-) molecules leading to a lower adsorption capacity. On the contrary, at acidic pH, some of the negatively charged ions are neutralized and the acetaminophen molecules also remain dissociated, leading to a higher adsorption capacity. Therefore, the adsorption efficiency of acetaminophen from synthetic wastewater can be improved in an acidic environment. In the present study, maximum acetaminophen adsorption was obtained for a synthetic wastewater pH of 6 pH units.

5.3.2. CONCLUSIONS

Two adsorbent materials (Fe_3O_4 and ZSM-5) were used to remove acetaminophen from synthetic wastewater. The highest adsorption capacity (68.9 mg/g) and the highest removal efficiency (84.6%) were obtained for Fe_3O_4 at a pH value of 6.

Using ZSM-5 as adsorbent material, an adsorption capacity of 49.5 mg/g and a removal efficiency of 75% for acetaminophen was obtained. The difference between the two adsorbent materials studied is based on the specific active surface, the porosity and the method by which the adsorbent materials were obtained.

Adsorption data were evaluated using two mathematical models (Langmuir and Freundlich). It was found that the adsorption was well described by the adsorption isotherm, Langmuir. Higher correlation (regression) coefficients were obtained for the Langmuir isotherm compared to the Frendlich isotherm, for both materials studied.

The equilibrium parameter (RL) for the Langmuir isotherm was in the range of 0 – 1 (0.79 for Fe_3O_4 and 0.86 for ZSM-5), indicating that the adsorption process is favorable for both adsorbent materials.

Adsorption studies for the removal of emerging pollutants from wastewater are limited and strategies could be developed to improve the efficiency of treatment plants.

The proposed method is simple, sensitive, fast, specific and could be applied to improve the monitoring of acetaminophen and its degradation products in wastewater, in sewage treatment plants.

5.4.1. Comparative studies of paracetamol adsorption in the presence of ZSM-5 and CNT

5.4.2. Adsorption study of paracetamol on ZSM-5

Adsorption studies were performed using synthetic wastewater solutions with paracetamol (100 ml) with an initial paracetamol concentration of 25mg/L and using ZSM-5 as adsorbent material. The amounts of adsorbent material used in these adsorption studies varied between 0.25g and 0.50g. The pH of the acetaminophen solutions was varied between 4 and 8 unit.pH

All solution concentrations were recorded with Specord 200Plus spectrophotometer, Analytic Jena. And the removal efficiency was calculated with the following formula:

$$\eta(\%) = \frac{C_i - C_f}{C_i} * 100 \qquad (9)$$

Where:

η =removal efficiency;

ci,cf – the initial concentration and the final concentration of paracetamol (mg/L).

The results obtained (Specord 200 Plus, Analytik Jena) were determined according to an internally validated method over a measurement range of 0.10 – 0.50mg/L for paracetamol at a wavelength of 302nm.

Using an amount of adsorbent of 0.25 and 0.50 g, a synthetic solution of 100 mL and an initial concentration of paracetamol of 15 mg/L, the following were obtained (table 35):

Table 35. Results of the paracetamol adsorption study using ZSM-5

Sample	pH	Quantity of ZSM-5 (mg) The first case	Removal efficiency(%)	Quantity of ZSM-5 (mg) The second case	Removal efficiency (%)
1	4	500	39,4	250	32,9
2	6	500	85,2	250	53,4
3	8	500	42,1	250	23,6

To achieve the adsorption process in optimal conditions: the influence of pH, contact time, amount of adsorbent material and the initial concentration of paracetamol on the entire adsorption process was investigated.

The amount of adsorbent material influences the adsorption efficiency of paracetamol on the surface of the adsorbent material (ZSM-5).

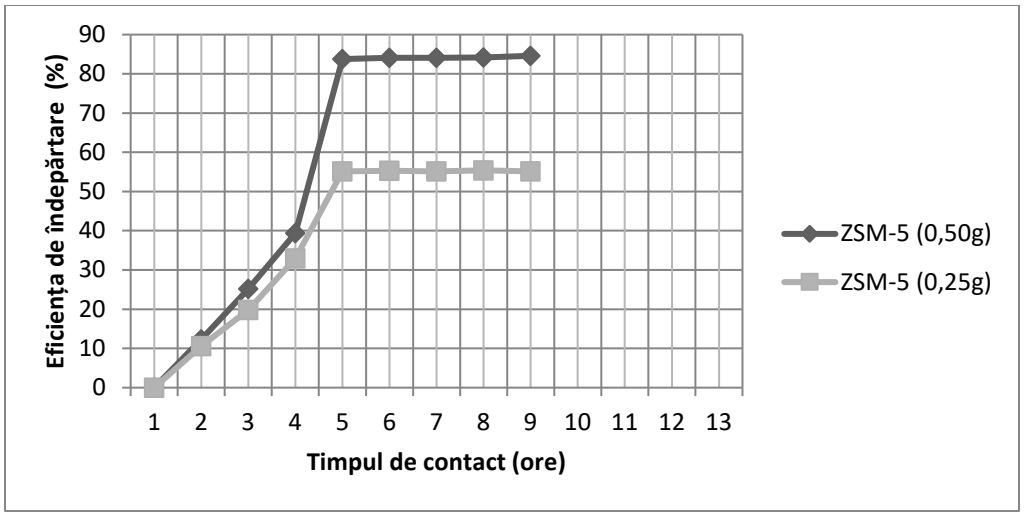


Figure 69. Acetaminophen removal efficiency using ZSM-5 as adsorbent material

The results of synthetic wastewater treatment using an amount of adsorbent between 0.25 and 0.50 g, a synthetic paracetamol solution of 100 ml and the initial concentration of 15 mg/L are shown in figure 69. It can be stated that the physical properties of the adsorbent ZSM-5 are suitable for the adsorption of acetaminophen from wastewater. After 9 hours of contact, it can be stated that the maximum removal efficiency of paracetamol was 84.6%, using 0.50 g of adsorbent, at a pH value equal to 6. And if 0.25 g of material was used adsorbent, the adsorption yield was only 53.4%, at a pH value equal to 6.

The proposed method for the adsorption of paracetamol on zeolites is simple, fast, specific for paracetamol and can be easily applied for monitoring paracetamol and pharmaceutical compounds in wastewater.

5.4.3. Study of paracetamol adsorption on CNTs (carbon nanotubes)

Adsorption experiments were carried out in 100 ml Erlenmeyer flasks with stoppers, using a horizontal shaker, at room temperature 20±20C, stirring speed of 100 rpm, for 8 hours. The amounts of adsorbent (CNT) varied between 0.25 g and 0.50 g and were contacted with synthetic wastewater with acetaminophen having a concentration of 25 mg/L.

Acetaminophen solutions were prepared in 2 both dissolution media:

a) 0.1 M HCl solution (HCl purchased from Sigma Aldrich)

b) 0.01 N KOH solution (KOH purchased from Sigma Aldrich).

The amounts of adsorbent material added were between 0.25 g and 0.50 g. All concentrations were read on a spectrophotometer (Specord 200Plus, Analytic Jena). SIPS, Langmuir, Redlich-Petersen Redlich adsorption isotherms were used for experimental data modeling.

The influence of pH on the adsorption capacity

All experiments were performed at ambient temperature. The adsorption studies followed three parameters: concentration at equilibrium, time and pH of the working solutions. Three adsorption isotherms were used to highlight the obtained results: SIPS, Langmuir and Redlich-Petterson.

Adsorption studies were performed using synthetic acetaminophen wastewater solutions (100 mL) with an initial paracetamol concentration of 25 mg/L and using carbon nanotubes (CNTs) as adsorbent material. The amounts of adsorbent material used in these adsorption studies varied between 0.25 g and 0.50 g. The pH of the acetaminophen solutions was varied between 4 and 8 pH units.

Table 36. Results obtained for the adsorption study of acetaminiphen in the presence of CNT and using 0.1N HCl as dissolution medium

Sample	pH	Quantity of CNT (mg) The first case	Removal efficiency (%)	Quantity of CNT (mg) The second case	Removal efficiency (%)
1	4	250	31.20	500	45.60
2	6	250	49.50	500	84.60
3	8	250	40.30	500	58.10

The highest removal efficiency was obtained at pH=6 and using 500 mg of adsorbent material (84.6%), as shown in table 36.

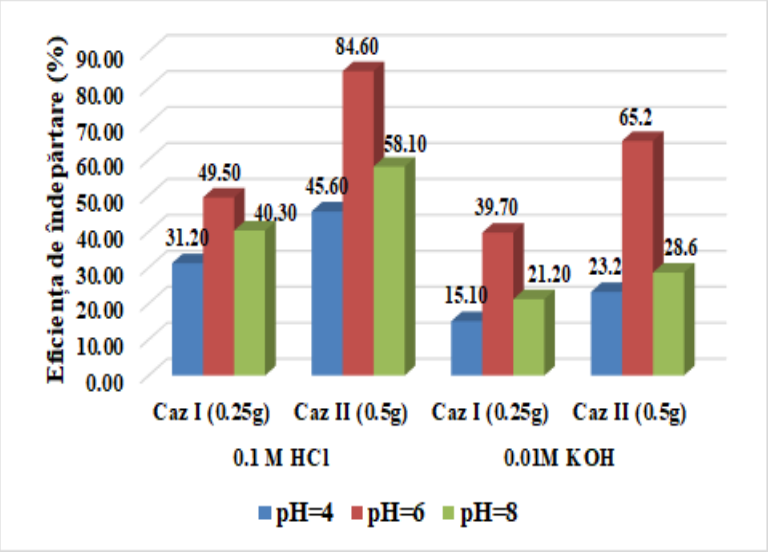


Figure 70. Adsorption capacity at three pH values (4, 6, 8) using 0.1M HCl and 0.01M KOH (case I – 0.25g and case II – 0.5g adsorbent dose)

Based on the results obtained, it can be stated that in the case of using the KOH 0.01M dissolution medium, the removal efficiency is lower (65.2%) compared to that of the HCl 0.1M dissolution medium (84.6%), as observed in figure 70.

Therefore, the adsorption capacity of acetaminophen can be improved in an acidic environment. In the present study, the maximum adsorption of acetaminophen was obtained at a value of 6 of the studied pH.

The influence of contact time on the removal efficiency of paracetamol using carbon nanoparticles

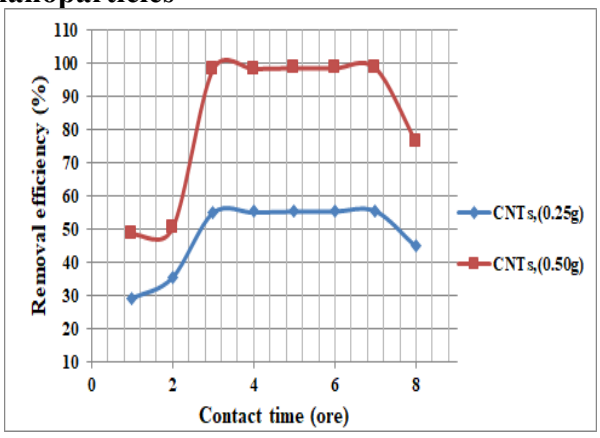


Figure 71. Removal efficiency as a function of contact time (case I-250mg and case II-500mg) in 0.1M HCl medium

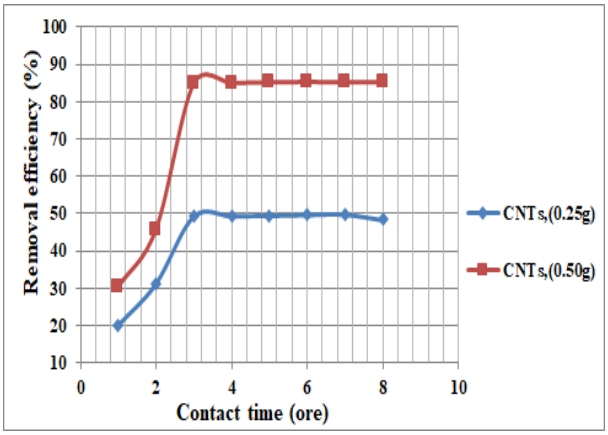


Figure 72. Removal efficiency versus contact time (case I-250mg and case II-500mg), in 0.01M KOH medium

In the case of using as a dissolution medium HCl 0.01 M and 0.5 g of adsorbent material, a removal efficiency of 84.60% was obtained compared to the use of 0.25g of adsorbent material where a removal efficiency of 49.50 was obtained % (figure 71).

As can be seen in figure 60, the removal efficiency is maximum in the first 3 hours, then it remains in equilibrium for 5 hours, and after 8 hours it starts to decrease. Contact time has a significant influence on the removal efficiency of paracetamol.

In the case of using KOH 0.01 M as a dissolution medium and 0.5 g of adsorbent material, a removal efficiency of 65.20% was obtained compared to the use of 0.25g of adsorbent material where a removal efficiency of 39.70 was obtained % (figure 72). In both cases, increasing the adsorbent dose in the range of 0.25 g to 0.5 g led to higher removal efficiency at pH=6.

5.4.4. Mathematical models applied to the adsorption process
SIPS, Langmuir, Redlich-Petersen adsorption isotherms

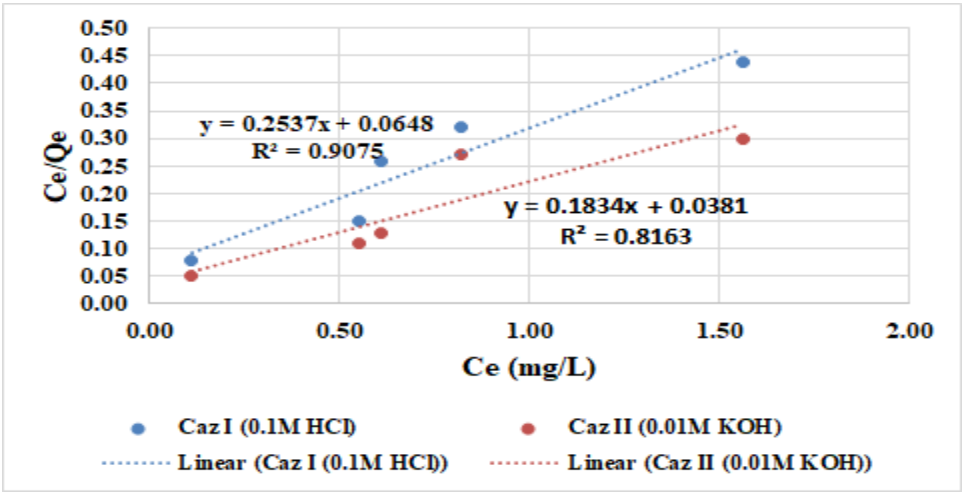


Figure 73. SIPS linearized isotherm for acetaminophen adsorption on CNTs

Table 37. SIPS adsorption parameters

Experimental conditions	SIPS parameters		
	Q_{max} (mg/g)	K_S (L/mg)	R^2
Acetaminophen dissolved in HCl 0,1M (Caz I)	85,5	0,162	0,9075
Acetaminophen dissolved in KOH 0,01M (Caz II)	62,6	0,149	0,8163

In Case I, the R^2 correlation factor is 0.9075, and in Case II, the R^2 correlation factor is 0.8163. The SIPS isotherm can be applied to the 2 dissolution media in the case of wastewater containing acetaminophen. The maximum adsorption capacity (85.5mg/g) is higher in acidic dissolution medium compared to alkaline dissolution medium (62.6mg/g), as shown in table 37 and figure 73.

Langmuir isotherm model

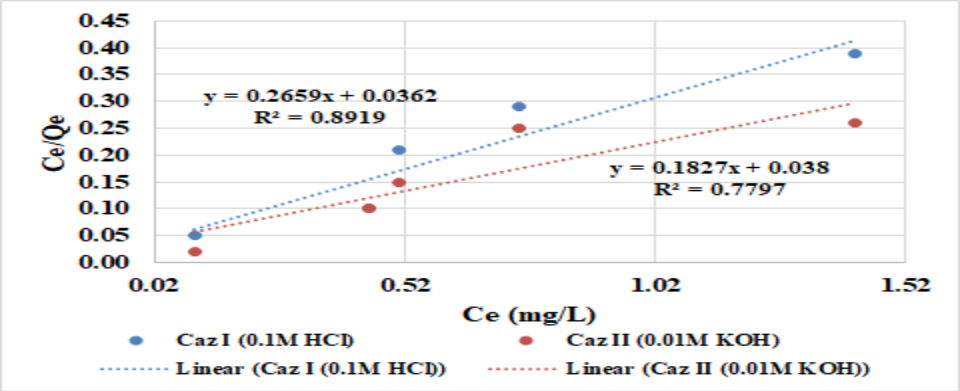


Figure 74. Linearized Langmuir isotherm for acetaminophen adsorption on CNTs

Table 38. Langmuir adsorption parameters

Experimental conditions	Langmuir parameters		
	Q_m (mg/g)	K_L (L/mg)	R^2
Acetaminophen dissolved in HCl 0,1M (Caz I)	80,2	0,151	0,8919
Acetaminophen dissolved in KOH 0,01M (Caz II)	54,9	0,130	0,7797

In case I, the R^2 correlation factor is 0.8919, and in case II, the R^2 correlation factor is 0.7797.

And in the case of the Langmuir model, maximum adsorption capacities were obtained in acidic medium (80.2mg/g), compared to alkaline medium (54.9mg/g) as shown in figure 74 and table 38.

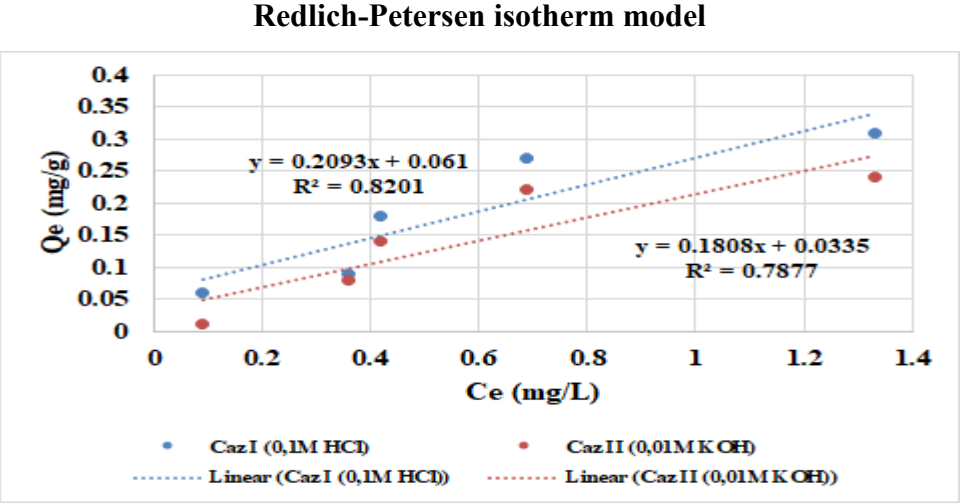


Figure 75. Redlich-Peterson linearized isotherm for acetaminophen adsorption on CNTs

Table 39. Redlich-Peterson adsorption parameters

Experimental conditions	**Redlich-Peterson parameters**		
	Q_m (mg/g)	**K_{RP}**	**R^2**
Acetaminophen dissolved in HCl 0,1M (Caz I)	78,1	0,146	0,8201
Acetaminophen dissolved in KOH 0,01M (Caz II)	50,7	0,118	0,7877

In Case I, the R^2 correlation factor is 0.8201, and in Case II, the R^2 correlation factor is 0.7877. And in the case of the Redlich-Peterson model, maximum adsorption capacities were obtained in the acid environment (78.1mg/g), compared to the alkaline environment (50.7mg/g), as shown in figure 75 and table 39.

Comparison between different mathematical models applied to describe the adsorption process of acetaminophen in 0.1 M HCl dissolution medium

The adsorption process of acetaminophen on carbon nanotubes was described with the help of three mathematical models: SIPS, Langmuir and Redlich-Peterson.

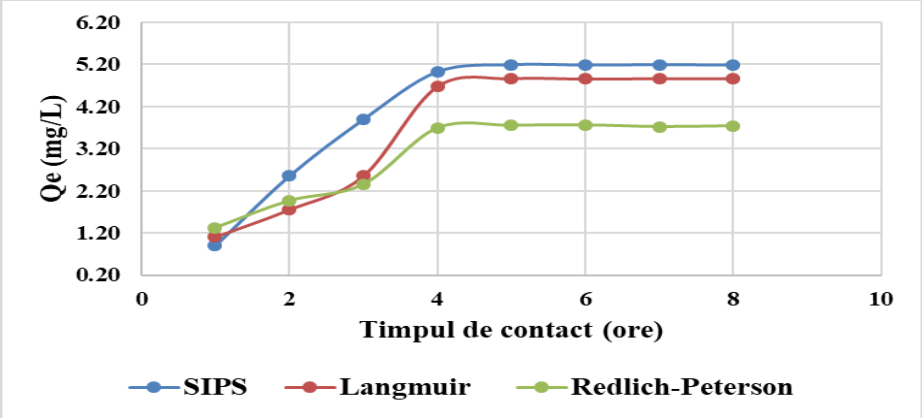


Figure 76. The maximum adsorbed concentration at equilibrium on CNTs in 0.1 M HCl medium as a function of contact time after applying the 3 mathematical models

The mathematical model that best describes the adsorption process is the SIPS model, as can be seen in figure 76. After 8 hours of contact between 0.5 g CNT and the acetaminophen solution dissolved in 0.1 M HCl, the following were obtained adsorption capacities at equilibrium after applying the 3 mathematical models: SIPS - Qe=5.19 mg/L; Langmuir - Qe=4.87mg/L; Redlich-Peterson - Qe=3.75mg/L.

Comparison of different mathematical models applied to describe the adsorption process of acetaminophen in 0.01 M KOH dissolution medium

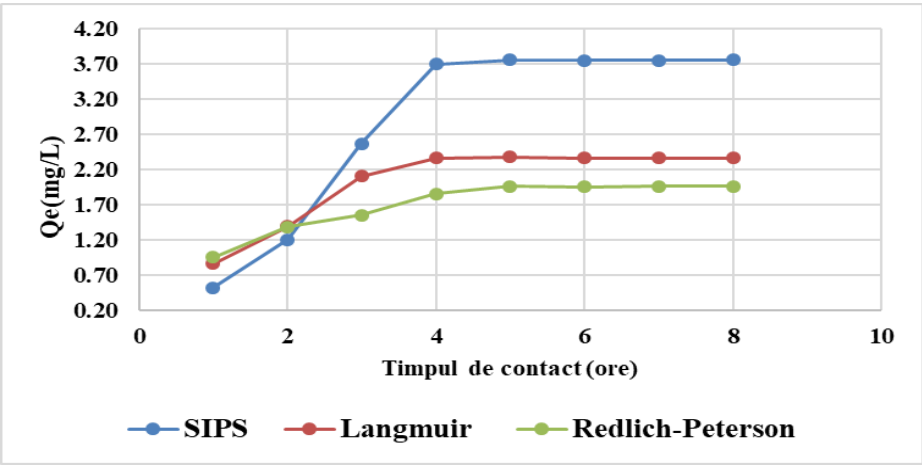


Figure 77. The maximum adsorbed concentration at equilibrium on CNT in 0.01 M KOH medium as a function of contact time after applying the 3 mathematical models

After 8 hours of contact between 0.5g of CNT and the acetaminophen solution dissolved in 0.01M KOH, the following equilibrium adsorption capacities were obtained after the application of the 3 mathematical models: SIPS-Qe=3.75mg/L; Langmuir-Qe=2.37mg/L; Redlich-Peterson - Qe=1.96mg/L

From the experimentally obtained results, it can be stated that the SIPS mathematical model can be successfully applied to describe the adsorption process in the case of wastewater containing acetaminophen (figure 77).

5.4.5. CONCLUSIONS

In this study, the following aspects were followed: the influence of the parameters (pH, contact time and dissolution medium) for the adsorption of acetaminophen from a synthetic wastewater solution, as well as different mathematical models (SIPS, Langmuir and Freundlich) to describe the process of acetaminophen adsorption (removal).

Before carrying out all the adsorption studies for acetaminophen, a method for its determination by the UV-VIS technique was developed internally. Internally validated method for the determination of paracetamol by UV-VIS proved to be simple, sensitive, rapid, specific and can be easily applied to the monitoring of paracetamol and degradation products in wastewater.

The removal efficiency of acetaminophen on carbon nanotubes was higher in 0.1M HCl medium compared to the removal efficiency obtained in 0.01M KOH medium. The adsorption process was evaluated using SIPS, Langmuir and Redlich Peterson isotherms.

The SIPS isotherm was successfully used for the adsorption process of acetaminophen in 0.1M HCl and 0.01M KOH medium. The results of this study were highlighted by the possible use of carbon nanotubes for the removal of acetaminophen and degradation products present in wastewater. Based on the correlation coefficients, it can be stated that the SIPS isotherm model characterizes the adsorption process in acidic medium very well. The order of the obtained correlation coefficients was as follows: SIPS (R^2=0.9075), Langmuir (R^2=0.8989) and Redlich-Peterson (R^2=0.8201), for 0.1M HCl medium. And for alkaline dissolution medium, the order of correlation factors was as follows: SIPS (R^2=0.8163), Langmuir (R^2=0.7797) and Redlich-Peterson (R^2=0.7877).

Therefore, the removal efficiency of paracetamol using carbon nanotubes can be improved in an acidic environment. In the study carried out, the maximum removal efficiency of acetaminophen was obtained at a pH equal to 6, a contact time of 8 hours and using an amount of 0.50 g of adsorbent material. Increasing the amount of adsorbent material significantly influenced the adsorption process. In 0.1M HCl dissolution medium, the removal efficiency increased from 49.50%, using 0.25g of adsorbent material to a value of 84.60% using 0.50g of adsorbent material. And in 0.01M KOH medium, the removal efficiency increased from 39.70% (with 0.25g adsorbent material) to a value of 65.20% (with 0.50g adsorbent material).

5.5.1. Comparison between mathematical models applied to the adsorption process of paracetamol for ZSM-5 and for CNT

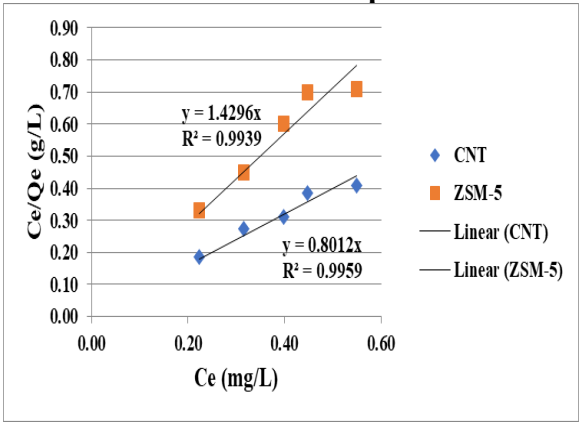


Figure 78. Linearized Langmuir isotherm applied to paracetamol adsorption on ZSM-5 and CNT

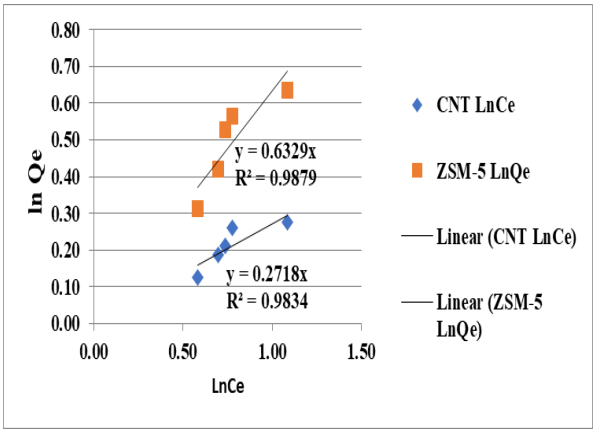


Figure 79. Linearized Freundlich isotherm for paracetamol adsorption on ZSM-5 and CNT

In figure 78, the linearized Langmuir isotherm describes very well the adsorption process of paracetamol and can be applied especially for CNT. The correlation factor (R^2) obtained for CNT is higher (R^2=0.9959) compared to the correlation factor of 0.9939 obtained for ZSM-5.

As can be seen from figure 79, the Freundlich isotherm describes very well the adsorption process of paracetamol on ZSM-5, because the correlation factor (R^2) obtained in this case is higher (R^2=0.9879) compared to the correlation factor obtained for CNT (R^2=0.9834).

Table 40. Langmuir and Freundlich adsorption parameters

Adsorbent material	Langmuir parameters			Freundlich parameters		
	Qmax (mg/g)	K_L (L/mg)	R^2	K_F (L/g)	1/n	R^2
CNT	88,9	0,156	0,9959	55,16	0,364	0,9834
ZSM-5	64,5	0,134	0,9939	41,21	0,320	0,9879

From the experimental data presented in figures 78 and 79, as well as from table 40, it is demonstrated that the Langmuir isotherm describes very well the experimental data ($RL^2 > RF^2$) for the two adsorbent materials tested.

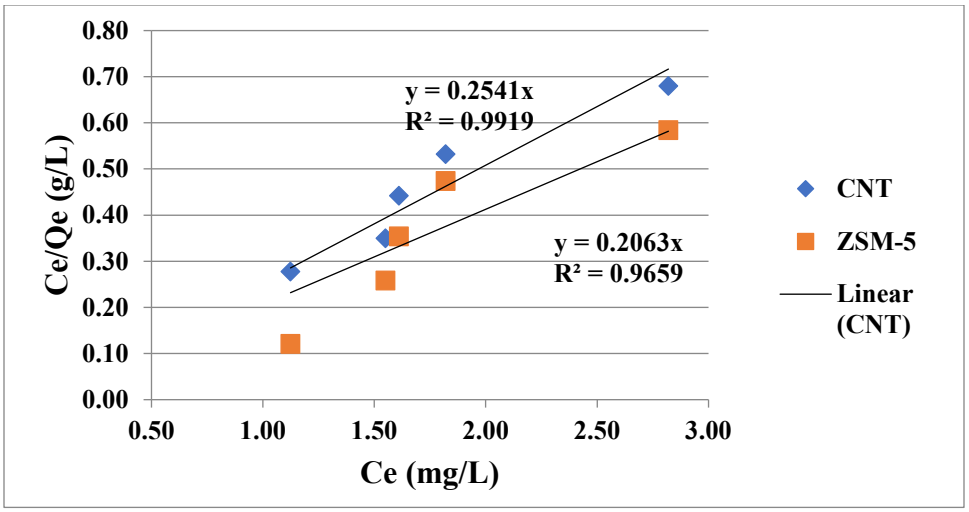


Figure 80. Linearized SIPS isotherm for paracetamol adsorption on ZSM-5 and CNT

Table 41. Adsorption parameters for the SIPS isotherm

Adsorbent material	SIPS parameters		
	Qmax (mg/g)	Ks (L/mg)	R^2
CNT	75.5	0.151	0.9919
ZSM-5	60,2	0.143	0.9659

The SIPS isotherm describes very well the adsorption process applied on ZSM-5 and CNT obtaining a correlation factor of 0.9919 compared to the correlation factor of 0.9659 obtained for the ZSM-5 zeolite (table 41).

5.5.2. CONCLUSIONS

Research into the adsorption of pharmaceutical compounds from the environment is on a continuous rise. It is desired to introduce new methodologies for a controlled monitoring and constant data collection of pharmaceutical compounds present in environmental matrices.

This study was carried out to find compatible adsorbent materials for the retention of acetaminophen from wastewater. Nano-structured materials such as zeolites and carbon nanotubes lend themselves to be used to retain paracetamol from wastewater.

The adsorption capacity of carbon nanotubes is higher (88.9mg/g) compared to the adsorption capacity of zeolites (64.5mg/g). The Langmuir isotherm better describes the experimental data obtained for the two adsorbent materials because higher adsorption capacities and correlation coefficients were obtained compared to the Freundlich and SIPS isotherms.

The in-house (internal) method for the determination of acetaminophen using the UV-VIS method is simple, sensitive, fast, specific and could be applied for the treatment of paracetamol and degradation products from wastewater. The results show that the adsorption capacity of CNT for acetaminophen in 0.1 M HCl medium was higher compared to the results obtained for acetaminophen dissolved in 0.01 M KOH.

5.6.1. Adsorption and desorption of the four studied drugs (acetaminophen, diclofenac, ibuprofen and ketoprofen) **using activated carbon by TOC determination**

The present study describes a method for removing anti-inflammatory drug residues from wastewater using an adsorbent material such as activated carbon. Four anti-inflammatory drugs were used for adsorption and desorption studies: acetaminophen, diclofenac, ibuprofen and ketoprofen. The physicochemical properties of the synthetic pollutants were studied and the pH, concentration and contact time of the drug residues were varied. Langmuir and Freundlich isotherms were used to highlight the mathematical model that best describes the adsorption processes. To demonstrate the presence of drug residues in wastewater, the total organic carbon (TOC) quantification technique was used. Three drug concentrations were experimented: 1mg/L, 5mg/L, and 10mg/L, and the amounts of activated carbon material used were 0.1g, 0.5g, and 1g.

Adsorption studies

Adsorption experiments were performed using wastewater with three different concentrations of anti-inflammatory drugs (1mg/L; 5mg/L and 10mg/L), at two different pH values (4 and 6) using three different amounts of material adsorbent for the adsorption process (0.1g, 0.5g and 1g). All experiments were performed in Erlenmeyer flasks (100ml) using a horizontal, temperature-controlled shaker (stirring speed of 100 rpm) for 120 min. The experiments were performed at room temperature (20 ± 2^0C).

The main results are shown in Figures 58a to 58d as well as in Figure 59.

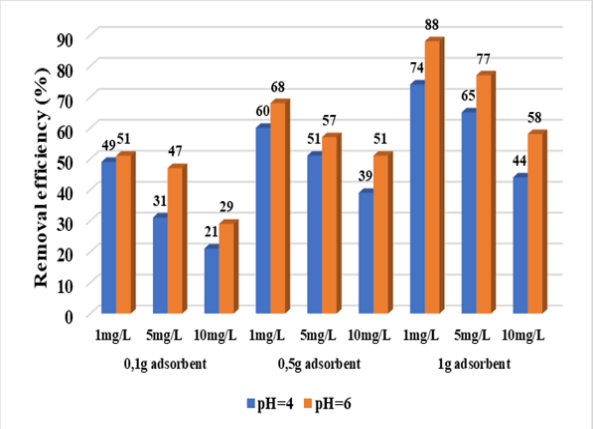


Figure 81a. Influence of pH on acetaminophen impurity removal efficiency at different adsorbent concentrations and dosages

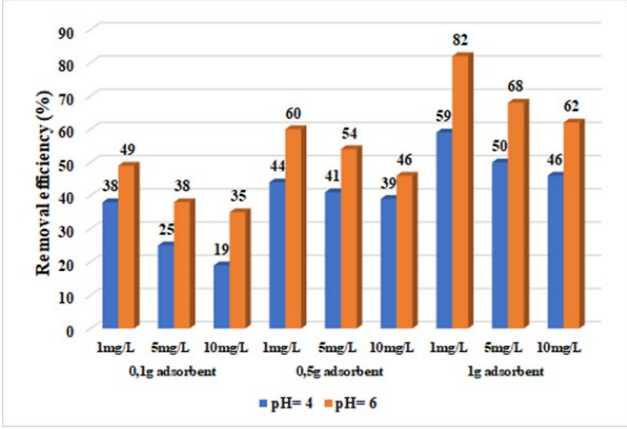


Figure 81b. Influence of pH on diclofenac impurity removal efficiency at different adsorbent concentrations and dosages

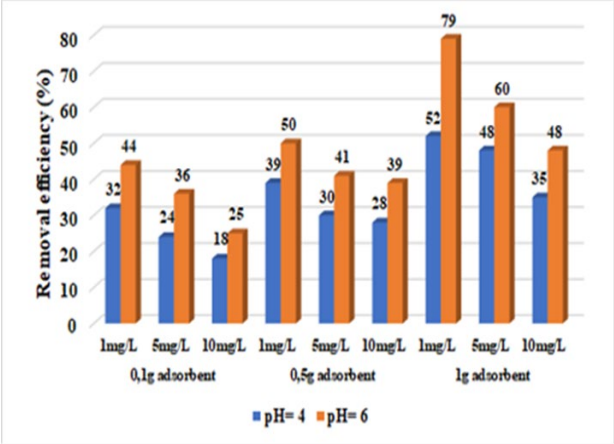


Figure 81c. Influence of pH on the impurity removal efficiency of ketoprofen at different adsorbent concentrations and dosages

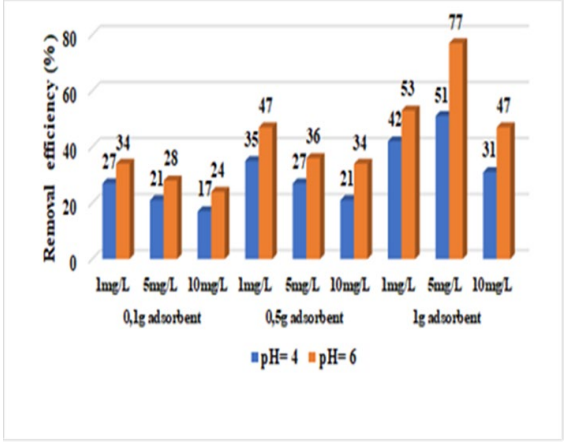


Figure 81d. Influence of pH on the impurity removal efficiency of ibuprofen at different adsorbent concentrations and dosages

Using 1g of adsorbent material and at a pH=6, the highest results for pollutant removal efficiency were obtained, compared to 0.1g of adsorbent material, also at pH=6, which showed the lowest removal efficiency as shown in Figures 81a through 81d.

The results obtained for the four drug residues using a quantity of 1g adsorbent material, at an initial concentration of 1mg/L pollutant and pH=6 were as follows: acetaminophen (88%) > diclofenac (82%) > ketoprofen (79%)) > ibuprofen (77%).

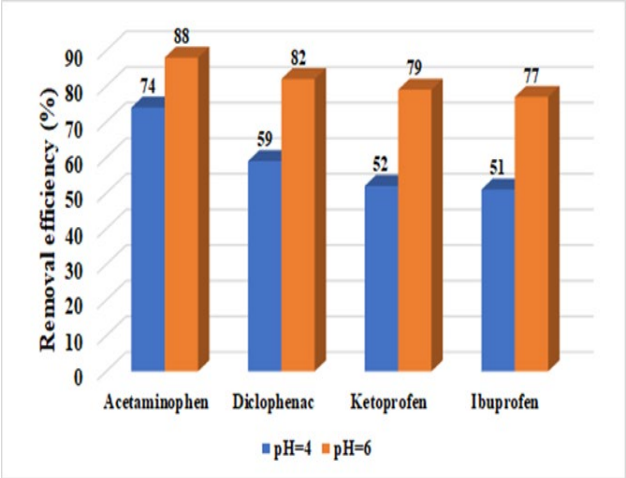


Figure 82. Influence of pH on impurity removal efficiency at different pollutant concentrations and adsorbent doses

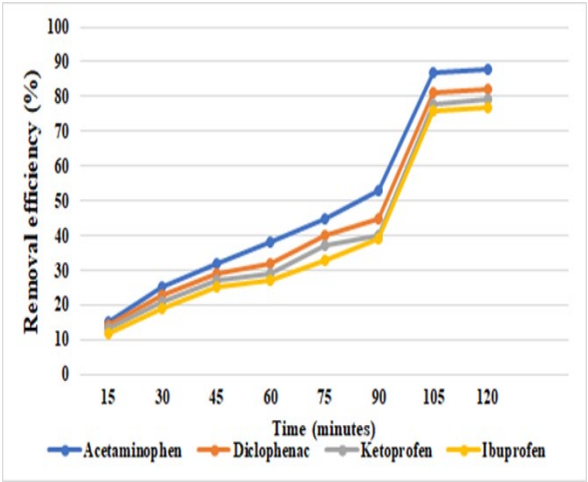


Figure 83. Pollutant removal efficiency as a function of contact time

Figure 82 shows the importance of the pH value of the wastewater in the treatment process. In all cases, the effectiveness of the treatment was maximal. However, the pH value of 6 is much more advantageous for the removal of drug impurities from wastewater, observing the shorter treatment time (120 minutes).

These studies want to highlight the importance of determining TOC (total organic carbon) for a small concentration of pollutants in wastewater. The effect of contact time on synthetic wastewater at a pH=6 was also studied, and the results are presented in figure 83.

A high removal efficiency for the four drug impurities was recorded at a pH value of 6 and low values at pH=4. After a contact time of 120 minutes, the adsorbent material becomes saturated and no longer absorbs drug impurities. The equivalence point is reached and the adsorption process ends.

Desorption studies

Desorption studies were applied to highlight the pollutants retained on the adsorbent material and the possibility of re-using this material in other adsorption studies. The desorption procedure was as follows: the adsorbent material loaded with 1mg/L synthetic pollutants was put in contact with 100 ml of HCl with different concentrations (0.1M; 0.3M and 0.5M) on a mechanical stirrer (90 minutes at 250 rpm). The obtained supernatant was centrifuged and analyzed using the TOC technique. Since the adsorption of acetaminophen, diclofenac, ketoprofen and ibuprofen on activated carbon was carried out separately, so was the desorption of these pollutants. It was possible to identify and quantify each impurity dissolved in hydrochloric acid of different concentrations.

The desorption of drug impurities from the surface of adsorbent materials was calculated using the following equation:

$$Desorption(\%) = \frac{C_d}{(Q*m)} * 100 \quad (10):$$

Where:
Cd is the concentration of drug residues desorbed from the adsorbent material (mg/L);
Q is the adsorption capacity (mg/g);
m(g) is the mass of the adsorbent material applied in the experiment

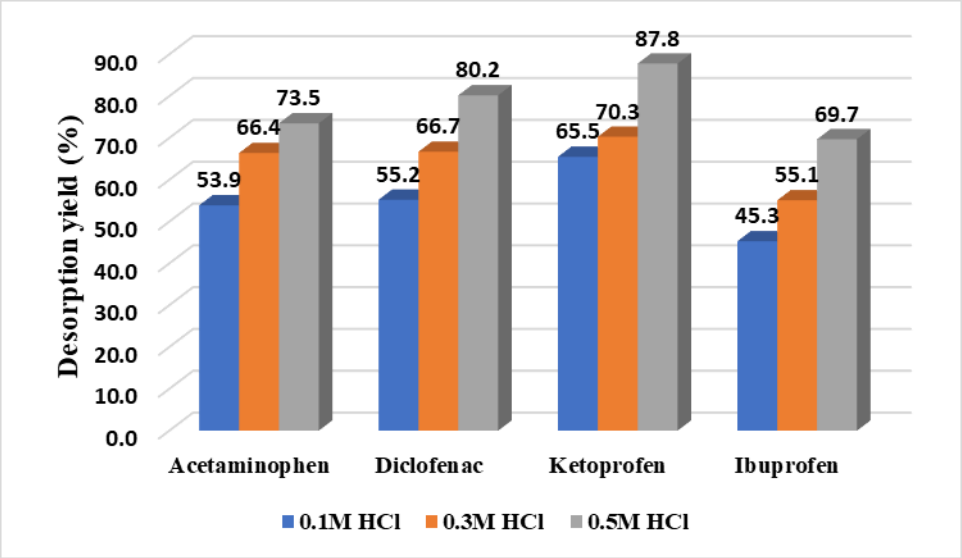


Figure 84. Desorption yields at different concentrations of HCl

The desorption yields of drug impurities retained on the activated carbon surface were influenced by the concentration of hydrochloric acid used, as can be seen in figure 61. The desorption order is not the same as the adsorption order on carbon, as can be seen in figure 73: 87.8% ketoprofen>80.2%diclofenac >73.5% acetaminophen>69.7% ibuprofen can be influenced by the dissociation constant and the solubility of the pollutants.

The highest drug impurity desorption efficiency was recorded at a concentration of 0.5M hydrochloric acid, as can be seen in figure 84. All experiments were performed using 1g of adsorbent material and 1mg/L synthetic pollutant solution .

The desorption time was 90 minutes for all pollutants tested in this study. All experiments revealed that the adsorbent material used in this study can be re-used in other adsorption studies.

5.6.2. CONCLUSIONS

This study aims to highlight the use of the TOC technique for the quantification of impurities and active substance in the case of acetaminophen, ibuprofen, ketoprofen and diclofenac from wastewater as total organic carbon (TOC) at very low concentrations of organic pollutants, as well as the use of activated carbon as adsorbent material for drug impurities.

Anti-inflammatory drugs such as paracetamol, diclofenac, ketoprofen and ibuprofen end up in city sewers and break down under certain environmental conditions. Drug impurities or their by-products formed in wastewater must be monitored according to the European legislation in force. At the national level, there is no legislation for monitoring these compounds in wastewater and drinking water.

Following the experiments presented in this article, it was found that the efficiency of drug impurity removal from synthetic wastewater was maximal. In the case of synthetic wastewater with pH= 6, using an amount of 1g of adsorbent material, the required treatment time was 120 minutes for initial drug impurity concentrations of 1 mg/L.

Two mathematical models were used to describe the adsorption processes. Based on the correlation factor (R^2) it can be concluded that the Langmuir model describes well the data for diclofenac and ketoprofen, and the Freundlich model describes well the data for acetaminophen and ibuprofen.

Desorption was carried out separately for each pollutant impurity, and the quantification of drug impurities was carried out by the TOC method. The results obtained in this study also indicate the feasibility of using activated carbon as an adsorbent material for the removal of drug impurities from wastewater.

Adsorption studies involved several parameters such as: pH, contact time, adsorbent dosage and initial acetaminophen concentration. The adsorption study was carried out by adding 0.1 g of adsorbent to a series of 100 ml stoppered Erlenmeyer flasks containing 50 ml of norfloxacin solution. The containers were shaken in a mechanical shaker and samples were collected at preset time intervals. The samples were centrifuged for 5 min and analyzed by HPLC to determine the concentration of norfloxacin in the wastewater solution.

This study consisted in determining the concentration of norfloxacin in a synthetic wastewater, applying the chromatographic method (HPLC) before and after adsorption studies.

The following experimental parameters were studied:

a) pH of the solutions (4, 6 and 8)

b) norfofloxacin concentration (0.5, 1 and 2 mg/L)

c) the amount of adsorbent material 0.1g zeolite

d) contact time (9h)

Influence of pH on the removal efficiency of norfloxacin in the presence of zeolite (ZSM-5)

The influence of pH on the removal efficiency of ZSM-5 was obtained using norfloxacin wastewater solutions adjusted to pH 4 to 8 (Figure 86). The adsorption process is influenced by a number of factors such as: pH value, contact time and initial concentration of norfloxacin.

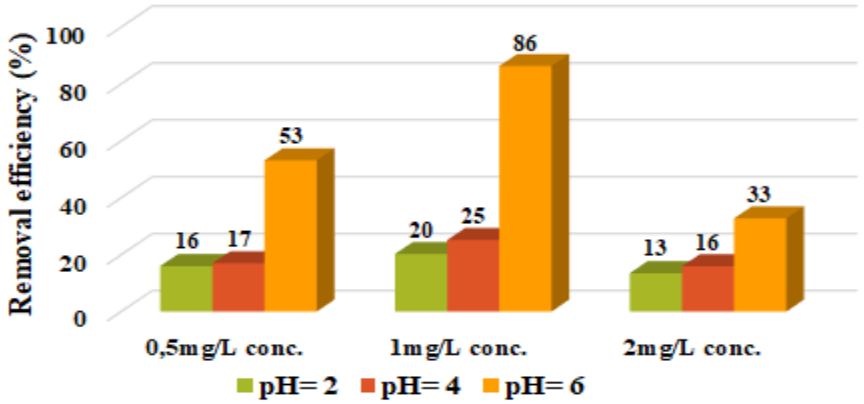


Figure 85. Influence of pH on the removal efficiency of norfloxacin

Using 0.1g of adsorbent material (ZSM-5) the best results were obtained for a removal efficiency of 86% at pH = 6 as seen in figure 85.

The pH effect is due to the fact that at different pH values, the surface of the adsorbent has a negative or positive charge, and the pH value affects the state of norfloxacin in aqueous solution.

Consequently, repulsive or attractive forces may occur between the pollutant and the adsorbent surface. In addition, most industrial effluents and wastewaters loaded with norfloxacin have different pH values depending on the type of industrial activities carried out. Thus, it is necessary to conduct studies to establish the influence of pH value on the adsorption of norfloxacin by ZSM-5 nanomaterial.

The influence of the amount of adsorbent material on the removal efficiency of norfloxacin in the presence of zeolite (ZSM-5)

Adsorbent dosage of 0.1 g (ZSM-5) was used in this study. Adsorption studies were performed using three initial norfloxacin concentrations: 0.5, 1, and 2 mg/L norfloxacin. Figure 87 plots the removal efficiency results.

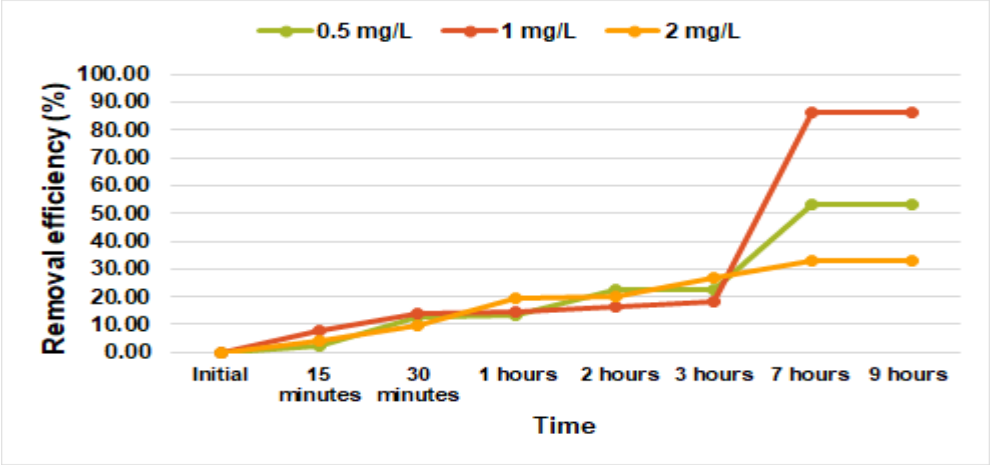


Figure 86. Norfloxacin removal efficiency versus contact time

Based on the results in figure 86, it can be stated that the optimal contact time was 9 hours, and the adsorption process was very fast in the first 7 hours.

After that, a slow increase in adsorption was observed until adsorption equilibrium was reached. In the first stage of rapid adsorption, all adsorption sites of the adsorbent material are available and will be occupied by norfloxacin. As the adsorption of norfloxacin continues, more adsorption sites are successively occupied and, consequently, the number of free sorption sites will decrease and adsorption will proceed more slowly.

The removal efficiency of norfloxacin showed high values at the concentration of 1 mg/L (86.33%) and had low values at the concentration of 2 mg/L (32.78%). At pH=6, a norfloxacin removal efficiency of 86% was recorded.

After a contact time of 9 hours, the zeolite becomes saturated and no longer absorbs norfloxacin. The equivalence point is reached and the adsorption process ends.

Langmuir and Freundlich adsorption isotherms

Figures 87 and 88 show the linear forms of the equilibrium isotherms. These were used to determine the values of the adsorption parameters.

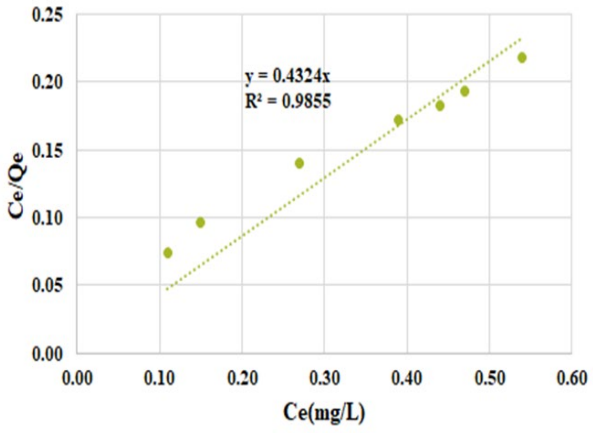


Figure 87. Langmuir linearized form

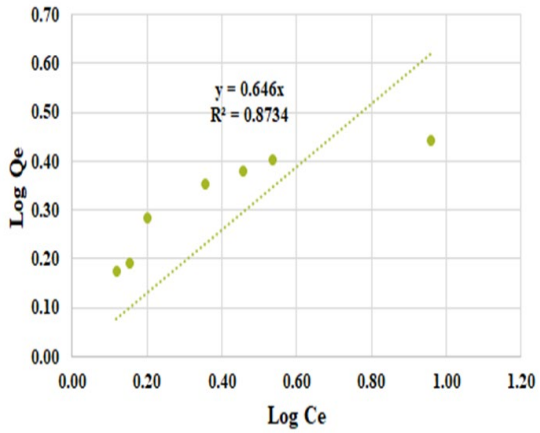


Figure 88. Linearized Freundlich form

The experimental data shown in Figures 87 and 88 and Table 42 showed that the Langmuir isotherm model describes very well the experimental data for the adsorption process of norfofloxacin on zeolite.

Table 42. Langmuir and Freundlich adsorption parameters

Adsorbent material	Langmuir parameters				Freundlich parameters		
	Qmax (mg/g)	**K_L (L/mg)**	**R_L**	**R^2**	**K_F (L/g)**	**1/n**	**R^2**
ZSM-5	74,2	0,119	0,69	0,9855	39,53	0,332	0,8734

The adsorption process of norfloxacin on ZSM-5 was described using the Langmuir and Freundlich equation. Optimizing operating conditions in wastewater treatment design is an important issue involving the adsorption process.

Therefore, establishing the adsorption capacity of adsorbent materials with different isotherm patterns allows the design of a treatment process and the appropriate optimization of operating conditions. The Langmuir equilibrium constant R_L is in the range of (0-1), indicating that the retention process of norfloxacin is favorable using this adsorbent material.

The Freundlich isotherm shows a much lower correlation coefficient (R^2=0.8734) than the Langmuir isotherm, as can be seen in Figures 87 and 88.

It can be seen from Figure 86 that the adsorption of norfloxacin decreases with increasing pH of the wastewater solution and the maximum adsorption of norfloxacin is obtained at a pH of 6.

Desorption studies

After 9 hours from the start of the adsorption studies, the adsorbent material reached the saturation threshold and it was necessary to regenerate it with a nitric acid solution of different concentrations (0.5M, 1M, 3M and 5M).

Parameters studied:

a) 0.1g zeolite saturated in norfloxacin

b) HNO_3 concentration (0.1M, 0.3M, 0.5M)

c) Contact time 45 minutes

d) Stirring speed 250rpm

The quantification of norfloxacin obtained after the desorption step was carried out using the TOC method.

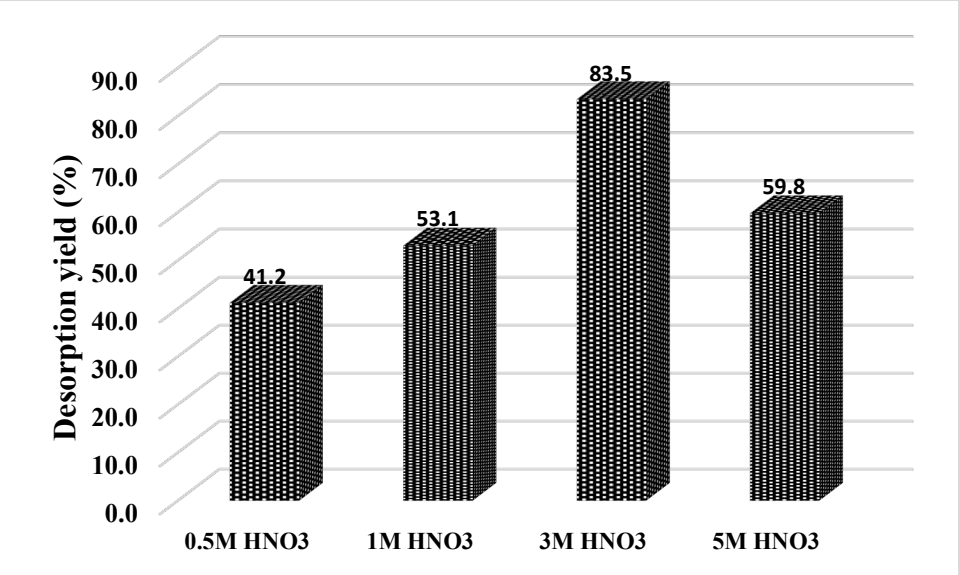


Figure 89. Desorption yield at different concentrations of nitric acid

Only a dose of 0.1 g of adsorbent was used in each experiment (figure 89).

The highest desorption yields (83.5%) were obtained using 3M HNO_3. The regeneration of zeolite (ZSM-5) indicates the feasibility of using this material for the removal of norfloxacin from wastewater and the possibility of re-using these materials. After 4 cycles of adsorption-desorption, the zeolites can no longer be reused. All site pores are occupied.

5.7.2. CONCLUSIONS

ZSM-5 adsorbent material was used to remove norfloxacin from wastewater. The highest removal efficiency (86%) of norfloxacin was obtained at a pH value of 6 and using 0.1g of adsorbent material and at a norfloxacin concentration of 1 mg/L

Adsorption data were evaluated using two mathematical models (Langmuir and Freundlich). It was found that, the adsorption process was well characterized by the Langmuir adsorption isotherm. The equilibrium parameter (RL) for the Langmuir isotherm was in the range of 0-1 (RL=0.69), which indicates that the adsorption process is favorable for this type of adsorbent material (ZSM-5).

Adsorption studies for the removal of emerging environmental pollutants are limited, and strategies could be developed to improve the efficiency of treatment plants. The zeolites can be reused after the desorption process (desorption yield 83.5%), in new adsorption studies.

The proposed HPLC method for the determination of norfofloxacin is simple, sensitive, rapid, specific, and could be applied to monitor norfloxacin and its secondary products in wastewater.

5.8.1. Inhibition of bacterial growth in the presence of norfloxacin after the adsorption step on zeolites

To understand how antibiotics can destroy the bacterial cell we can focus on the cellular function inhibited by the main drug-target interaction. Antibiotics can be classified, according to the cellular system they affect, if they induce cell destruction, bactericidal drugs, or only inhibit cell growth, bacteriostatic drugs.

Most current bactericidal antimicrobials inhibit DNA synthesis, RNA synthesis, cell wall synthesis or protein synthesis. The removal of antibiotics can be achieved by adsorption with zeolites or by bacterial biodegradation. The combination should be investigated for further application in the elimination of antibiotics. The study investigated the effect of zeolite antibiotic adsorption on bacterial growth by disc diffusiometric method using the tested Gram-negative reference strain, Escherichia Coli - ATCC 25922 (E. coli). The growth of the bacterial strain (Escherichia. Coli) was carried out on Mueller Hinton Agar (MH) nutrient medium. A pure culture of E. coli was distributed in a petri dish with MH medium using the tissue seeding technique. Qualitative sensitivity screening was performed by a diffusion-adapted method on Mueller Hinton solid medium previously seeded with a bacterial inoculum adjusted to a density corresponding to the McFarland standard of 0.5.

The adsorption study was performed by adding 0.1 g of zeolites to a solution of 0.5 mg/L and 2 mg/L norfloxacin for 5 minutes at room temperature (approximately 22°C) in a series of conical flasks of 250 mL. After incubation, the supernatant was used in the disk diffusion assay.
Disc diffusion testing is performed according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines. The disk diffusion test was the main method of antimicrobial susceptibility testing (AST) in this study. Diffusion discs were soaked in the supernatant resulting from adsorption tests between zeolites and antibiotics.
The soaked discs were applied to the petri dish at a distance of approximately 2 cm from the edge of the petri dish and at a distance of 3 cm from each other, then incubated at 35°C for 22 hours. A microorganism is considered sensitive or resistant depending on the diameter of the zone of inhibition of culture growth.

The diffusimetric method of testing the sensitivity to antibacterial substances is a semi-quantitative evaluation of the sensitivity to antimicrobial substances of aerobic bacteria, of fast-growing pathogenic bacteria, and of some bacteria that are more pretentious to the cultivation conditions (Escherichia coli.), using in our case solutions with content of antibacterial substances in certain concentrations, applied on the surface of Mueller Hinton agar, previously inoculated with bacterial suspension from the strain under test. The existing antimicrobial substance (norfloxacin) diffuses into the environment previously inoculated with the bacterial strain to be tested, the consequence being the appearance of a circular zone of inhibition, the diameter of which is dependent on the sensitivity of the tested bacterial strain.

5.8.2. CONCLUSIONS

Zeolites can be used to adsorb drug compounds, fluoroquinolones in this study, norfloxacin. The mechanism of norfloxacin adsorption on zeolites and inhibition of E. coli growth may involve several steps:

Physical adsorption: Norfloxacin binds to the zeolite surface through physical bonds such as van der Waals forces or hydrogen bonds. This process may allow the norfloxacin molecule to remain on the zeolite surface.

Ion exchange: Zeolites are known for their ion exchange properties. Norfloxacin can enter the zeolite structure replacing ions in the zeolite structure. This ion exchange can be an effective way to adsorb the antibiotic.

Chemical interaction: Norfloxacin can form chemical bonds with functional groups present on zeolites, such as hydroxyl or silanol groups, through covalent or ionic chemical bonds. One of these steps or a combination of them may be responsible for the adsorption of norfloxacin on the zeolites. Once norfloxacin is adsorbed by zeolites, its concentration in solution can be reduced, which can lead to the inhibition of bacterial growth of E. coli. Antibiotics adsorbed on zeolites are no longer available to affect the bacterial strain, and these processes may vary depending on the experimental conditions.

The removal efficiency of antibiotics by zeolites was tested by the disk diffusion method. In the first step, it was shown that the bacterial inhibition was directly related to the concentration of the antibiotic from 2 mg/L to 0.5 mg/L. Bacterial inhibition was directly related to antibiotic concentration 2 mg/L versus 0.5 mg/L (Figure 90), the inhibitory effect of antibiotics on bacterial strains decreased. The results showed that the addition of zeolites to the antibiotic solutions reduced the inhibitory effect on the bacterial strains of the 2mg/L antibiotic. The removal of antibiotics by adsorption on zeolites led to a decrease in the antibiotic concentration and, implicitly, to a decrease in the inhibition of bacterial growth.

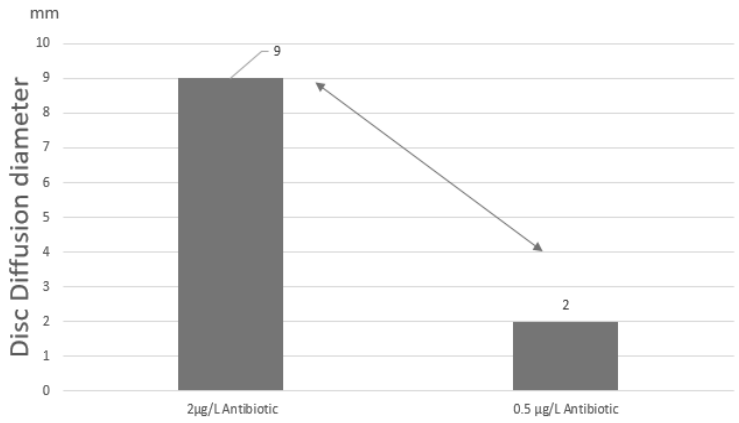


Figure 90. Inhibition of bacterial growth

Adding 0.1 g of zeolite to the test solution moderates the effect of the antibiotic at a concentration of 2 mg/L on bacterial growth, so that the inhibition effect of bacteria decreased (Figure 90) The inhibition zones appear around the discs, which means no growth bacterial. The diameters of the inhibition zones are read in millimeters (mm) and are compared to the other discs (figure 91).

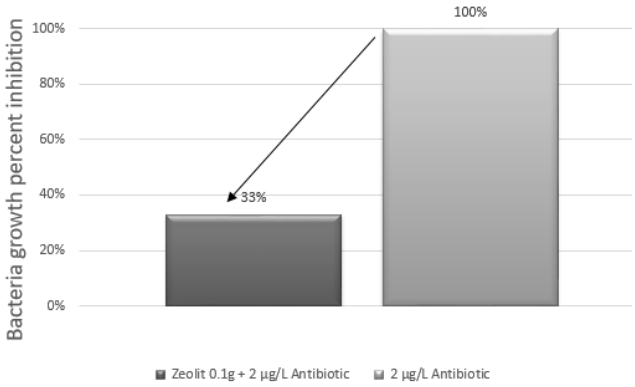


Figure 91. Zeolite modulated the effect on bacterial growth under the influence of the antibiotic

Zeolites exhibit intrinsic properties such as size, pore uniformity, thermal stability, mobile cations and surface properties, hydrophobicity and hydrophilicity. These characteristics lead to a number of applications of zeolites, such as catalysis, ion exchange, water treatment, as well as various applications in the medical field.

5.9. Comparison of adsorbent materials used in adsorption study from the treatment technique

For the comparison of adsorbent materials (activated carbon, ZSM-5, Fe_3O_4 and carbon nanotubes), the results obtained from the application of mathematical models were used to describe the optimal adsorption process of the 5 drugs and their use in the treatment of water containing compounds pharmaceuticals.

Next, the most relevant obtained will be presented, based on the obtained regression coefficients (R^2).

Table 43. Results obtained for regression coefficients after applying mathematical models

Adsorbent material	**Pollutant**	**Adsorption isotherms**	
		Langmuir (R^2)	**Freundlich (R^2)**
Active coal	Acetaminophen	0,9826	0,9996
	Ibuprofen	0,9655	0,9991
	Diclofenac	0,9874	0,9491
	Ketoprofen	0,9999	0,8598
Zeolites (ZSM-5)	Acetaminophen	0,9510	0,9204
	Ibuprofen	0,9193	0,8958
	Diclofenac	0,9011	0,7583
	Ketoprofen	0,8909	0,7452
	Norfofloxacina	0,9855	0,8734
Carbon nanotubes(CNT)	Acetaminophen	0,9959	0,9834
Magnetite (Fe_3O_4)	Acetaminophen	0,9604	0,9325

As can be seen from the table above, the Langmuir isotherm best characterizes the adsorption process of the 5 pollutants. Regression coefficients for the Langmuir isotherm range from 0.8909 to 0.9999 compared to regression coefficients for the Freundlich isotherm which range from 0.7452 to 0.9996.

Table 44. Factors influencing pollutant removal efficiency

Adsorbent material	**Pollutant**	**pH**	**Contact time**	**The concentration of the pollutant (mg/L)**	**The amount of adsorbent material (d)**	**Maximum removal efficiency (%)**
Active coal	Acetaminophen	6	10 min	1 mg/L	1g	98 %
	Ibuprofen	6	10 min	1 mg/L	1g	96 %
	Diclofenac	6	10 min	1 mg/L	1g	92 %
	Ketoprofen	6	10 min	1 mg/L	1g	88 %
Zeolites (ZSM-5)	Acetaminophen	4	110 min	1 mg/L	0,2g	81 %
	Ibuprofen	4	110 min	1 mg/L	0,2g	70 %
	Diclofenac	4	110 min	1 mg/L	0,2g	72 %
	Ketoprofen	4	110 min	1 mg/L	0,2g	57 %
	Norfofloxacina	6	7 h	1 mg/L	0,1g	86 %
Carbon nanotubes (CNT)	Acetaminophen	6	3h	25 mg/L	0,5g	85%
Magnetite (Fe_3O_4)	Acetaminophen	6	200 min	50 mg/L	0,5g	84%

As can be seen from table 44, the four factors (pH, contact time, initial pollutant concentration, as well as the amount of adsorbent material significantly influence the adsorption process. Based on the characteristics of the adsorbent material (specific surface area, pore size and the functional groups it has), the pollutants were selected and the adsorption studies were carried out.

Based on the results obtained in this study, it can be said, however, that activated carbon is the best adsorbent material for anti-inflammatory compounds. Zeolite type adsorbent material is suitable for the adsorption of norfloxacin from wastewater.

6. FINAL CONCLUSIONS. ORIGINAL CONTRIBUTIONS. PERSPECTIVES

6.1. FINAL CONCLUSIONS

The objectives of the doctoral thesis, **"TREATMENT OF WATER CONTAINING PHARMACEUTICAL COMPOUNDS"** were achieved by implementing an experimental program designed in such a way that the research is carried out in a unitary and coherent manner for the study of the treatment process of the elimination of non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics (norfloxacin) from wastewater with the help of nanomaterials and materials with adsorbent role: Fe_3O_4, zeolites, carbon nanotubes and activated carbon.

The choice of these classes of drugs was not accidental. Taking into account the type of active substances found in many medicinal products, as well as their large-scale use in hospitals

as well as by the population frequently, due to their pharmacokinetic action they can end up in considerable quantities in sewage treatment plants.

Current technologies have limitations: incomplete removal of pollutants, high energy consumption, obtaining toxic sludge during the wastewater treatment process, which can lead to expensive systems and implicitly to increase the price of drinking water. Technological advances in studying the properties of nanomaterials may highlight opportunities to harness them for sustainable water management. The treatment processes of the drugs encountered in the literature are varied, but the choice of the adsorbent material is very important in these studies which may depend both on the characteristics of the adsorbent material and on the physico-chemical properties of the medicinal product studied.

In order to fulfill the objectives of the doctoral thesis, methods for the determination of NSAIDs and FQs were developed and validated in the laboratory, as well as their removal studies characterized mostly based on Langmuir and Freundlich isotherms.

6.2. ORIGINAL CONTRIBUTIONS

In the conducted study, an HPLC-DAD method was developed and validated for the detection and quantification of acetaminophen (ACF), ketoprofen (KTF), diclofenac (DCF) and ibuprofen (IBU) in wastewater samples. This method, with a separation time of only 10 min, was optimized to rapidly separate these four analytes at concentrations of the order of µg/L in complex wastewater matrices.

The HPLC-DAD method had an average recovery yield of 95.99% for ACF, 83.92% for KTF, 81.45% for DCF, and 90.22% for IBU. In terms of precision, the RSD was between 0.11% and 0.29% for repeatability and between 0.23% and 0.40% for intermediate precision in the case of the direct injection method. Limits of quantification were 0.20 µg/L for acetaminophen, 0.46 µg/L for ketoprofen, 0.60 µg/L for diclofenac, and 0.14 µg/L for ibuprofen. The expanded uncertainty of the analytical method was 12%.

In addition, the performance parameters of a method for the determination of acetaminophen using UV-VIS spectroscopy were determined in two different media: 0.1 M HCl and 0.01 M KOH. For 0.1 M HCl, the LOD and LOQ have were 0.04 and 0.12, respectively, and for 0.01 M KOH, the limit of detection was 0.06 and the limit of quantification was 0.18.

Using zeolites as adsorbent materials, the highest adsorption capacity was recorded in the order: ibuprofen (21.9 mg/g) > acetaminophen (20.5 mg/g) > diclofenac (16.7 mg/g) > ketoprofen (15 .9 mg/g) when 200 mg of zeolite was used.

The developed and validated HPLC method was also applied in adsorption tests on activated carbon for the four pharmaceutical compounds. Parameters such as pH (4 and 6), contact time (0-120 minutes), initial concentration of pollutants (1, 5 and 10 mg/L), amount of adsorbent (0.1 g, 0, 5 g and 1 g) and stirring speed (150 rpm and 250 rpm).

The highest removal efficiency (%) was recorded for acetaminophen (98%) > ibuprofen (96%) > diclofenac (92%) > ketoprofen (88%) when an amount of 1 g of adsorbent material was used, a concentration of pollutants of 1 mg/L and a stirring speed of 250 rpm at a pH of 6.

To characterize the adsorption process, Langmuir and Freundlich mathematical models were applied. The results obtained indicated that the Langmuir model (R^2=0.99) presented higher correlation coefficients (R^2) compared to the Freundlich model (R^2=0.98). Thus, the Langmuir model is suitable for describing the complete adsorption process on activated carbon.

In addition to the analysis of the compounds of interest, their degradation products in the form of total organic carbon (TOC) were also quantified. TOC values varied depending on the tested compound and its concentration (1, 5, 10 and 20 mg/L). The TOC results for the certified reference materials (CRMs) were consistent with the uncertainty of the analytical method. HPLC and TOC methods for the quantification of analytes and their impurities can be useful in evaluating the removal of the four anti-inflammatories from the wastewater matrix.

Two adsorbent materials, Fe_3O_4 and ZSM-5, were used to remove acetaminophen from wastewater. Fe_3O_4 showed the highest adsorption capacity (68.9 mg/g) and the best removal efficiency (84.6%) at pH 6.

Adsorption studies for the removal of emergent pollutants from wastewater are still limited, and the development of strategies to improve the efficiency of treatment plants would be beneficial. The proposed method for monitoring acetaminophen and its degradation products in wastewater is simple, sensitive, rapid and specific.

The influence of parameters (pH, contact time and dissolution medium) on the adsorption of acetaminophen from wastewater was investigated, as well as different mathematical models (SIPS, Langmuir and Freundlich) to describe its adsorption process. Prior to adsorption studies for acetaminophen, a method for its determination by UV-VIS was developed in-house. This internally validated method has been shown to be simple, sensitive, rapid and specific and can be effectively used in the monitoring of acetaminophen.

The removal of acetaminophen using carbon nanotubes was more efficient in 0.1M HCl medium compared to 0.01M KOH medium. The adsorption process was analyzed using SIPS, Langmuir and Redlich Peterson isotherms. The SIPS isotherm was successfully applied to the adsorption of acetaminophen in 0.1M HCl and 0.01M KOH media. The results of this study highlighted the effectiveness of carbon nanotubes in removing acetaminophen and its degradation products from wastewater. Based on the correlation coefficients, the SIPS isotherm model highlighted the adsorption process in acidic medium very well. The order of correlation coefficients was as follows for 0.1M HCl medium: SIPS (R^2=0.9075), Langmuir (R^2=0.8989) and Redlich-Peterson (R^2=0.8201). For 0.01M KOH medium, the order of correlation coefficients was: SIPS (R^2=0.8163), Langmuir (R^2=0.7797) and Redlich-Peterson (R^2=0.7877).

In conclusion, the removal efficiency of paracetamol by using carbon nanotubes can be improved in an acidic environment. In this study, the maximum removal efficiency of acetaminophen was achieved at a pH of 6 with a contact time of 8 h and using 0.50 g of adsorbent material. The increased amount of adsorbent material had a significant impact on the adsorption process. In 0.1M HCl medium, the removal efficiency increased from 49.50% with 0.25g adsorbent material to 84.60% with 0.50g adsorbent material. In 0.01M KOH medium, the removal efficiency increased from 39.70% (with 0.25g adsorbent material) to 65.20% (with 0.50g adsorbent material).

Research on the adsorption of pharmaceutical compounds from the environment is constantly on the rise. It is desired to introduce new techniques for the treatment and constant data collection of pharmaceutical compounds present in environmental matrices.

This study was carried out to find compatible adsorbent materials for the retention of paracetamol from wastewater. Nano-structured materials such as zeolites and carbon nanotubes can be used to retain paracetamol from wastewater.

The adsorption capacity of carbon nanotubes is higher (88.9mg/g) compared to the adsorption capacity of zeolites (64.5mg/g). The Langmuir isotherm better describes the experimental data obtained for the two adsorbent materials because higher adsorption capacities and correlation factors were obtained compared to the Freundlich and SIPS isotherms.

The method for the determination of paracetamol by the UV-VIS method is simple, sensitive, fast, specific and could be applied for the monitoring of paracetamol and its degradation products in wastewater. The results show that the CNT adsorption capacity for paracetamol in 0.1 M HCl medium was higher compared to the results obtained for paracetamol dissolved in 0.01 M KOH.

As part of the doctoral thesis, an HPLC-DAD method was also developed and validated for the determination of norfloxacin and ciprofloxacin in wastewater samples by an HPLC-FLD method with a separation time of only 7 minutes.

All liquid-chromatographic conditions (nature and composition of the mobile phase, injection volume, detection wavelength, column temperature, etc.) were optimized for the rapid separation of the 2 analytes with high sensitivity for the determination of these antibiotics in complex matrices of waste water. The recovery yields of the HPLC-FLD method were 87.2% for norfloxacin and 90.3% for ciprofloxacin.

Regarding the precision of the developed and validated HPLC method, RSD values of 4.17% for norfloxacin and 3.14% for ciprofloxacin were obtained for repeatability and 6.87% RSD for norfloxacin and 7.03 % for ciprofloxacin, intermediate precision performed on repeated surface water samples. The wastewater precision data obtained for norfloxacin were: RSD % repeatability 7.78 and RSD% intermediate precision 11.20, and in the case of ciprofloxacin were: RSD % repeatability 6.71 and RSD% intermediate precision, 13.20%.

ZSM-5 adsorbent material was used to remove norfloxacin from wastewater.

The highest removal efficiency (86%) of norfloxacin was obtained at a pH value of 6 and using 0.1g of adsorbent material and at a norfloxacin concentration of 1 mg/L

Adsorption data were evaluated using two mathematical models (Langmuir and Freundlich). It was found that, the adsorption process was well characterized by the Langmuir adsorption isotherm. The equilibrium parameter (R_L) for the Langmuir isotherm was in the range of 0-1 (R_L=0.69), which indicates that the adsorption process is favorable for this type of adsorbent material (ZSM-5).

Adsorption studies for the removal of emerging environmental pollutants are limited, and strategies could be developed to improve the efficiency of treatment plants. The zeolites can be reused after the desorption process (desorption yield 83.5%), in new adsorption studies.

The proposed HPLC-FLD method for the determination of norfloxacin is simple, sensitive, rapid, specific and could be applied to improve the treatment technique of norfloxacin and its secondary products from existing environmental waters.

Zeolites can be used to absorb drug compounds such as fluoroquinolones, in this case, norfloxacin, according to this study. The mechanism of norfloxacin adsorption on zeolites and inhibition of E. coli growth may involve several steps:

Physical adsorption: Norfloxacin attaches to the zeolite surface by physical forces such as Van der Waals forces or hydrogen bonding. This process allows the norfloxacin molecules to remain on the zeolite surface.

Ion exchange: Zeolites are known for their ion exchange capabilities. Norfloxacin can enter the zeolite structure replacing ions from this structure. This ion exchange can be an effective way to adsorb the antibiotic.

Chemical interaction: Norfloxacin can form chemical bonds with functional groups present on zeolites, such as hydroxyl or silanol groups, through covalent or ionic chemical bonds. One of these mechanisms or a combination of them could be responsible for the adsorption of norfloxacin on zeolites.

Once norfloxacin is adsorbed by zeolites, its concentration in solution can be reduced, which can lead to the inhibition of bacterial growth of E.coli. Antibiotics adsorbed on zeolites are no longer available to affect the bacterial strain, and the efficiency of these processes can vary depending on the experimental conditions. The removal efficiency of antibiotics by zeolites was evaluated by the disk diffusion method. In a first step, it was shown that the bacterial inhibition is directly related to the concentration of the antibiotic, going from 2 mg/L to 0.5 mg/L. The amount of antibiotic (norfloxacin) adsorbed on zeolite is detailed in chapter 5.8.9. Bacterial inhibition was directly related to the antibiotic concentration of 2 mg/L compared to 0.5 mg/L (Figure 91), showing a decrease in the inhibitory effect of antibiotics on bacterial strains.

The results showed that the addition of zeolites to the antibiotic solutions reduced the inhibitory effect on the bacterial strains of the 2mg/L antibiotic. The removal of antibiotics by adsorption on zeolites led to a decrease in the antibiotic concentration and, implicitly, to a decrease in the inhibition of bacterial growth.

The experimental studies carried out will be able to constitute a necessary database for the improvement of the treatment techniques of pharmaceutical pollutants from different water matrices in the environment.

PERSPECTIVES

- Adsorption studies will be continued for compounds from the class of antibiotics (norfloxacin, ciprofloxacin, etc.), for other types of adsorbent materials (CNT, active carbon, magnetite)

- On the basis of the obtained results, the submission of proposals for demonstrative experimental projects will be attempted.

RESEARCH ACTIVITY
PUBLISHED ARTICLES

1. **F. Pirvu**, C. I. Covaliu-Mierla, I. Paun, G, Paraschiv and V. Iancu, (2022), Treatment of Wastewater Containing Nonsteroidal Anti-Inflammatory Drugs Using Activated Carbon Material, Materials 2022, 15, 559. https://doi.org/10.3390/ma15020559, Impact Factor: 3.4;

2. **F. Pirvu**, C. I. Covaliu-Mierla, and G. A. Catrina, (2023), Removal of Acetaminophen Drug from Wastewater by Fe3O4 and ZSM-5 Materials, Nanomaterials 2023, 13, 1745. https://doi.org/10.3390/nano13111745, Impact Factor: 5.3;

3. L. Covaliu, **F. Pirvu**, C. I. Covaliu-Mierla (2023), Ecotechnology removal from wastewater of four anti-inflammatory drugs using activated carbon material, Scientific Bulletin, Series B Chemistry and Materials Science, ISSN 1454-2331, recenzat in curs de publicare .

SCIENTIFIC COMMUNICATIONS

1. F. Pîrvu, A G Catrina, C. I. Butnariu, G. Paraschiv, *Treatment of wastewater containing pharmaceutical products using nanoadsorbants,* 1 st International Conference on Emerging Technologies in Materials Engineering EmergeMAT 4 th International Workshop on Materials under Extreme Conditions SUPERMAT 14-16 November 2018 – Bucharest, Romania

2**. Pîrvu F,** Butnariu C. I., Catrina G. A., Paraschiv G., *Pharmaceutical products removal from wastewater,* International Symposium ISB-INMA TEH' 2018, "Agricultural and Mechanical Engineering" - co-organizator împreună cu INMA București 1-3 november –Bucharest, Romania

3.C. I. Butnariu, A. Mircea, **F. Pîrvu**, A. G. Catrina, G. Paraschiv, *Nanoparticles application in wastewater treatment by flotation*, 1 st International Conference on Emerging Technologies in Materials Engineering EmergeMAT 4 th International Workshop on Materials under Extreme Conditions SUPERMAT 14-16 November 2018 – Bucharest, Romania;

4.C. I. Butnariu, M. Turcan, E. Matei, **F. Pîrvu**, A. G. Catrina, G. Paraschiv, *Depollution of wastewaters using nanobiotechnology based on new TiO2 photocatalyst*, 1 st International Conference on Emerging Technologies in Materials Engineering EmergeMAT 4 th International Workshop on Materials under Extreme Conditions SUPERMAT 14-16 November 2018 – Bucharest, Romania

5.C. I. Butnariu, O. Stoian, A. G. Catrina, **F. Pîrvu**, G. Paraschiv, *Wastewater treatment using zeolite*, 1 st International Conference on Emerging Technologies in Materials Engineering EmergeMAT 4 th International Workshop on Materials under Extreme Conditions SUPERMAT 14 -16 November 2018 – Bucharest, Romania

6.C.I. Covaliu, G. Paraschiv, G.A. Catrina (Traistaru), **F. Pirvu**, *Magnetite and zeolite nanomaterials for removal of acetaminophen from wastewater*, 12thInternational Conference on Materials Science & Engineering BraMat 2019, 13-16 martie Brasov, Romania, pp.22, http://www.bramat.ro/uploads/7/7/4/0/77408170/program_bramat2019m.pdf

8. **F. Pirvu**, C. I. Covaliu, G. Paraschiv, I. Paun, G. A. Catrina (Traistaru), *Preliminary study of THE removal of acetaminophen from wastewater*, International Symposium "The Environment and the Industry", E-SIMI 2020, http://doi.org/10.21698/simi.2020.ab17

10. **F. Pirvu**, C. I. Covaliu, I. Paun, V. Iancu, N. Vasilache, M. Niculescu, G. Serban, F. L. Chiriac, *Adsorption of Non-Steroidal Anti-inflammatory Pharmaceutical Residues using Activated Carbon from* Wastewater, International Symposium "The Environment and the Industry", E-SIMI 2021, http://doi.org/10.21698/simi.2021.ab19

11. **F. Pirvu**, C. I. Covaliu-Mierlă, C. Cîrtoaje, I. Jipa, G. Paraschiv, (2023), Acetaminophen drug removal from wastewater using carbon nanotubes nanomaterials, **ICIR Euroinvent 2023,**

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