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TETRACYCLINE (TC) ADSORPTION FROM AQUEOUS SOLUTIONS

By

Sorelis Nieto Zambrano

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Approved by:

Oscar Perales-Pérez, PhD
Member, Graduate Committee

Date

Aidalu Joubert, PhD
Member, Graduate Committee

Date

Félix R. Román, PhD
President, Graduate Committee

Date

Carlos Velázquez Figueroa, PhD
Representative of Graduate Studies

Date

Francis Patrón, PhD
Interim Chairperson of the Chemistry Department

Date

ABSTRACT

The adsorption behavior of tetracycline (the most common antibiotic in veterinary) onto carbon black and different size of tires crumb rubber in aqueous medium was evaluated. Tetracycline samples at pH 3.80 with exposition times up to 168 hours at room temperature were placed in contact with crumb rubber, carbon black in the presence and absence of Zn and EDTA. The samples were removed at different time intervals and analyzed by UV-Vis spectrophotometer, liquid chromatography coupled to mass spectrometer (LC-MS) and diode array detection. The results showed that the crumb rubber adsorbed tetracycline (TC) however the sorption capacity was dependent on mesh size, presence of Zn in solution, acid wash and the addition of EDTA as a metal complexing agent. TC removal was variable. At 168 h, the TC removal by the tires crumb rubber of mesh 14-20 and mesh 30 were 48.22 % and 59.80 %, respectively. These results were compared with carbon black present in crumb rubber matrix at 10.97 – 45.60 %, w/w. TC was completely removed from aqueous solution placed in contact with carbon black after 24 hours suggesting its involvement in the TC adsorption by crumb rubber. The analysis also suggested that metals (mainly Zn) present in tires crumb rubber influence the TC adsorption process.

We consider that this work will contribute to expand the tires recycling options using the tires crumb rubber or carbon black to remove TC from aqueous medium.

RESUMEN

El comportamiento de adsorción de tetraciclina (el antibiótico más común en veterinaria) sobre carbón black y diferentes tamaños de partículas de caucho de llantas fue evaluado. Muestras de tetraciclina a pH 3.80 con tiempos de exposición hasta 168 horas a temperatura de laboratorio fueron colocadas en contacto con partículas de caucho, carbón black en presencia y ausencia de Zn y EDTA. Las muestras fueron removidas a diferentes intervalos de tiempo y analizadas por detección con arreglo de diodo, cromatografía líquida acoplada a espectrómetro de masas (LC/MS) y espectrofotómetro UV-Vis. Los resultados mostraron que las partículas de caucho adsorbieron tetraciclina (TC), sin embargo, la capacidad de absorción dependió del tamaño de malla, presencia de Zn en solución, lavado ácido y la adición de EDTA como un agente acomplejante de metales. La remoción de TC fue variable. A 168 horas, la remoción de TC por partículas de caucho de llantas con malla 14-20 y malla 30 fueron 48.22% y 59.80 %, respectivamente. Esos resultados fueron comparados con carbón black presente en la matriz de las partículas de caucho en 10.97 - 45.60 %, P/P. La TC fue completamente removida de la solución acuosa colocada en contacto con carbón black después de 24 horas, sugiriendo su participación en la adsorción de TC por las partículas de caucho. El análisis sugirió que metales (principalmente Zn) presentes en las partículas de caucho de llantas influyen el proceso de absorción de TC. Consideramos que este trabajo contribuirá a expandir las opciones de reciclaje de llantas para remover TC de medio acuoso.

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To God,

To my mom, to my family

When you decide change the things, it's because you learn that dreams are only for becoming reality.

ACKNOWLEDGEMENTS

I will start by giving enormous thanks to God because He has been my fortress during these years of study, my helper and my faithful friend. Thanks to my mother for her patience and love: “because the good times will arrive”. I want to express sincere gratefulness to my advisor, Dr. Félix R. Román because he gave me the opportunity to do research in his group. I received much encouragement and support from his during all this investigation. I will always appreciate that. I am also very grateful to Dr. Oscar Perales- Pérez for his support and for being another advisor to me. He was very interested in my research until its completion. Thanks to Dra. Aidalu Joubert for accept to be part of my graduate committee.

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CHAPTER I

1. INTRODUCTION

In recent years, there has been growing interest in the occurrence, fate and possible effects of human and veterinary drugs residues in the environment (e.g. antimicrobials) that affect the water quality and soils. Today, up to 80 pharmaceuticals compounds have been identified and quantified in the range of nanograms to micrograms per liter including tetracyclines¹⁻². Studies performed in the United Kingdom, Denmark, Germany, and the United States reveal that these agents represent a new class of environmental contaminants worldwide³⁻⁷. There is concern about effects resulting from the entry of these compounds into the environment, including the link between antibacterial use in agriculture and antibacterial – resistance infections and possible effects on the endocrine system⁷⁻¹⁰. Recently, sophisticated analytical technique, liquid chromatography combined with tandem mass spectrometry (LC/MS/MS), has led to the detection of tetracyclines (TC) on farmed land at concentrations of up to 300 µg/Kg soil, which demonstrated that this group of antibiotics (specially TC) are persistent and can accumulate in soil after repeat fertilization with liquid manure from intensive animal farming⁷. Several field studies gave no proof of TC leaching of these compounds into deeper soil segments or into groundwater because of the strong sorption of the drugs in topsoil, thus, it is very probable to find them in superficial water bodies¹¹. In 1981 in Great Britain

revealed that pharmaceuticals was present in rivers up to 1 µg/L³. It has been identified on Iona Island (Vancouver, Canada), ibuprofen and naproxen in waste water⁷. Recent investigations showed the exposure of a wide range of pharmaceuticals (e.g., betablockers, sympathomimetics, antiprogestics, lipid regulators, antiepileptics, antibiotics, vasodilators) to rivers and creeks. Ternes et al (2002) reported the identification of pharmaceuticals in the aquatic environments. Further, a reconnaissance study by the United States Geological Survey (USGS)² reported detectable levels of tetracyclines in several rivers and streams from many parts of the USA. Tetracycline was detected between 0.05 µg/L to 0.10 µg/L, oxytetracycline at 0.10 µg/L and chlortetracycline in 0.05 to 0.10 µg/L.^{2, 4, 6}

Actually, there are no regulations for antibiotics in water; however the USGS investigations may lead to this direction. The European Commission had set maximum residues limits (MRL) for tetracyclines in muscle (100 µg/Kg), kidney (600 µg/Kg), egg (200 µg/Kg) and milk (100 µg/Kg)¹². The MRL are the sum of oxytetracycline, tetracycline, chlortetracycline and their 4-epimers. In the USA exists a similar MRL for kidney (600 µg/L), liver (300 µg/L), eggs (200 µg/L), muscle (100 µg/L) and milk (100 µg/L).^{12, 13, 14}

The use of these antibiotics in agriculture began in 1950 with oxytetracycline and chlortetracycline. Environmental evaluations of these drugs have been required by Food and Drugs Administration (FDA), since 1980 and in the Europe Community since 1997.¹⁵

1.1 STATEMENT OF THE PROBLEM

It is estimated that the use of antibacterial in the agriculture in the USA, oscillate from 92,500 to 196,400 kg/year¹⁵. On to ground have been found up to 300 µg/Kg soil⁷ and in municipal waste-water have been detected up to 4,00 µg/L¹⁶. The interest in these compounds is related with the increase in antibacterial resistance and/or possible effects on the endocrine system. These antibacterials have been classified as new environmental micro-contaminants⁷⁻¹⁰.

It is considered that 50 to 80 percentages of the original dosed tetracyclines are recoverable in the animal urine due to several factors which can be influence the renal elimination, including the animal age, the administration route, the urine pH and the tetracycline that was used. Large-scale use of tetracyclines and several other veterinary drugs (e.g. various sulphonamides, tylosin) in cow, hen, chicken, pig production is common not only within the European Union but also in the United States, China, Southeast Asia and Russia. Theses drugs are in use or have been in use for many years as feed additives that allow animals to be brought to market faster at a lower cost because they serve as growth promoters and for prophylactic, metaphylactic and therapeutic purposes¹⁵.

On the other hand, the disposal of tires represents a major environmental issue throughout the world, because the same properties that make them desirable, most notably the durability, complicate their disposal and reprocessing. They are almost immune to biological degradation. In order to prevent waste rubber, especially discarded automobile tires from damaging the environment, it is highly desirable to find ways to recycle this material. According with EPA waste management¹⁷, in the United States, were discarded approximately 290.000.000 tires/year in 2003. Around 80.4 % of 290.000.000 discarded, 44.7% were used as fuel, 19.4% recycled or used in civil engineering projects, 7.8 % converted into ground rubber and recycled, 4.3 % used in rubber-modified asphalt and 3.1 % were exported. In Puerto Rico are discarded annually 4 –million of tires and less than 7 % are recycled^{18, 19}.

Our project addresses the issue of antimicrobials in the environment and the environmental concern related to the waste tires. The first one is to find effective adsorbents that are likely to be put in place to reduce the probability of tetracycline's making their way into water bodies. The second one makes adequately waste tire disposal and management of the millions of tires discarded in the United States that cause many public health and aesthetic problems. The disposal of waste tire and its proper management is a prime issue for environmental discussion. Legislation is important in managing the waste tire problem; however alternative use to disposal represents best long-term solutions.

1.2 RESEARCH HYPOTHESIS

The purpose of this research was to assess the potential use of tires crumb rubber as adsorbent for tetracycline antibiotic from aqueous solutions.

The potential advantage of our research project includes:

- a) To expand the use tires recycling options
- b) The use of crumb rubber as cheap adsorbent, which can be used to remove environmental contaminants including tetracycline.

The specific objectives of the study were to:

Evaluate the absorptive capacity of the crumb rubber for removal of tetracycline from aqueous solutions as function of particles sizes of crumb rubber, antimicrobial concentration, and pH of solution.

1.3 LITERATURE REVIEW

The use of antibiotics in agriculture began in the 1950 with the use of oxytetracycline and chlortetracycline as feed additives. Environmental assessments of veterinary medicines have been required by the U.S Food and Drug Administration (FDA) since 1980 and in the European Union since 1997. Actually, there is a available information on the direct effects of these drugs on soil biota and the accelerating of antimicrobial - resistance bacteria and/or effects on the endocrine system (endocrine-disrupting compounds, ECDs)^{7,8,9,10,11}. Although the antimicrobials given to humans often are not the same as those used in animals, the structures can be similar enough that antimicrobials used for animals can cause resistance to those used by humans^{6,8,9}.

Several investigations were considered in order to support our hypothesis:

Recently, Nwosu V.C. et al (2001)⁸ reported that the antibiotics of greater use, which have development antibacterial resistance in USA are tetracyclines (figure 1).

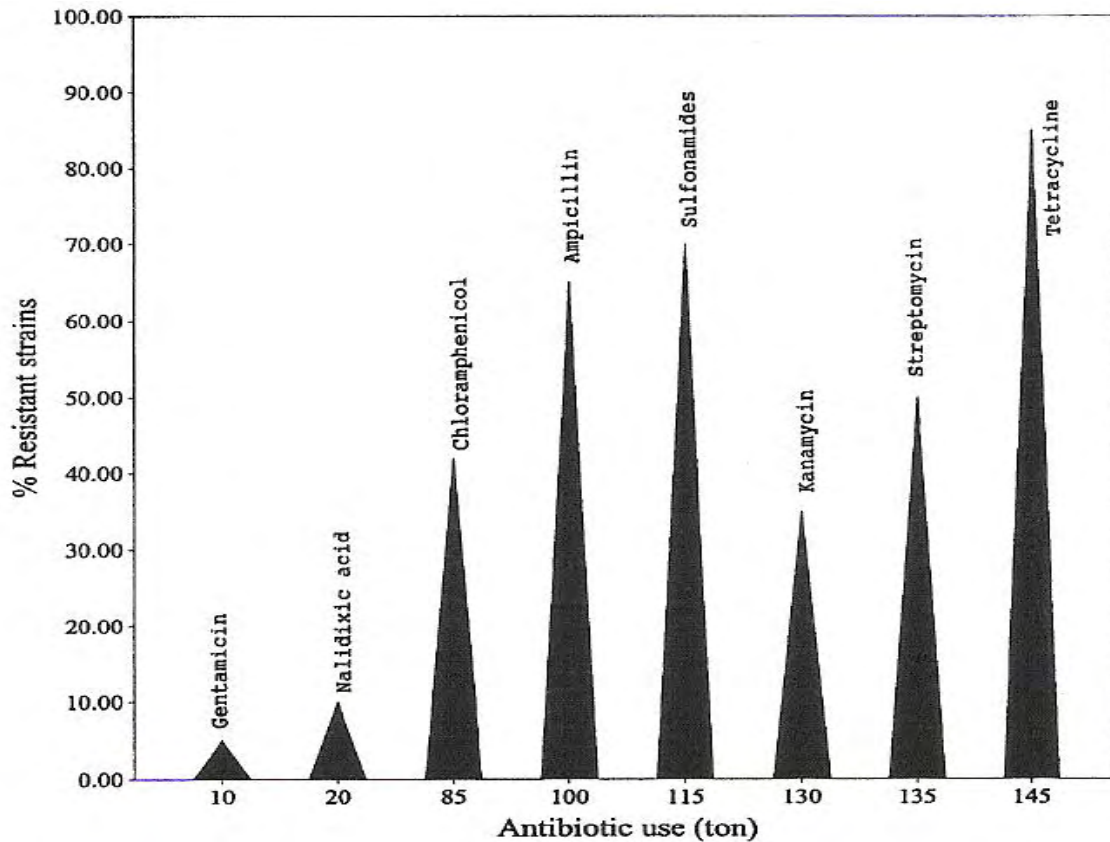


Figure 1. Relationship Between Antibiotic Use and Increase in Antibacterial Resistance⁸

According to Park J.K. et al (1991),²⁰ the rubber is a good sorbent for organic compounds. They investigated the permeation of organic compounds through rubber gaskets commonly found in portable water containers and they found that these were capable of adsorbing significant amounts of organic compounds.

Kim Y. et al (1997),²¹ published a work using batch sorption test to investigate the sorption capacity of organic compounds (ethylbenzene, toluene, trichloroethylene, 1,1,1-trichloroethane, chloroform and methylene chloride) by ground tire. They used the linear model of partition

coefficient to explain the sorption of organic compounds and concluded that the organic compound sorption mechanism onto tires appears to be caused by first sorption onto polymeric materials surface of tire rubber followed by diffusion onto the tire rubber matrix.

Johnson T. and T. Sabu (1999),²² investigated the sorption and diffusion of benzene and methyl-substituted benzenes through epoxidized natural rubber mixed with four types of carbon black. Diffusion constant was found to decrease with increase in the degree of reinforcement. Rate constant decrease with an increase in particle size and the interaction parameters depend on both the type and amount of carbon black in the mix.

Gunasekara A.S. et al (2000),²³ performed a study to determine the sorption of organic contaminants onto ground discarded rubber, such as: naphthalene, toluene and inorganic contaminants as well mercury from water. Their sorption properties and kinetics were determined by batch equilibration techniques at 20 °C. Freundlich isotherms were linear for naphthalene and toluene. Sorption of the organic compounds by the ground rubber particles was relatively fast. However, the mercury isotherms were non-linear, and its sorption was slow as compared to the sorption of the organics. Naphthalene and toluene presented sorption affinities with high values.

Hartig. C. et al (2001),²⁴ presented the adsorption behavior of two polar organic micropollutants (n-buthylbenzenesulphonamide and sulphamethoxazole) onto powdered activated carbon under competitive conditions prior to and after filtration with a tight

ultrafiltration membrane. The sulphonamides were spiked into microfiltered tertiary municipal effluent in $\mu\text{g/L}$ quantities. Ultrafiltration of these effluents resulted in better absorbability permeates compared to the feed waters. This behavior seemed to be caused by a reduced blocking of microspores by lower concentrations of high molecular weight compounds in membrane filtrates.

Thomas Ternes (2002)³ of Germany Institute for Water Research and Water Technology (ESWE) in a work about removal of pharmaceuticals in drinking water treatment, explained that Granulate Activated Carbon (GAC) is an effective absorbent because carbon can be interact a broad category of compounds. Many pharmaceuticals have components like benzene rings or amine groups that enhance their ability to be taken up by the activated carbon.

Adams. C. et al (2002),²⁵ found that the conventional drinking water treatment process (DWTP) removed effectively sulfonamides from deionized and Missouri River water. They tested several processes used in DWTP and concluded that powder activated carbon (PAC), oxidation and reverse osmosis was effective.

Kim. S. et al (2005),¹⁶ conducted experiments to examine the influence of hydraulic retention and solid retention time (HRT and SRT) on the removal of tetracyclines in the activated sludge process. They used lab-scale batch reactors. The removal efficiency of TC was

calculated for 3 phases, which were identified by the different HRT and SRT. The removal in phase 3 (HRT: 7.4h/SRT: 3 days) was significantly lower than that observed in phase 1 (HRT: 24h/SRT: 10 d) with 86.4 % of TC removal.

Kumar. K. et al (2005),²⁶ conducted studies to determine whether or not plant grows in manure-applied soil absorb the antibiotics present in manure. The test crops were corn (*Zea Mays*), gree onion (*Allium Cepa*) and cabbage (*Brassica oleracea*). All three crops absorbed chlortetracycline. The concentration of CTC in plant tissues were 2-17 ng/L fresh weight and the concentration increased with increasing amount of antibiotic present in manure.

CHAPTER II

2 THEORETICAL BACKGROUND

2.1 The Antibiotics

Antibiotics are low molecular weight microbial metabolites that at low concentrations inhibit the growth of other microorganism. The inhibition of the growth of other microorganisms by an antibiotic means either temporary or permanent affecting the capacity of the microorganisms to reproduce and, consequently, inhibition of the growth of the bacterial population rather than of an individual cell. When the inhibition is permanent, the antibiotic activity is termed bactericidal. If the inhibition is lost when the antibiotic is removed from the medium, the antibiotic is a bacteriostatic²⁷.

2.1.1 Tetracyclines

Eight TCs are now commercially available since the first member of the tetracycline family, chlortetracycline was discovered in 1948. The tetracyclines are antibiotic groups that contain a hydronaftacene structure with four rings of six members fusions. They are products of different strains as Aureofaciens and Rimosus Streptomyces

They act by preventing ribosomal protein synthesis and are bacteriostatic agents. Their activity spectrum is particularly broad, including Gram-positive and Gram-negative bacteria, rickettsiae, chlamydiae, and some protozoa. The specificity of the toxicity of tetracyclines for bacteria resides in their ability to accumulate it^{13, 28}.

2.1.1.1 Chemistry of Tetracyclines

The basic structure of tetracycline class is:

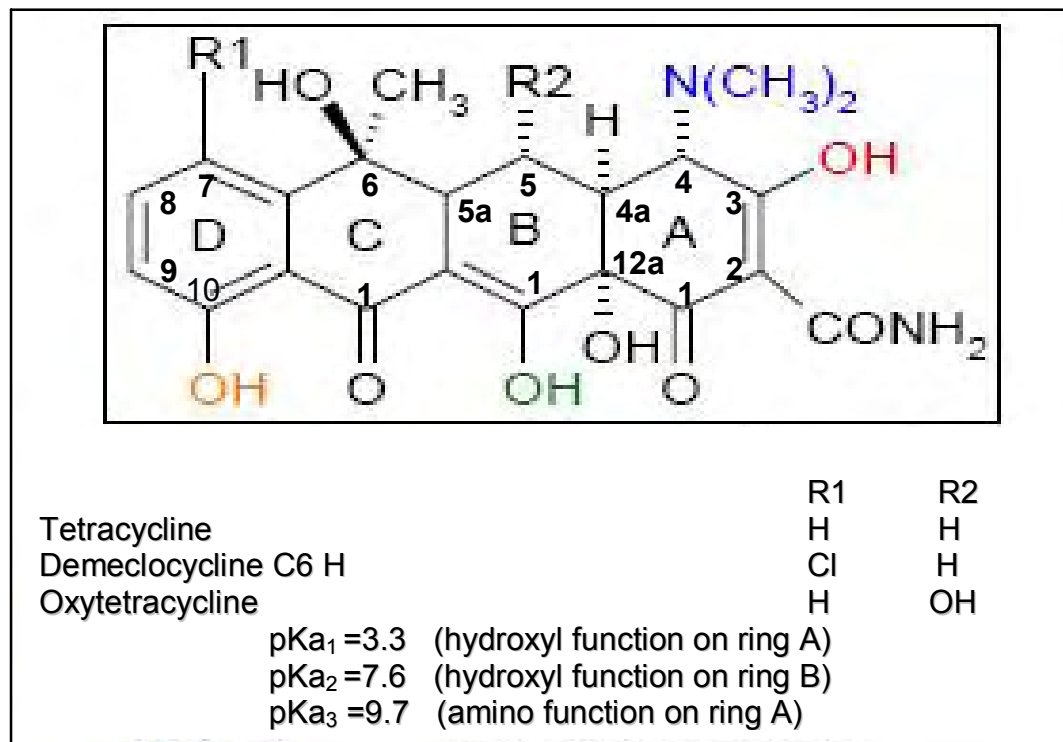


Figure 2. General Structure of Tetracyclines (TCs)

In general, the TCs have three different pKa. The first pKa is associated with the deprotonation of C₃ hydroxyl. Loss of protons from O₁₂ and dimethylammonium constitutes pKa₂ and pKa₃. As indicated by their acid dissociation constants, the TCs contain localized charges across all pH values and only achieve an overall neutral state as zwitterions^{29, 30}.

TCs are generally fairly stable to acids but quite sensitive to alkali. The TCs can be reversibly epimerized at position C₄ to form the correspondent 4-epimers. This type of reaction has been found to be favorable in the pH range from 2 to 6 but at neutral pH the epi-tetracycline (ETC) is also reported to be formed. Strong Acidic conditions facilitate the dehydration of the hydrogen at position C_{5a} and the hydroxyl group at position C₆. The resulting products are anhydrotetracycline (ATC), epi-anhydrotetracycline (EATC), apo-tetracycline (apo-TC) and finally terrinolide.

Profound structural changes occur on treatment with alkali. Tetracyclines having a hydroxyl group at C₆ cleave readily to their respective isotetracyclines. Desmethyl or didesmethyl analogs of the isotetracyclines may be formed under alkaline conditions if oxygen is present.^{28, 30, 31}

2.1.1.2 Photolysis of Tetracyclines

Tetracyclines photodecomposes and may be converted to several products. In previous studies^{30, 31} three major photodecomposition products of TC were reported: 4-dedimethylaminotetracycline, 5a, 6-anhydro-tetracyclines, and a quinone. Oka et al (1989)³⁰, studied the photodecomposition of TC in water of a culture pond and identified seven different photodegradation products that all had lost their tetracycline structure.

2.1.1.3 Tetracyclines Complexes

Tetracycline possesses a great tendency to form complexes with a number of chemical species, due to its phenolic β -dicetone group, dicarbonyl system and amino-alcohol group, which are their chromophoric groups, shown in figure 3.

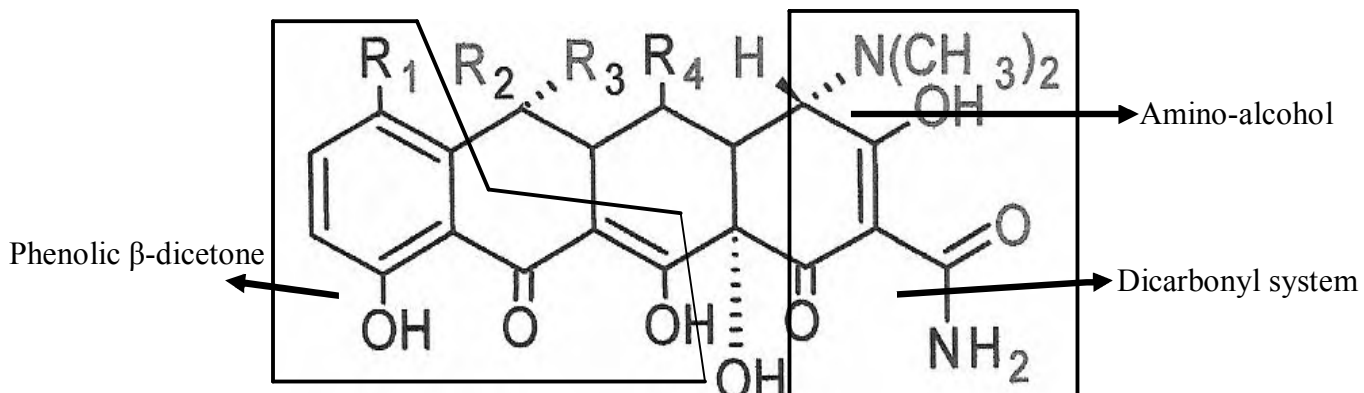


Figure 3. Metal Coordination and Chromophoric Group of TCs³¹

It complexes most readily with Fe^{3+} , Fe^{2+} , Cu^{2+} , Ni^{2+} , Co^{2+} , Zn^{2+} , Mn^{2+} , Mg^{2+} , Ca^{2+} , Be^{2+} , Al^{3+} among metal ions. This complexing is further supported by the observed dramatic changes in UV spectral bands from the chromophoric regions of TC upon interaction with complexing agents. Multiples species of chelated TCs can co-exist in solution. The number and kind of species can change depending on pH or which metal is present. Additionally, ternary complexes of a TC+metal+ligand are possible, and can affect differences in TCs protonation states depending on choice of metal and ligand present^{28, 31, 33-34}.

Accordingly, the complexation behavior is therefore an important factor to consider understanding the presence of tetracyclines in the environment. This fact, is related whereupon complexation explains^{7, 11} the field studies that have been made by investigators, which, do not give test of tetracyclines leaching in deep ground segments or underground water, due to strong adsorption of this drugs in the topsoil and therefore its higher probability of being in superficial water bodies than subterraneous waters.

The presence of these compounds in the environment is because they are partially metabolized after its administration, mainly in the agriculture field and a significant portion of the antibiotic can be excreted as father compound or conjugated forms that can be reverted to the original antibiotic.

The more important route of entrance to the environment is outlined in the figure 4. It is possible to be considered that the unloading of water effluents of remainders originating from

agriculture activities, the excretion of substances in the urine and manure of animals for food production and waste from treatments for animals and hospitals are the main sources¹⁵.

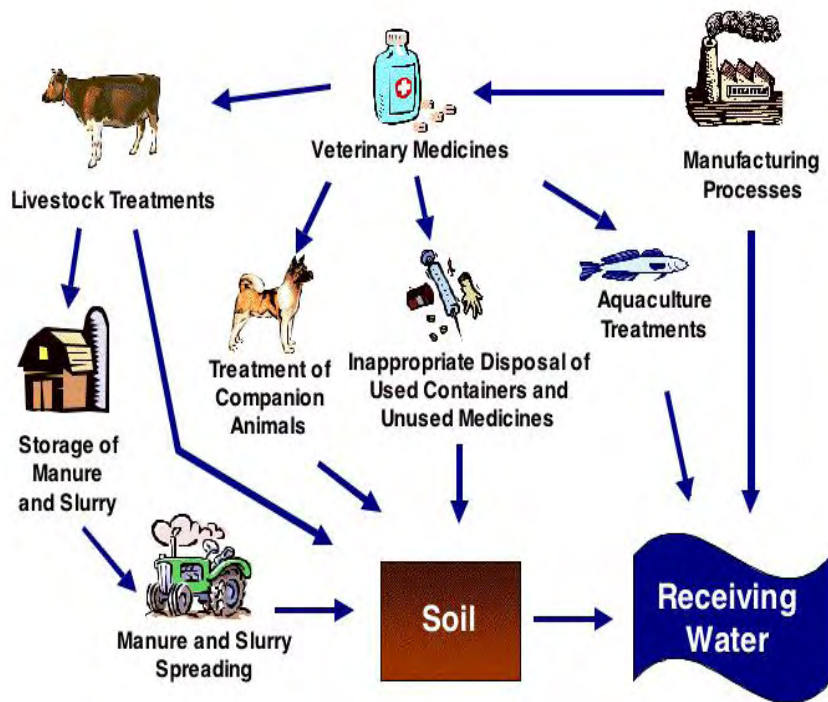


Figure 4. Pathways into Environment for Antibiotics¹⁵

The tetracyclines analysis has reported low spike recoveries due to their trend to form complexes. However, recently the liquid chromatography coupled to MS has lead to tetracyclines detection with better recoveries. Besides, the lack of volatility and thermal stability of many antibiotics make LC-MS the method of choice for their analysis.¹²⁻¹⁴ Table 1 shows some conditions of HPLC used for analysis of tetracyclines. It has been used different stationary phases with several conditions of mobile phases and C8 and C18 stationary phases, which have given acceptable results.

Table 1. HPLC Conditions Summary²⁸

Tetracycline	Sample Type	Stationary Phase	Mobile Phase	Extraction and/or Clean process	λ(nm)	Concentration Range
OTC,TC,CTC,DC, ETC, MTC,DMCTC,PRMTC	-	C8	MeOH-MeCN-0.01 M oxalic acid (pH3.0) (1:1.5:7)	-	350 nm	-
OTC,TC,CTC,DC,MTC,DMCTC,	Honey	C8	MeOH-MeCN-0.01 M oxalic acid (pH3.0) (1:1.5:7)	C ₁₈ cartridge pretreated with EDTA and COOH cartridge clean up	350 nm	0.05–0.10 ppm
OTC,EOTC,AOTC, apo-OTC	-	PolymerL.PLRP-	tert-BuOH-0.2Mphosphate buffer (pH8.0)-0.02MTBA-0.0001M EDTA(55:100:50:10)	-	254 nm	-
OTC,TC,CTC,DC	Animal tissues	LiChrosorb RP-8	MeOH-MeCN-0.01 M oxalic acid (1:1.5:3)	Extraction with McIlVaine buffer(pH4.0) containing 0.1 M EDTA. C ₁₈ cartridge pretreated with EDTA clean up	350 nm	0.01 ppm
OTC	Fish tissues	Hypersyl ODS	0.5% (NH ₄) ₂ HPO ₄ -0.5% DEA-MeCN-DMF (40.5:40.5:19.6), pH2.5	Extraction with 1M HCl-50% TCA (15:1)	365 nm	0.005 ppm
OTC,TC, CTC	Feeds	Novapak C ₁₈	MeOH-MeCN-0.01 M oxalic acid (pH3.0) (1:1.5:6.5)	Extraction with McIlVaine buffer(pH4.0) containing EDTA. Sephadex LH-20 clean up	370 nm	-
OTC	Fish tissues	Supelcosil LC18DB	0.005M phosphate buffer (pH 2.0). MeCN-THF (81:10:9)	Extraction with phosphate buffer pH 4.2 containing 0.1 M EDTA.. C ₁₈ Cartridge clean up	357 nm	0.005 ppm
OTC,TC,CTC and DC	Fish tissues	Spheri C ₁₈	MeCN-DMF-0.01 M oxalic acid (22:6:72)	Extraction with McIlVaine buffer (pH 4.0). C ₁₈ Cartridge pretreated with EDTA clean up	355 nm	0.05-0.1 ppm

OTC: oxytetracycline
MTC: methacycline
PRMTC: rolitetracycline

TC:tetracycline
EOTC: epioxytetracycline
apo-OTC: apo-oxytetracycline

CTC: chlortetracycline
AOTC: apo-oxytetracycline

DC: doxycycline
ETC: 4-epitetracycline

DMCTC: demeclocycline
EDTA: disodium ethylenediaminetetraacetate

Table 2. Summary of Physico-Chemical Characteristics of Tetracycline^{29, 35}

Antibiotic	Tetracycline
Chemistry Name	4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1
Condensed Formula	C ₂₂ H ₂₄ N ₂ O ₈
Molecular Weight	444.44 g/mol
pKa	pKa ₁ = 3.30 ; pKa ₂ = 7.68 ; pKa ₃ = 9.69 / isoelectric point ~5
CAS Number	60-54-8
Appearance and Odor	Yellow Crystalline Powder; odorless
Melting Point	214 – 215 °C
Solubility in water	Freely soluble, freely soluble in alkali hydroxide and carbonates solutions; sparingly soluble in alcohol; practically insoluble in chloroform and in ether.
Stability	Is stable in air, but exposure to strong sunlight causes it darkening. It loses potency in solutions of pH below 2 and is rapidly destroyed by alkali hydroxide solutions.

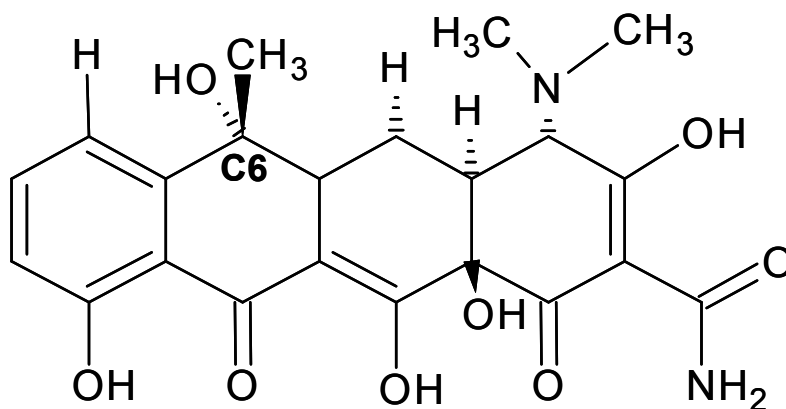
**Figure 5. Structure of Tetracycline**

Table 3. Summary of Physico-Chemical Characteristics of Demeclocycline^{29, 35}

Antibiotic	Demeclocycline
Chemistry Name	7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-110
Condensed Formula	C ₂₁ H ₂₁ ClN ₂ O ₈
Molecular Weight	465.31 g/mol
pKa	pKa ₁ = 3.30; pKa ₂ = 7.40 ; pKa ₃ = 9.30
CAS Number	64-73-3 as Demeclocycline Hydrochloride
Appearance and Odor	Yellow Crystalline Powder; odorless
Solubility in water	Sparingly soluble in water; soluble in solutions of alkali hydroxides and carbonates.
Stability	DMC is the more stable than other TCs; stable in air, but exposure to strong sunlight causes it darkens. It loses potency in solutions of pH below 2 and is rapidly destroyed by alkali hydroxide solutions.

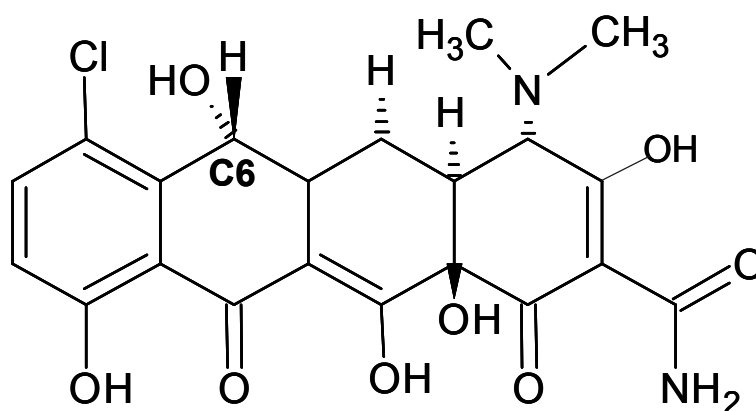


Figure 6. Structure of Demeclocycline

2.2 Crumb Rubber

2.2.1 General Properties

The tire rubber is a mixture of elastomers, especially natural rubber, carbon black, sulfur, fibers, zinc oxide, stainless steel, stearic acid and process oil. Natural rubber is a polymer formed by isoprene units. The major components of the tires are vulcanized rubber with sulfur (0.07 to 2.22 %), stainless steel, (0.55 to 2.79 %) zinc oxide and 10.97 to 45.60 % of carbon black¹⁹.

Passenger cars and trucks represent about 85 % of the total number of tires manufactured. Depending on their size and utilizations, tires vary in design, construction and total weight³⁶.

2.2.2 Rubber Composition

From an engineering point of view, crumb rubber has a number of special thermo-mechanical and physico-chemical properties (appendix A). The size of the rubber particles is graded with the finest one can be as small as about 0.2mm (Mesh #80). The grade commonly used in rubberized asphalt pavement is between about 2.0 mm to 0.5 mm (Mesh #10 to Mesh #40). Crumb rubber is light in weight with durability for a long period of time in a natural environment. From a safety consideration point of view, crumb rubber is a non-toxic and inert material³⁶. The table 4 shows the material composition of passenger car and truck tires. Tires contain about 1.5 % by weight of elements or compounds listed in table 4 according to the Basel Convention³⁶. These are encased in the rubber compound or present as an alloying element.

The natural rubber is a polyisoprene polymer (figure 7) and may be obtained from a number of species of plants, the primary one being the tree *Hevea Brasiliensis*. Chemical and environmental resistance and mechanical properties are improved through cross-linking (vulcanizing), usually by treatment with sulfur⁴².

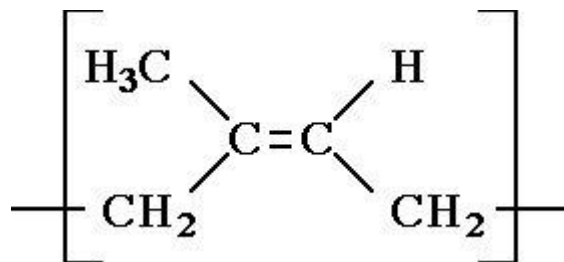


Figure 7. Structure of polyisoprene unit, C₅H₈

Table 4. Comparison of material average composition of passenger car and truck tire, US³⁶

Material	Passenger Car	Truck Car
Rubber / Elastomers	47 %	45 %
Carbon Black	21.5 %	22 %
Metal	16.5 %	25 %
Textile	5.5 %	--
Zinc Oxide	1 %	2 %
Sulfur	1 %	1 %
Additives	7.5 %	5 %

Table 5. Hazardous Waste Constituents³⁶

Chemical Name	Remarks	Content (% weight)
Copper compounds	Alloying constituent of the metallic reinforced material (steel cord)	~ 0.02 %
Zinc Compounds	Zinc Oxide, retained in the rubber matrix	~ 1 %
Cadmium	At trace levels, as cadmium compounds attendant substance of the zinc oxide	Max. 0.001 %
Lead Lead Compounds	At trace levels, as attendant substance of the zinc oxide	Max. 0.005 %
Acidic solutions or acids in solid form	Stearic acid, in solid form	~ 0.3 %
Organo-halogens compounds	Halogen butyl rubber	Content of halogens max. 0.10 %

2.2.3 Rubber Thermal Properties

The net calorific value of a tire is between 32 and 34 MJ / Kg. A ton of tires is equivalent to a ton of good quality coal or 0.7 ton of fuel oil. It has therefore, an excellent potential as a fuel, which is not surprising since tires are mainly composed of oil products.

Table 6. Comparison of the Energy Content Tire Derived Fuel and Other Sources³⁶

Fuel	Grade	Heat Content
Gas	Natural	1.000 BTU / ft ³
TDF	Tire Derived Fuel	15.500 BTU / lb
Coal	Sub-bituminous	10.500 BTU / lb
Coal	Bituminous	12.700 BTU / lb

Nevertheless, a tire is very difficult to ignite. The temperature at which the ignition induced by the pilot flame may be maintained was 33° – 350°. There is no possibility of self ignition for tires³⁶.

2.2.4 Rubber Potential Risk to the Environmental

The lack capacity to identify and dispose of end-of-life tires can lead to serious health and environmental problems. Despite the obvious stability of tires, due to the fact that the different components of the rubber mixtures are trapped in the three-dimensional grid of the polymer, it is essential to ensure that tires are not treated in a way that may cause harm to the environment. Several studies have been carried to assess the impact of tires rubber to the environment³⁶.

2.2.4.1 Ecotoxicity

At the request of BLIC (European Association of the Tire Industry) the following tests were performed, using ISO standard test methods on powdered rubber from tire tread.

- In 1995, studies were carried out at the Pasteur Institute in Lille using rubber powder generated from tire tread (on Alga: *S. Capricornutum* and crustacean: *Daphnia magna* and Fish *Brachydanio rerio*) as per norms ISO 8692, 6341 and 7346.
- In 1996, a supplemental study was done: “Determination of Acute Toxicity as per ISO 11268/1 –Observation of effect of tire powder rubber on a population of earthworm

placed in a definite substratum” – at the Paster Institute in Lyon. They used standardized norms and showed no toxicity³⁶.

2.2.4.2 Leaching

In 1989, the Minnesota Pollution Control Agency studied the leachate from waste tire samples to determine detrimental effects to the environment. Soil and groundwater samples were taken from two existing shredded tire fill sites and stockpile site were analyzed and the results compared with other laboratory. The following points summarize the findings of the study³⁶:

- Tires samples exposed to acidic solutions leach higher concentrations of metals than those subjected to neutral or basic solutions.
- In neutral solutions (pH 7.00) tires samples did not leach any contaminants of concern.
- Samples subject to a pH of 3.5 produced leachate at metal concentrations that exceeded the Minnesota Department of Health Recommended Allowable Limits for drinking water standards.
- Metals detected in the highest concentrations included barium, cadmium, chromium, lead, selenium and zinc.
- Soil samples taken from shredded tire field sites displayed constituent concentrations comparable to those found in natural settings³⁶.

2.2.4.3 Waste Tires Mismanagement

2.2.4.3.1 Risks due to Uncontrolled Open-Air Burning: Uncontrolled open-air burning of the tires is not an environmentally sound or acceptable management practice. Such practices can release potentially hazardous levels of carbon monoxide and mono – and poly-aromatic hydrocarbon in the smoke plume. After open-air burning, organic compounds, like pyrolytic oils, rest in the soil and can cause environmental damages to the flora and fauna³⁶.

2.2.4.3.2 Risks due to Uncontrolled Stockpiling or Land Filing: under certain specifically defined climatic conditions waste tire dumps or stockpiles can become the breeding grounds for insects, such as mosquitoes, which are capable of transmitting diseases to humans. This is of particular concern in tropical or sub-tropical regions³⁶.

2.2.4.4 Management of Part-Worn Tires

In this section the following treatments are considered to prolong the life of a used tire:

- regrooving (truck tires only): it can prolong the life of truck tires. This practice is not admissible for car tires because the tread depth in the grooves is not adequate.
- Rethreading (all tires): it maximizes the tire utilization and is considered desirable to the extent the life of tire. 80 % of the original material value is available for reuse, after a rethreading. Selected undamaged car and truck tire casing are sold to rethreading companies. Whatever is left

of the original tread is removed by a buffing process and a fresh, patterned tread is vulcanized to the casing³⁶.

2.2.4.4.1 The Management of end-of-life Tires

When tires have reached the end of their useful life and re-use as a part-worn tire is not possible, the tires enter a waste management system. The recovery and eventual final disposal should take place in an environmentally sound manner. This means that an appropriate collection system should take care of these tires. In most cases the separately collected end-of-life tires are still valuable for other applications and not necessarily for land filing. According to the assessment made by each country, the lawful execution of these applications may vary. The management of end-of life tires depends very much on local economic and industrial conditions:

- Product Recycling
- Material Recycling
- Energy Recovery
- Landfill

The direct disposal of end-of-tires in landfills is to be used only where economically viable alternatives for the materials or energy recovery are not available³⁶.

2.2.4.4.2 Product Recycling

End-of-tires in whole, cut or stamped form can be used in many environmentally sound applications to take advantage of their shape, sound and impact adsorption properties and another

material characteristics. Machinery used to produce cut or stamped pieces of rubber is readily available.

2.2.4.4.3 Material Recycling

2.2.4.4.3.1 Shred: end-of-life tires can be shredded to facilitate transport, as a first step towards granulation, or for use in several applications. The tires are fed into a shredder and in most circumstances the steel and textile are not removed, but may include the additional process of material separation. The shredder can be mobile or fixed. Mobile shredders are used to facilitate the movement of end-of-life tires from place to place for another treatment. Regions which permit land filling with end-of-life tires often require that they are shredded in order to minimize the space requirements and to reduce the potential of the tires rising to the surface once the landfill has been capped. Tire shred can be used as a secondary fuel for incineration or as a first step in the granulation process³⁶.

2.2.4.4.3.2 Granulate: end-of-life tires and tire buffing can be used to produce rubber crumb or granulate. There are two principal methods of granulating end-of-life tires.

2.2.4.4.3.2.1 Grinding at ambient temperature: end-of-life tires are shredded and then fed into a grinding mill. After grinding, the material is separated into rubber granulate, steel and textile; granulate can be sieved into different particle sizes. Peels and buffing obtained from

rethreaded tire manufacturing processes are currently ground and the rubber obtained can be directly recycled into compounds used to produce new and rethreaded tires³⁶.

2.2.4.4.3.2 Grinding at very low temperature (cryogenic method): in this process the end-of-life tire and chopped rubber has to be cooled to below the freezing point, after which it is ground in a hammer mill. This process enables rapid separation of fibers, metal and rubber. ASTM standard D5603 gives a classification for Recycled Vulcanized Particulate Rubber. Total cost for unit management also takes into account the labor and power costs on the basis of local prices. Ratio between income and cost is calculated considering the current price for selling the ground rubber or other outputs from the unit³⁶.

2.2.4.4.3.3 Reclaim: reclaim rubber is produced by the chemical processing of a mixture of size reduced end-of-life tires, oil, water and chemicals. The resulting compound is submitted to a further thermo mechanical process where additives can be incorporate depending on the final product requirements. The material is extruded into slabs, cut and wrapped for shipment. Reclaim rubber blends in with virgin compounds can be used in a wide range of molded articles³⁶.

2.2.4.4.3.4 Pyrolysis: chemical conversion or breakdown of organic compounds by heating in the total or partial absence of oxygen. Activated carbon, Carbon black, oil (which must be refined) and scrap steel can be obtained from the pyrolysis of end-of-life tires. The “pyro-oil”

may be used as fuel or mixed in equal proportion with diesel oil. After refining, the “pyro-carbon” may be used as semi-reinforced filler or as an active carbon. Even if recent technological advances have improved product quality, it is still unclear whether there is a market demand for this product³⁶.

2.2.4.4.3.5 Energy Recovery

There are several controlled energy recovery methods which are environmentally sound. End-of-life tires represent an alternative supplementary non-fossil fuel. End-of-life tires provide the same heat energy commonly achieved by coal. Whole or shredded end-of-life tires can be used as a principal or secondary fuel source in the production of steam, electricity, cement, lime, and paper, steel and in the incineration of garbage. The addition of end-of-life tires is environmentally safe and does not release additional emissions in the atmosphere of sulfur oxides or nitrogen oxides when appropriate emission control device are properly installed and maintained³⁶.

2.3 Liquid Chromatography/Mass spectrometry

Liquid chromatography/mass spectrometry (LC/MS) is an analytical technique by which the separation of an analyte mixture in solution is effected and individual components (as ions or molecules) are caused to go into the gas phase at atmospheric pressure. Molecules are then ionized, and ions are desolvated followed (under some circumstances) by reproducible

fragmentation to produce gas-phase ions under reduced pressure (vacuum) that can be analyzed according to their mass-to-charge ratios and abundances to produce spectra that represent near-pure substances. The technique of LC/MS generates only mass spectral data. The presentation of these data can be in the form of a chromatogram, a spectrum, or a three-dimensional presentation including both. For most compounds, a mass spectrometer is more sensitive and far more specific than all LC detectors. It can analyze compounds that lack a suitable chromophore. It can identify components in unresolved chromatographic peak, reducing the need for perfect chromatography³⁷.

The mass spectrometer works by ionizing molecules and then sorting and identifying the ions according to their mass-to-charge (m/z) ratios. Two key components in this process are the ions source, which generates the ions, and the mass analyzer, which sorts the ions. The introduction of Atmospheric Pressure Ionization Techniques greatly expanded the number of compounds that can be successfully analyzed by LC/MS³⁷.

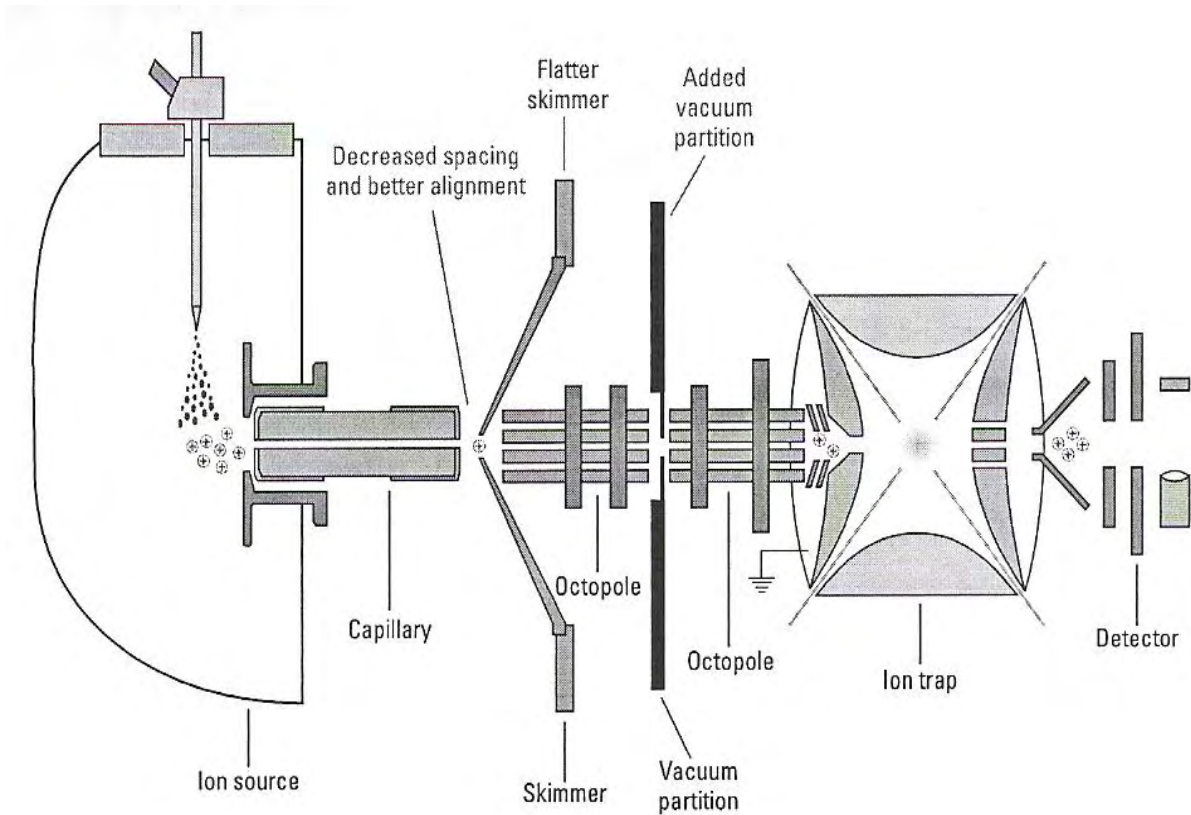


Figure 8. Schematic diagram of the Bruker-Daltonic Mass Spectrometer³⁸

2.3.1 Mass Analyzer

Although in theory any type of mass analyzer could be used for LC/MS, four types are used most often:

- Quadrupole
- Time-of Flight
- Ion Trap
- Fourier Transform-ion cyclotron resonance³⁹

2.3.1.1 Ion Trap

An ion trap mass analyzer consists of a circular ring electrode plus two end caps that together form a chamber. Ions entering the chamber are “trapped” by electromagnetic fields. Another field can be applied to selectively eject ions from the trap. The ion trap has the advantage of being able to perform multiple stages of mass spectrometry without additional mass analyzers. Figure 8 shows the schematic diagram of a Bruker Daltonics Ion Trap Mass Spectrometer equipped with electrospray ionization (ESI) such as the system that was used in this work.

2.3.2 Ion Source

Earlier LC/MS systems used interfaces that did not separate the mobile phase molecules from the analyte molecules (direct liquid inlet, thermospray) or before ionization such as the particle beam. In the particle beam interface, the analyte molecules were then ionized in the mass spectrometer under vacuum, often by traditional electron impact ionization.

The introduction atmospheric pressure ionization (API) techniques greatly expanded the number of compounds that can be successfully analyzed by LC/MS. In atmospheric pressure ionization, the analyte molecules are ionized first at atmospheric pressure, followed by mechanically and electrostatically separation from neutral molecules.

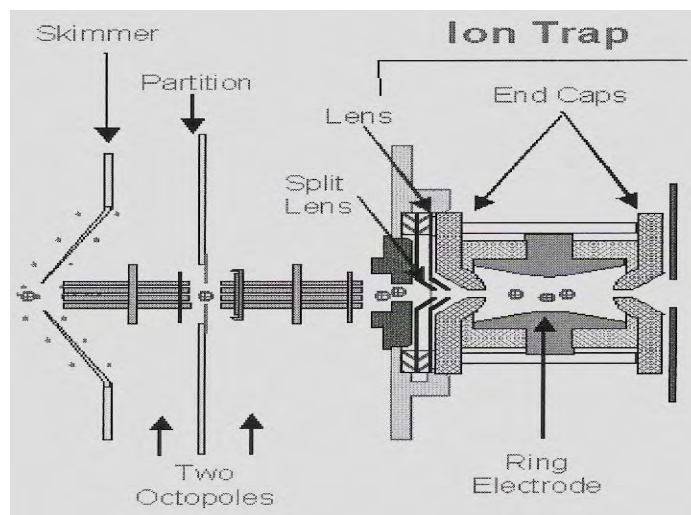


Figure 9. Scheme of Ion Trap³⁸

Common atmospheric pressure ionization techniques are³⁸:

- Electrospray Ionization (ESI)
- Atmospheric Pressure Chemical Ionization (APCI)
- Atmospheric Pressure Photoionization (APPI)

2.3.2.1 Electrospray Ionization

Electrospray relies in part on chemistry to generate analyte ions in solution before the analyte reaches the mass spectrometer. The LC eluent is sprayed (nebulized) into a chamber at atmospheric pressure in the presence of a strong electrostatic field and heated drying gas. The electrostatic field causes further dissociation of the analyte molecules. The heated drying gas causes the solvent in the droplets to evaporate and as the droplets shrink the charge concentration

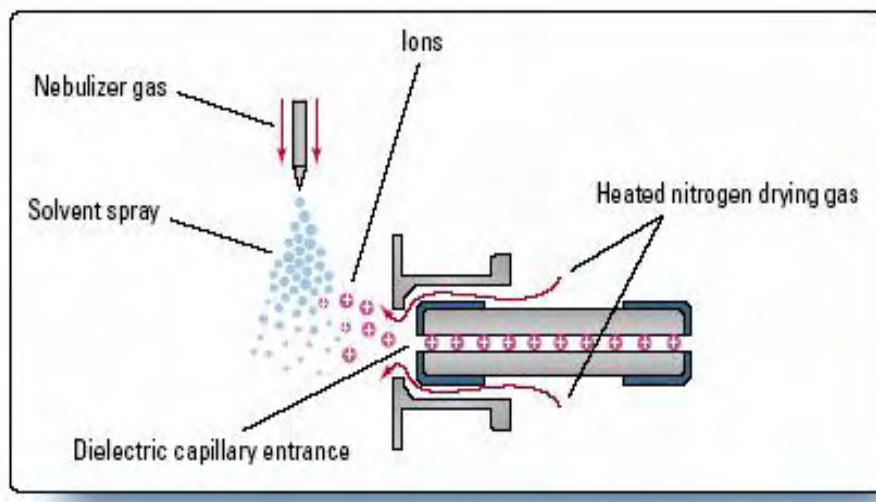


Figure 10. Electro spray Process³⁸

in the droplets increases. Eventually, the repulsive forces between ions with like charges exceed the cohesive forces and ions are ejected (desorbed) into the gas phase. The ions are attracted to and pass through a capillary sampling orifice into the mass analyzer³⁸. Figure 10 shows the schematic diagram of an electro spray ionization source.

2.4 Adsorption Background

Adsorption is a process in which, a soluble chemical (the adsorbate) is removed from a fluid by contact with a solid surface (the adsorbent). It is used in industry for product separation and waste treatment. In adsorption the forces of interaction between surface atoms and the adsorbate molecules depend on the distance between the surface of the adsorbent and the adsorbate molecule. In general, the adsorption is the process by which a component moves from one phase to another while crossing some boundary. Experiments by several scientists including Brunauer,

Emmett and Teller, McBain and Langmuir focused on the manner in which adsorbents removed adsorbates from both gases and liquids. It was found that the observed effect of adsorption was achieved within porous solids and that it was the result of interactive forces of physical attraction between the surface of porous solids and component molecules being removed from the bulk phase.

Adsorption can be classified as either physical or chemical. Physical adsorption involves weak forces, and is, therefore, reversible. Physical adsorption occurs at low temperatures. Physical adsorption is very similar to a condensation process, and thus, it is exothermic with a heat of adsorption similar to that of the latent heat of condensation. Chemical adsorption or chemisorption is important in gas-phase catalysis, but is not generally relevant to liquid-solid adsorption at ordinary temperatures. Chemisorption occurs at high temperatures with a significant activation energy, which involves strong bonds and is not reversible. The heat of adsorption is typically high in chemisorption and is similar to heat generated during a chemical reaction.

There are several factors that impacts physical adsorption. The major factors which affect physical adsorption include the surface area of the adsorbent, pore structure of the adsorbent, surface chemistry of the adsorbent, pH of the solution, and the presence of competing adsorbates. It is due to these factors, physical adsorption is considered to be a complex phenomenon. The surface area of the adsorbent is one of the most important factors on which adsorption greatly

depend. The surface area is comprised of two types, the external surface area and the internal surface area (pore walls). The pores of the adsorbent contribute largely to the internal surface area. Since physical adsorption greatly depends upon the surface area, the greater the surface area of the adsorbent, the greater the capacity for adsorption. The pore structure of the adsorbent material is almost as important as the surface area. The pore diameter for most media ranges from less than 10 to over 100,000 Angstroms. The pore structure should be such that the adsorbate molecule enters the pores and adsorb onto the inner surface. If the adsorbate molecules are larger than the pore diameter, lesser adsorption would take place due steric hindrances.

The surface of an adsorbent is typically composed of various surface functional groups. Adsorption of organic adsorbates is greatly dependent on the amount and nature of surface oxide groups. Strongly dissociated adsorbates are weakly adsorbed when compared to non-dissociated adsorbates. The more non polar an adsorbate, the higher the adsorption capacity. This is attributed to the fact these adsorbate molecules tend to prefer the adsorbent surface rather than being in the solution. It has also been shown that an increase in the molecular weight of the adsorbate will generally enhance adsorption until the size of the adsorbate is larger than the pore size of the adsorbent. Typically, aromatic compounds are more adsorbable than similar aliphatic compounds and branched-chain molecules are generally more adsorbable than straight-chain molecules. Double and triple carbon bond organics tend to adsorb better than single carbon bond organics. In addition, solubility of the adsorbate is also an important factor. In general, the

lower the solubility of the adsorbate, the higher the adsorption capacity since the forces of attraction between the adsorbate molecules and the adsorbent surface molecules will be greater than the forces of attraction between the adsorbate and the solvent molecules⁴⁰. The pH of the solution is a major factor in determining adsorption. Generally, acidic species adsorb better at low pH, while basic species adsorb better at higher pH⁴¹. The presence of competing adsorbate increases the competition for available adsorption sites. In general, the presence of competing adsorbates will reduce the adsorption capacity of all adsorbates onto the adsorbent. Equilibrium occurs when the adsorption sites are filled. Equilibrium is a phenomenon when the rate of adsorption and the rate of desorption are equal⁴⁰.

CHAPTER III

3 EXPERIMENTAL SECTIONS

3.1 Materials and Equipments

The following are a list of the materials used in the project:

- The granular crumb rubber screened at 14-20 (1.00 – 1.43 mm) and 30 (0.67 mm) mesh size was provided by REMA Corporation located in Caguas, Puerto Rico. The crumb rubber was washed twice with deionized water and left in agitation into an erlenmeyer flask using a magnetic stirrer for 24 hours; it was allowed to drying completely a room temperature on absorbent paper and placed into ziploc bags until its use.
- Carbon Black N 330 from Sir Richardson Carbon Company, which is used by tire manufacturing companies.
- Carbon Black provided by FISHER
- 3510 Branson Ultrasonic Bath.
- The 300 μ L plastic vials manufactured by Agilent, (USA) cat. No. 5188-2788 with snap cap 5182-0540 caps. Plastic flasks of 60 mL for batch experiments were obtained from Fisher (USA).

- Analytical balance from Mettler Toledo XS205, weighting capacity in mg with two significant decimal values and readability of 0.01 mg, repeatability of 0.02 mg and linearity of 0.2 mg.
- Stir bar of Fisherbrand PTFE (polytetrafluoroethylene), USA with diameter 3/16 “(14 mm) and 1/2 “(12 mm) long.
- Laboratory stirrer/hot plate Corning (USA), range 0 – 1100 rpm.
- Plastic Syringes Cat. No 8939-N01, 1 mL of Thomas Scientific, USA.
- The glass volumetric flask of 1 to 500 mL of Pyrex , USA
- Micropipettes of 0.50 μL – 100 μL and 100 μL – 1000 μL Eppendorf Research, provided by Fisher, USA.
- Millex syringe filter units of 4 mm, with hydrophilic PVDF (polyvinylidene difluoride) membrane, pore size 0.22 μm and filtration area of 0.10 cm^2 were used for filtration of the samples (100 μL) and 13 mm syringe filter with hydrophilic PVDF membrane, pore size 0.45 μm .
- pH Meter OAKLON pH 510 Benchtop, 0.00 – 14.00 pH range, \pm 0.01 pH accuracy with electrode holder, combination pH.
- Barnstead NANOpure DiamondTM Deionizer (18.1 $\text{m}\Omega\text{-cm}$)

- General filtration 0.45 μm membrane MCE (cellulose acetate and cellulose nitrate), diameter 47 mm for filtration of aqueous mobile phase and ICE 450 unsupported medium – hydrophilic cationic polyethersulfone was used for filtration of organic mobile phase.

3.2 Reagents and Chemicals

The test adsorbate used in this study was the antibiotic: Tetracycline Hydrochloride reference standard. Tetracycline Hydrochloride was obtained from U.S. Pharmacopeia, USP (Rockville, MD) in solid form as pure material. Test solutions were prepared by accurately weighing of the tetracyclines (TCs) and dissolving into deionized water from **Barnstead** NANOpure Diamond™ deionizer (USA). All water used in this work was from a **Barnstead** NANOpure Diamond™ (USA) deionized water system.

The antibiotics were kept in the refrigerator under 4 °C and sealed until its use. Acetonitrile, methanol, isopropanol were HPLC grade. Formic acid was sequencing grade and EDTA, HCl and NaOH were analytical grade and were provided by Fisher, (USA).

3.3 Instrumentation

3.3.1 Liquid Chromatography, HPLC

We used Liquid Chromatography (LC) from Agilent LC 1100 (Palo Alto, USA) coupled to Mass Spectrometry (MS) with Bruker Daltonics Esquire 6000 Mass Spectrometer (LC/MS) and UV-Vis Diode Array Detector. Our LC/MS system can be operated in several modes including standard LC and without LC separation analysis (direct infusion analysis using a low flow syringe pump). LC instrument is divided in control modules as is showed in figure 11. At first, the chromatographic analyses were carried out on Agilent LC 1100 with a UV-Vis diode - array detector. In this case, a reverse phase with isocratic conditions, and a column of 150x4.6 mm Zorbax Eclipse XDB – C8 with a 5 μm particle size from Agilent Technologies, (USA) were used. The Eclipse XDB – C8 had a packing made of dimethyl-n-octylsilane (C8) stationary phase onto a porous silica support.

Once the parameters were establishing and optimized, the LC was operated coupled MS with a reverse phase system and under isocratic conditions, using a LUNA C8 column (Phenomenex) of 100x4.6mm and pore size 3 μm , and with low metals concentration.

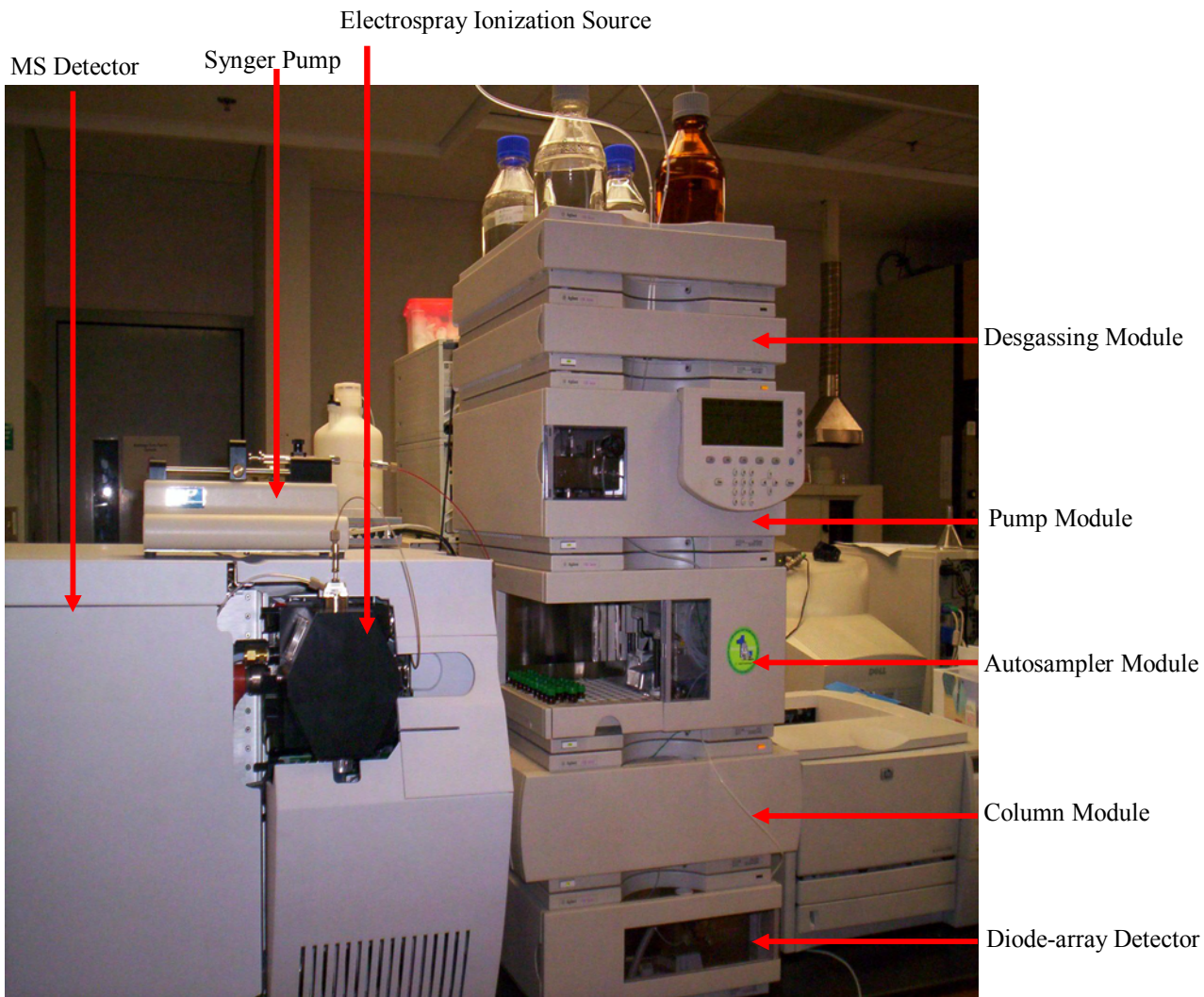


Figure 11. LC/MS Instrument in the Laboratory, Esquire 6000 from Brucker Daltonics

3.3.2 MS Spectrometry

Mass spectra were acquired in positive ion mode electrospray (ESI +) with a Bruker Daltonic Esquire 6000 Mass Spectrometer coupled to Agilent 1100 LC (Agilent, Palo Alto). The nebulization process was assisted with nitrogen nebulization and countercurrent drying gas. The drying gas was operated at a flow rate of 9.80 L/min at 350 °C. The nebulizer pressure was 48.00 psi, the capillary was set at 3180 volts and the fragmentor was set at 40 V. All parameters of the ion trap were optimizing individually and shown below.

MS Optimization Conditions

Ion Source Type: ESI
Ion Polarity: Positive
Scan Range: 400 – 520 m/z
Max. Accumulation Time: 50000 μ s
Average: 3 spectra

Ionization Camera Optimal Conditions

Dry Temp. : 350 °C
Dry Gas : 9.80 L/min
Nebulizer P: 48.00 psi

Ion Tramp Optimal Conditions

Trap Drive: 51.2 volt
Octopole RF Amplitude: 178.4 Vpp
Lens 2: 60.0 Volt
Capillary Exit: 124.4 Volt
HV Capillary: 3180 Volt
HV End Plate Offset: -500 Volt
Fragmentor: 40 eV

Direct Infusion Pumped Flow Conditions

Flow: 3.00 μ L/min
Nebulizer P: 10.00 psi
Drying Gas: 3 L/min
Dry Temp: 350 °C

3.4 Analytical Methods

3.4.1 Tetracycline Analysis (TC)

3.4.1.1 UV-Vis Spectrophotometry

Preliminary measures were performed using a UV-Vis Beckmann spectrophotometer. The experiments were conducted at laboratory temperature (25 °C) under acidic, neutral and alkaline pH (1.80, 3.80, 7.00, and 10.00) according to their pKa, for a suitable time while continually agitation at 250 rpm. Three replicas of the sample, a control (without crumb rubber) and a blank (deionized water with crumb rubber) were analyzed as part of the batches. The batches concentrations were 75.00 - 100.00 ppm TC and the exposure time of TC to crumb rubber particles was 76 hours. During the 76 hours were draw samples at 0, 0.5, 2, 4, 7, 10, 24, 48, 76 hours. The preliminary experiments were used to decide at which pH the preceding experiments were to be performed. In order to observe any degradation of TC liquid chromatography was later used.

3.4.1.2 UV-Vis Liquid Chromatography

TC was also analyzed by liquid chromatography. Using on the HPLC 1100 Agilent instrument equipped with the UV-Vis Diode Array detector under isocratic conditions at a flow rate of 0.5 mL/min. The mobile phase A contained 0.20 % of formic acid in deionized water (conductivity = 18.1 $\mu\Omega$ /cm) and mobile phase B consisted of HPLC grade acetonitrile at mixing proportions of 50 % A and 50% B. The TC was detected at λ_{average} of 360 nm and $\lambda_{\text{reference}}$ of 250 nm. TC eluted within 5 minutes with a 2 minutes post time allowed re-equilibration of the column. The

batches concentration was 100.00 ppm at pH 10.00 with exposure time up to 96 hours. A nine levels calibration curve was used in which the concentration range varied from 0.00 ppm to 120.00 ppm. The calibration curve was checked periodically with QC samples and recalibrated if the control did not pass.

3.4.1.3 LC/MS/MS Analysis

The LC-MS/MS analysis were performed using a LUNA C8 column (Phenomenex) of 100x4.6mm with 3 µm diameter packing using the mass detector and seven levels calibration curve. The concentration range was from 0.10 to 3.00 ppm. The working batches concentration were 10.00 ppm at pH 3.80 with exposure time up to 168 hours. LC/MS/MS run time was 4.00 minutes and 2 minutes of equilibration post-time. A new calibration curve was used for each complete set of sample. The samples were analyzed in duplicates with two replicas of each. The final pH of samples was also measured. The quantification was performed with the mode of selected reaction monitoring (SRM), which requires MS/MS fragmentation.

3.4.1.3.1 LC/MS/MS Conditions

- HP 1100 LC (Agilent) coupled to a MS detector (Brucker Daltonics Esquire 6000)
- Reversed-phase system, under isocratic conditions
- A column of 100x4.6 mm Luna C8 with a 3 μm particle size (Phenomenex)
- Mobile phase 40 % acetonitrile in deionized water, 1% formic acid, pH 2.43
- Selected reaction monitoring (SRM) with monitoring 410.20 and 427.17 m/z daughter ions from 445.31 m/z TC parent ion and 448.06 and 430.03 m/z daughter ions from 465.19 m/z DMC Internal Standard (IS) parent ion.
- Run time was 4 min
- 2-min post time allowed re-equilibrium of the column.

3.5 Samples Preparation

3.5.1 LC/MS/MS Adsorption Batch Experiments Samples Preparation

One-hundred mL solutions of TC were prepared starting from the pure solid TC for each sample and the pH measured. The pHs were adjusted with trace metal concentrated HCl and a 200.00 mL aliquots were withdrawn and labeled at initial time (t_0) and stored for later analysis. Starting from each original TC solutions, 60.00 mL were transferred to 60.00 mL flasks and labeled sample one (S1), sample two (S2) and control (no crumb rubber added) followed by adding 0.6000 g of crumb rubber to each and placed in a magnetic stirrer at 250 rpm. A blank was prepared using only 0.6000 g of crumb rubber in deionized water. All samples were covered to avoid light exposure. Aliquots of 250.00 mL of each were withdrawn from original solutions at

different time intervals (0.5, 1, 2, 4, 8, 12, 24, 48, 92, 120, 168 hours). Each sample was filtered using a syringe filter 0.22 μm and kept in the refrigerator at 4 °C until analysis. A summarized schematic diagram is shown bellow in figure 12.

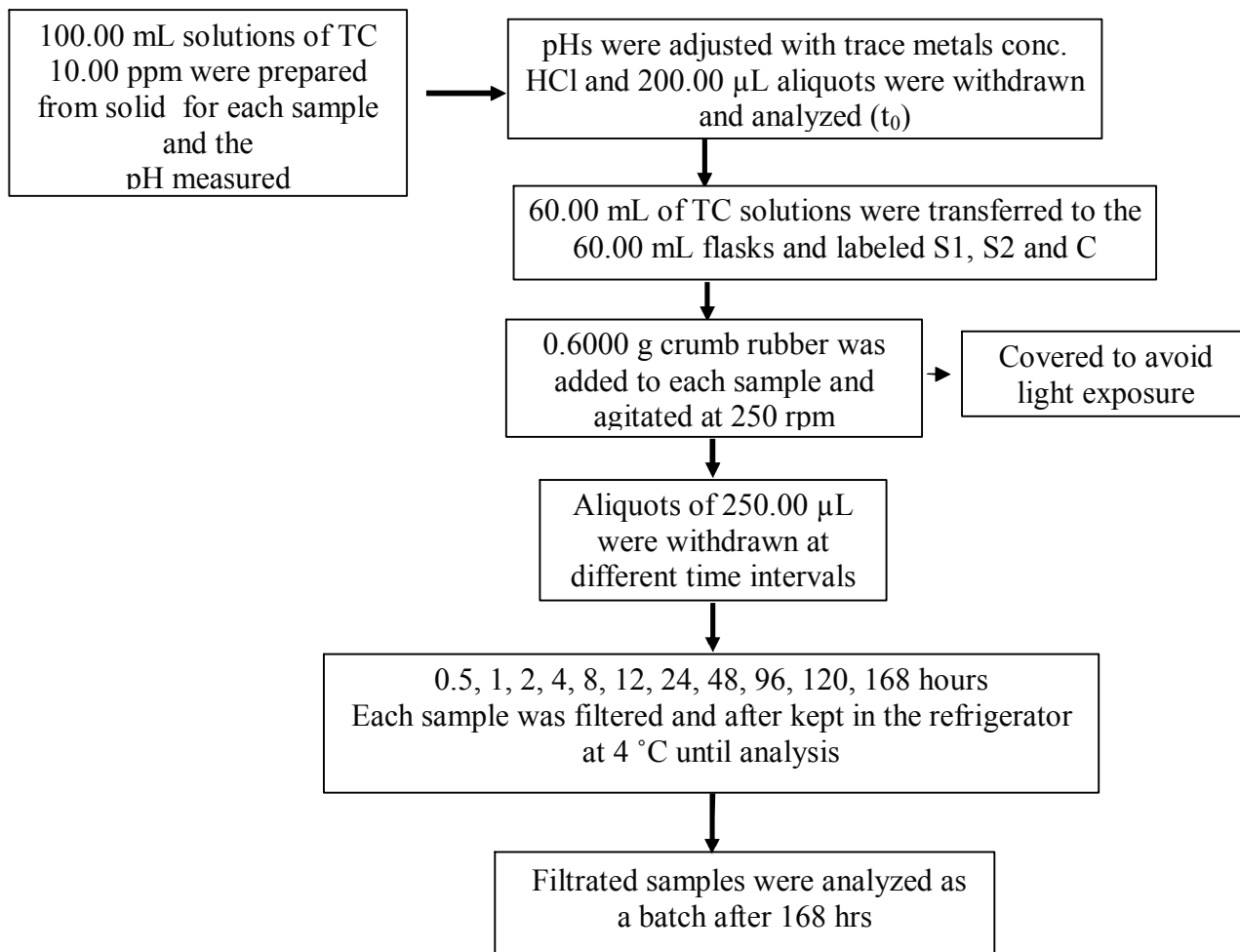


Figure 12. Diagram of LC/MS/MS Batch Experiments Preparation

3.5.2 UV-Vis Adsorption Batch Experiments Samples Preparation

The sample preparation for the UV-Vis experiments was similar for the procedure described in section 3.5.1 for the LC/MS/MS analysis however different pHs (1.80, 3.80, 7.00, 10.00) were evaluated with exposure time up to 76 hours. Another experiment was performed at pH 10.00 and the exposure time extended for up to 96 hours. Ten ml 100.00 ppm TC solutions were prepared and placed in 30 mL flasks and 0.1000 g of crumb rubber added and agitated at 250 rpm in a magnetic stirrer. Aliquots of 50.00 μ L were transferred and diluted to 500.00 μ L in an 800 μ L quartz cells and the absorbance of the solution measured. A summary of the procedure is shown in figure 13.

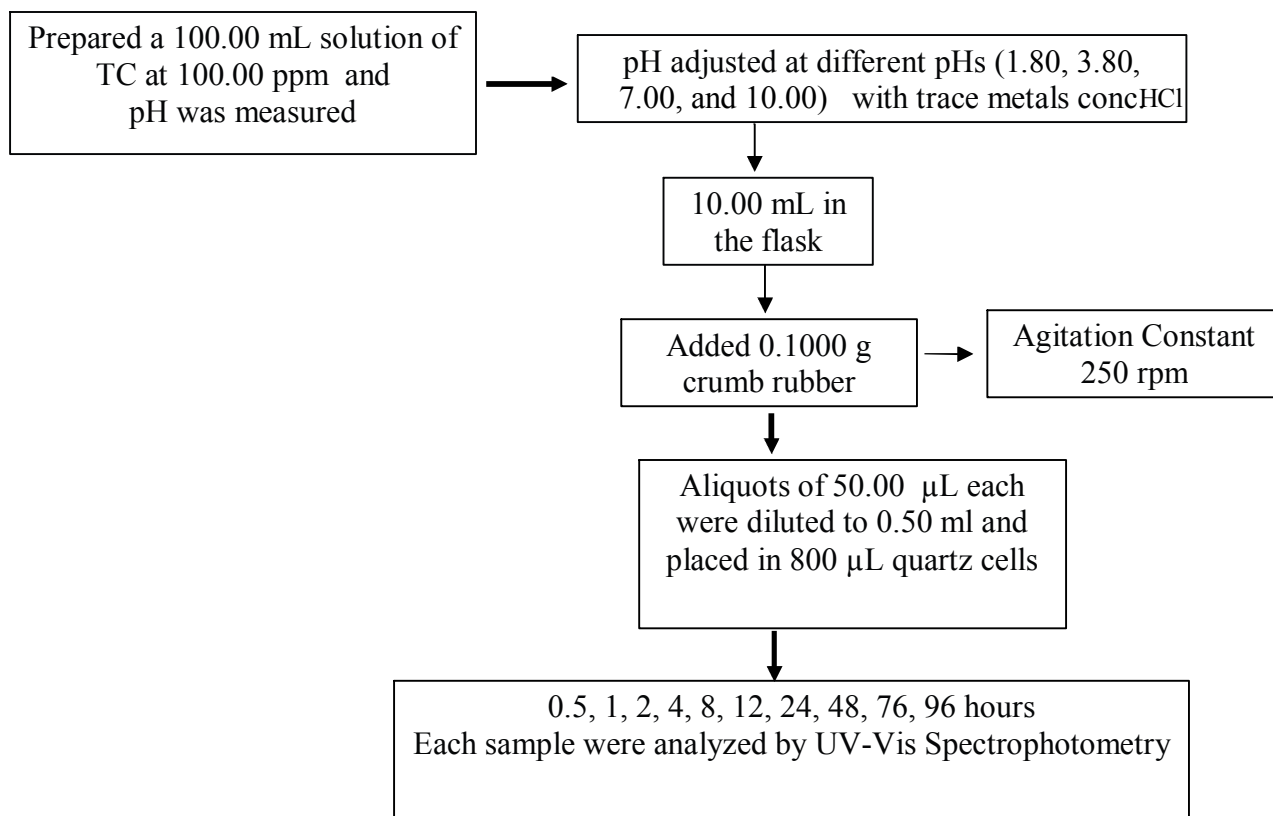


Figure 13. Diagram of UV-Vis Batch Experiments Preparation

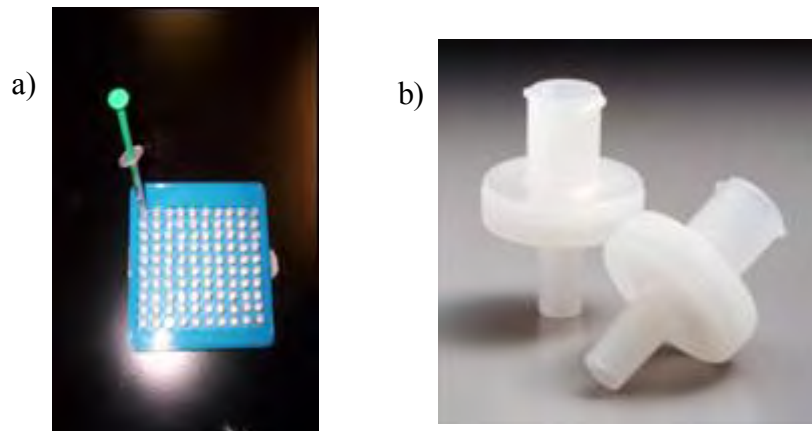


Figure 14. Samples Filtration

- a) Filtering samples using syringe filter**
- b) Syringe filters**

3.5.3 Samples Preparation for LC/MS/MS Analysis

The samples preparation was carried out of the following way:

An aliquot of 100.00 μL was taken from the 250.00 μL of sample stored and added 300.00 μL of deionized water. 1.20 μL of internal standard of 500.00 ppm demeclocycline (DMC) solution was added to the aliquot and 1.20 μL of 500.00 ppm TC solution resulting spikes of 1.50 ppm TC for percent recoveries calculate (figure 15). It was analyzed 400.00 μL of 1.50 ppm TC solution and considered as independent quality control (QC) solution, to which, 1.20 μL of 500.00 ppm DMC IS, were added. QC concentration was at the mid point of the calibration curve. The independent QC and two spikes were analyzed each twenty samples. Quality controls were measured periodically by injecting of recently prepared solutions. This procedure is summarized in figure 15.

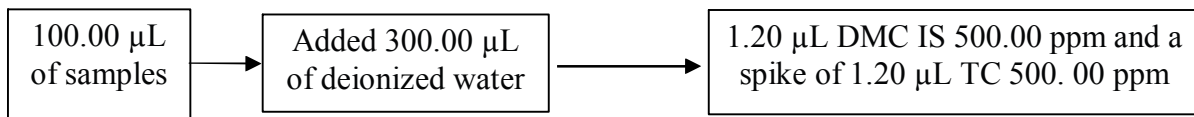


Figure 15. Sample Preparation for LC/MS/MS Analysis

3.5.4 Material Cleaning

Plastic and glass materials were immersed in a 5.00 % nitric acid (ACS Certified, Fisher) solution for 24 hours and followed by contact with a 0.05 M ethylenediamine tetracetate (EDTA) solutions. The EDTA is used to chelate metals that may be present on the surface of the material. After 24 hours of soaking, they were rinsed with deionized water three times and placed in the oven until dry.

CHAPTER VI

4 RESULTS AND DISCUSSION

The results were divided in preliminary UV-Vis Measurements, LC Diode-Array Detector and LC-MS/MS results.

4.1 Preliminary UV-Vis Measurements Results

The results were obtained with a BECKMAN UV-Vis spectrophotometer. Batch experiments were performed at pH values of 1.80, 3.80, 7.00 and 10.00. The last three are approximately the three different pKa values ($pK_{a1} = 3.30$; $pK_{a2} = 7.68$; $pK_{a3} = 9.69$) of tetracycline. The contact time was 76 hours at room temperature conditions using 10.00 g/L of crumb rubber mesh 14-20 without additional treatment.

The UV-Vis spectrum corresponding to a 50.00 ppm TC solution is showed in figure 16. Two absorption peaks were centered at 260 nm and 360 nm. These absorption maxima wavelengths are consistent with the values reported in the literature for TC^{12, 28}.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated in according with the formula $LOD = 3s_b/A_m$ and $LOQ = 10s_b/A_m$, where s_b is average standard deviation of the intercept and m is the average slope of three measurements of the standards. The calibration

curves were constructed from 0.00 to 120.00 ppm. The LOD for the λ of 360 nm was lower than that of $\lambda = 260$ nm.

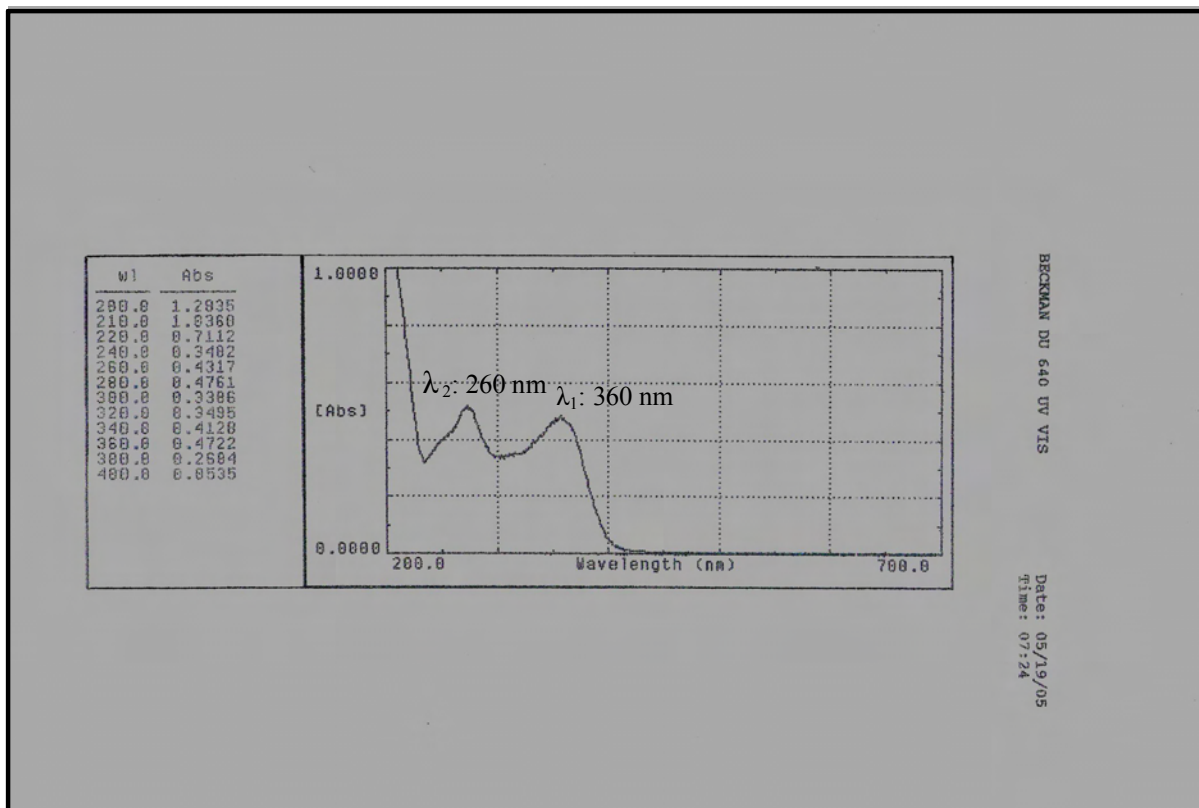


Figure 16. UV-Vis Spectrum, 50.00 ppm TC

LOD = 0.52 λ_1 : 360 nm
LOQ = 1.73

LOD = 0.88 λ_2 : 260 nm
LOQ = 2.92

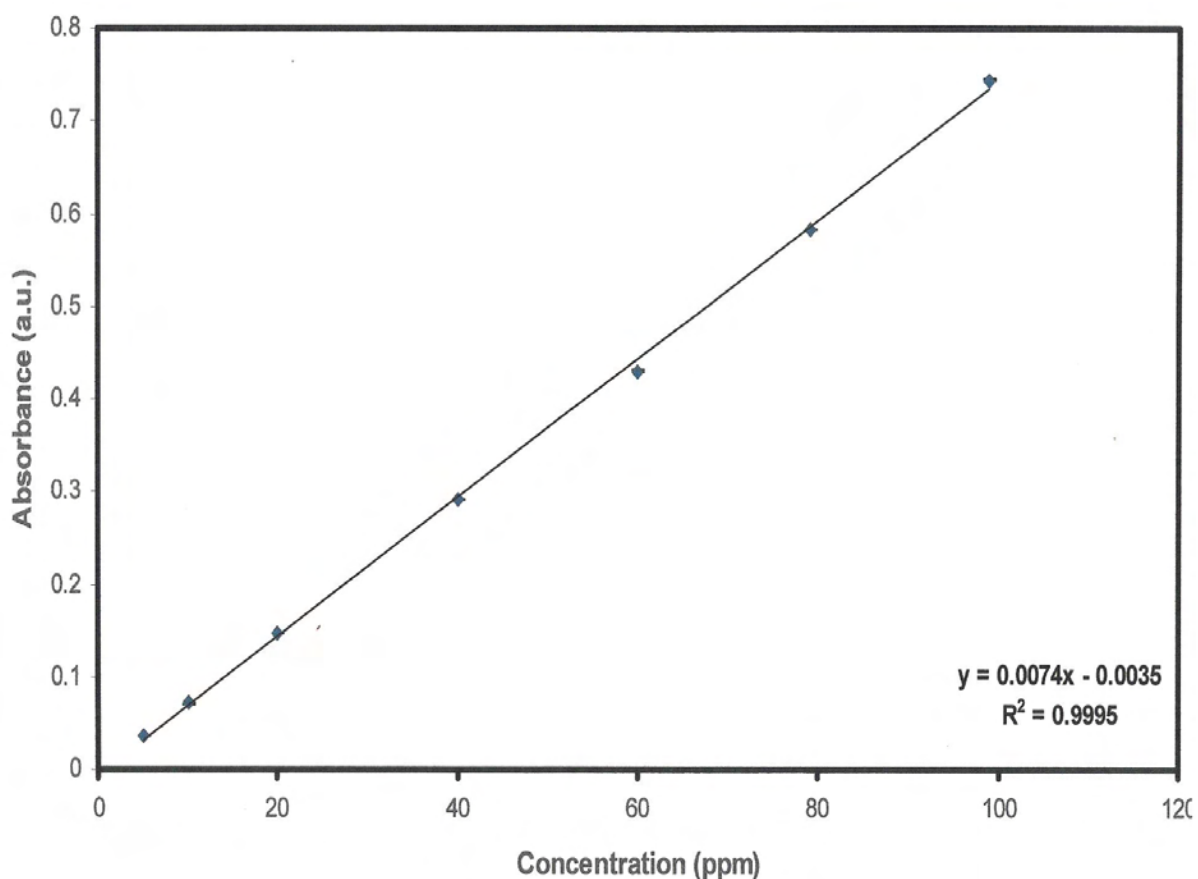


Figure 17. TC Calibration Curve for UV-Vis Measurements

The calibration curve (figure 17) showed a correlation coefficient of 0.9995 with an excellent linearity suitable for quantification using a linear model.

4.1.2 Preliminary Sorption Tests

Figure 18 shows the sorption behavior of TC at 95.00 ppm when placed in contact with 14-20 mesh crumb rubber at pH 3.80. Solutions were analyzed by UV-Vis spectrophotometry. Attained data presented a standard deviation varying from 0.00 to 0.70. It was observed a

particular behavior at earlier contact times (first 10 hours), as show in figure 18. There was observed a clear drop during first 2 hours and after that time was observed an increase in the absorbance. However, after a rapid adsorption of TC in the first two hours, there is an abrupt desorption until 5 hours. Then a slow sorption process begins until it reaches apparent equilibrium after 48 hours. Similar behavior was also observed at pH 7.00 and pH 10.00 (figures 19 and 20). At pH 3.80 and after 76 hours was observed a net decrease in concentration from 95.00 to 83.00 ppm, which represents a net removal of 11.89%

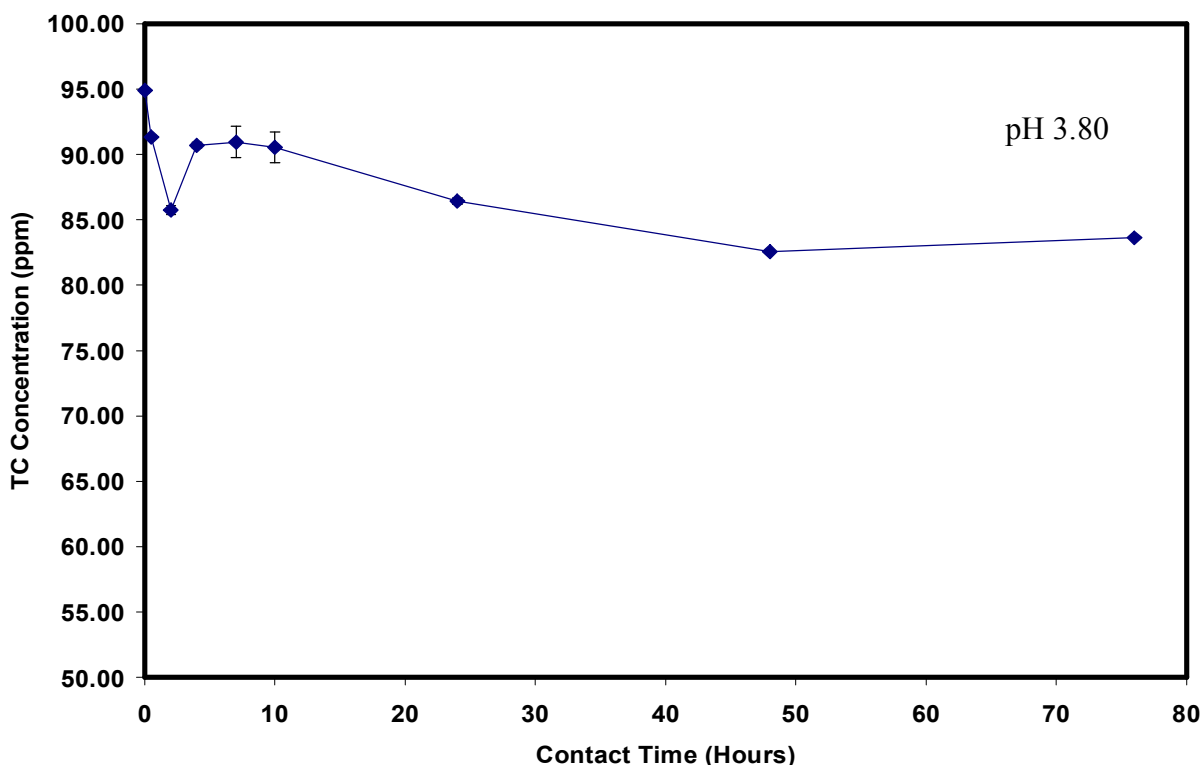


Figure 18. Sorption Behavior for TC contacted with 10.00 g/L 14-20 Mesh Crumb Rubber and 95.00 ppm TC initial concentration, pH 3.80

Figure 19, illustrates the behavior at pH 7.00. This data presented a standard deviation between 0.00 and 0.55. The TC concentration decreased rapidly from 74.00 ppm initial concentration

down to 65.00 ppm during the first two hours; however a rapid release occurs and then slowly adsorbs until reaching 60.00 ppm at 72 hours without reaching equilibrium. A similar behavior was observed at pH 10.00, as show the figure 20. At 12 hours the TC concentration went up to 79.00 ppm, suggesting its release to the solution. A gradual adsorption begins after 12 hours until the end of the experiment at 72 hours without reaching equilibrium. In this case, the net TC concentration decrease dropped from 81.00 ppm down to 65.00 ppm, representing a net adsorption of 19.45 %. The measurements precision were between 0.00 – 0.60.

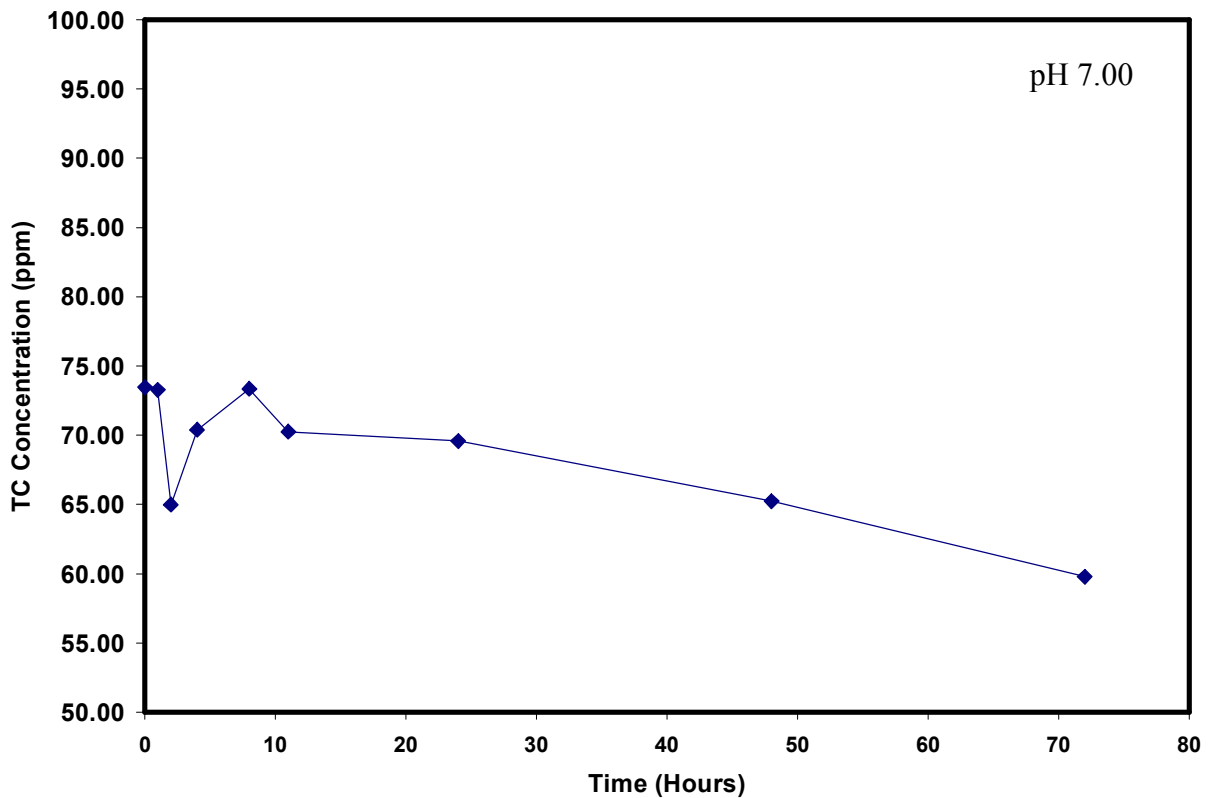


Figure 19. Sorption Behavior for TC contacted with 10.00 g/L 14-20 Mesh Crumb Rubber and 74.00 ppm TC initial concentration, pH 7.00

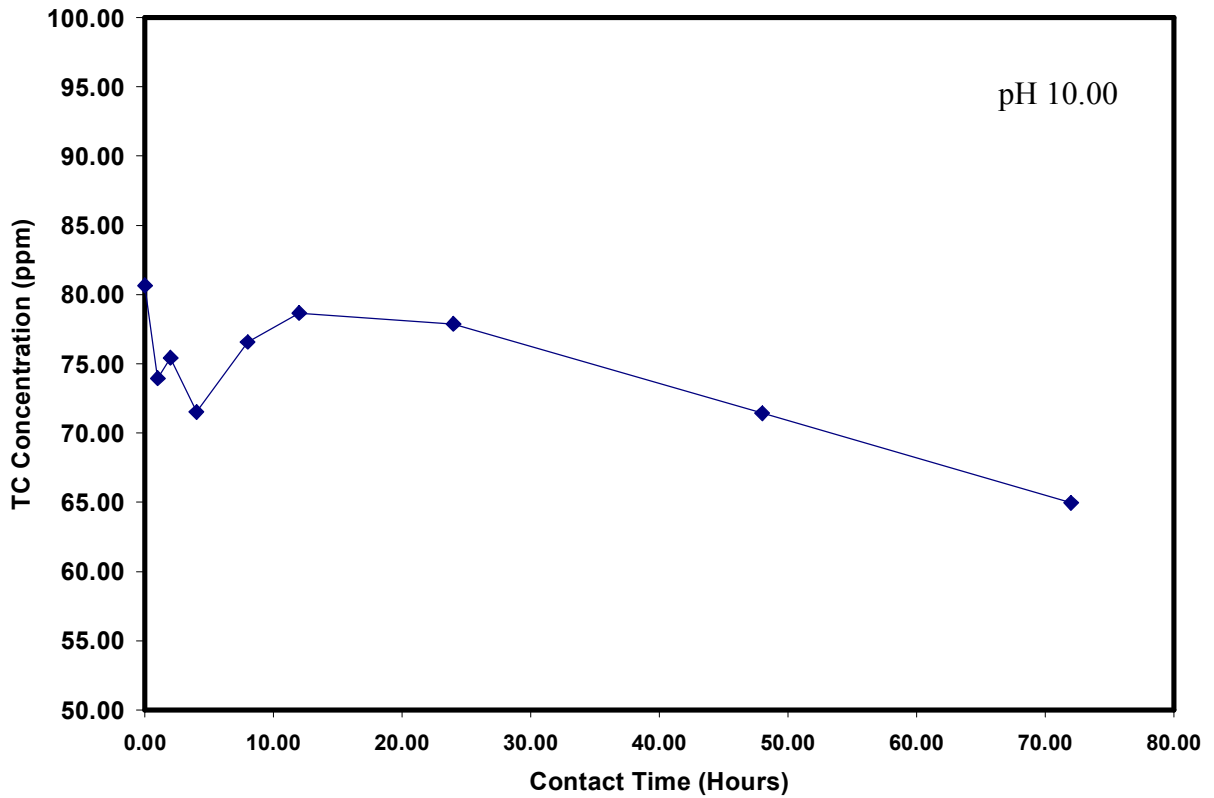


Figure 20. Sorption Behavior for TC contacted with 10.00 g/L 14-20 Mesh Crumb Rubber and 81.00 ppm initial concentration, pH 10.00

At pH 1.80 (figure 21) a similar behavior was observed during the first hours of the experiment. A fast adsorption took place during the first 2 hours. TC concentration decreased from 96.00 to 82.00 ppm within this period. However, this stage was followed by a fast release of the TC reaching the initial concentration. Not net adsorption was observed at this pH.

This lack of adsorption can be explained due to TC conjugated form that exists at pH 1.80 has a net positive charge, which may experience repulsion from charged species in the medium. It is

also possible that at very low pH the tire rubber suffers physical changes. Also, at low pH the tire rubber matrix can break the cross- links and decrease the swelling capacity⁴¹ and affecting -

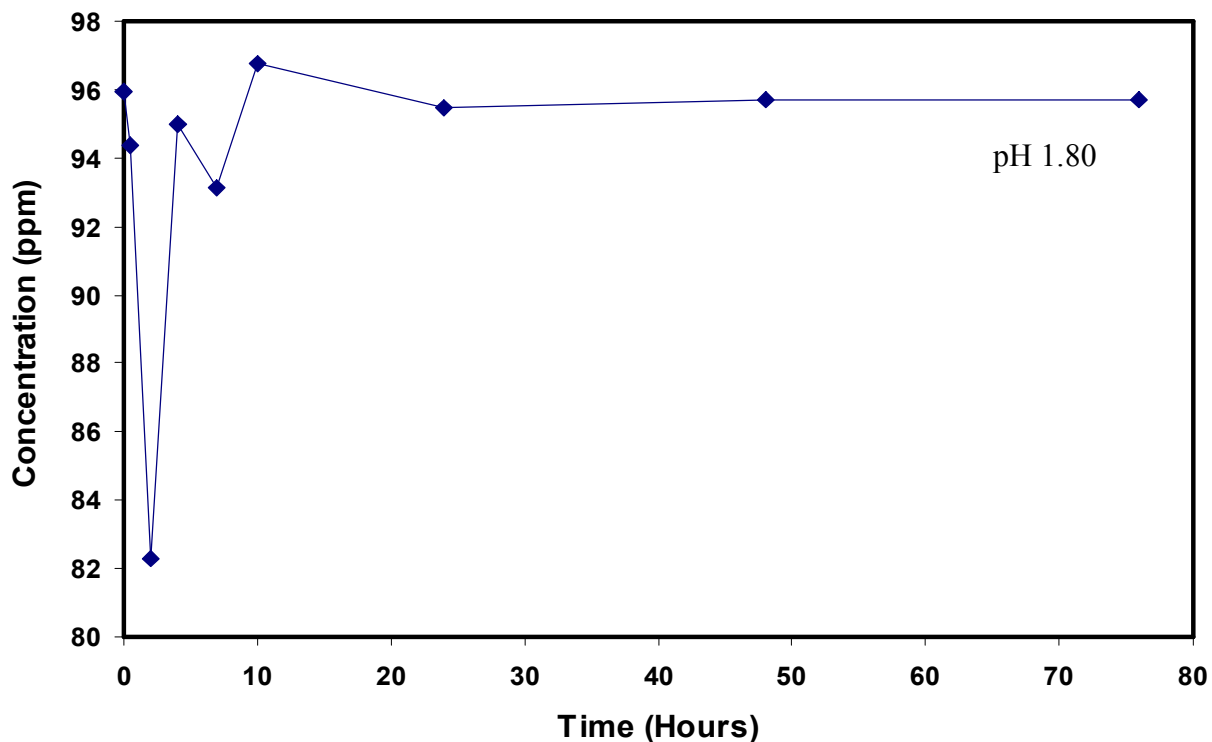


Figure 21. Sorption Behavior for TC contacted with 10.00 g/L 14-20 Mesh Crumb Rubber and 96.00 ppm initial concentration, pH 1.80

the diffusion of the adsorbate. The diffusion could be important for the adsorption if we consider that the carbon black present in the tire rubber is not completely exposed for the adsorption.

According to different pKa of TC shown in table 2, it can be predicted the conjugated forms that will exist at experimental pH ranges as shown in figure 22.

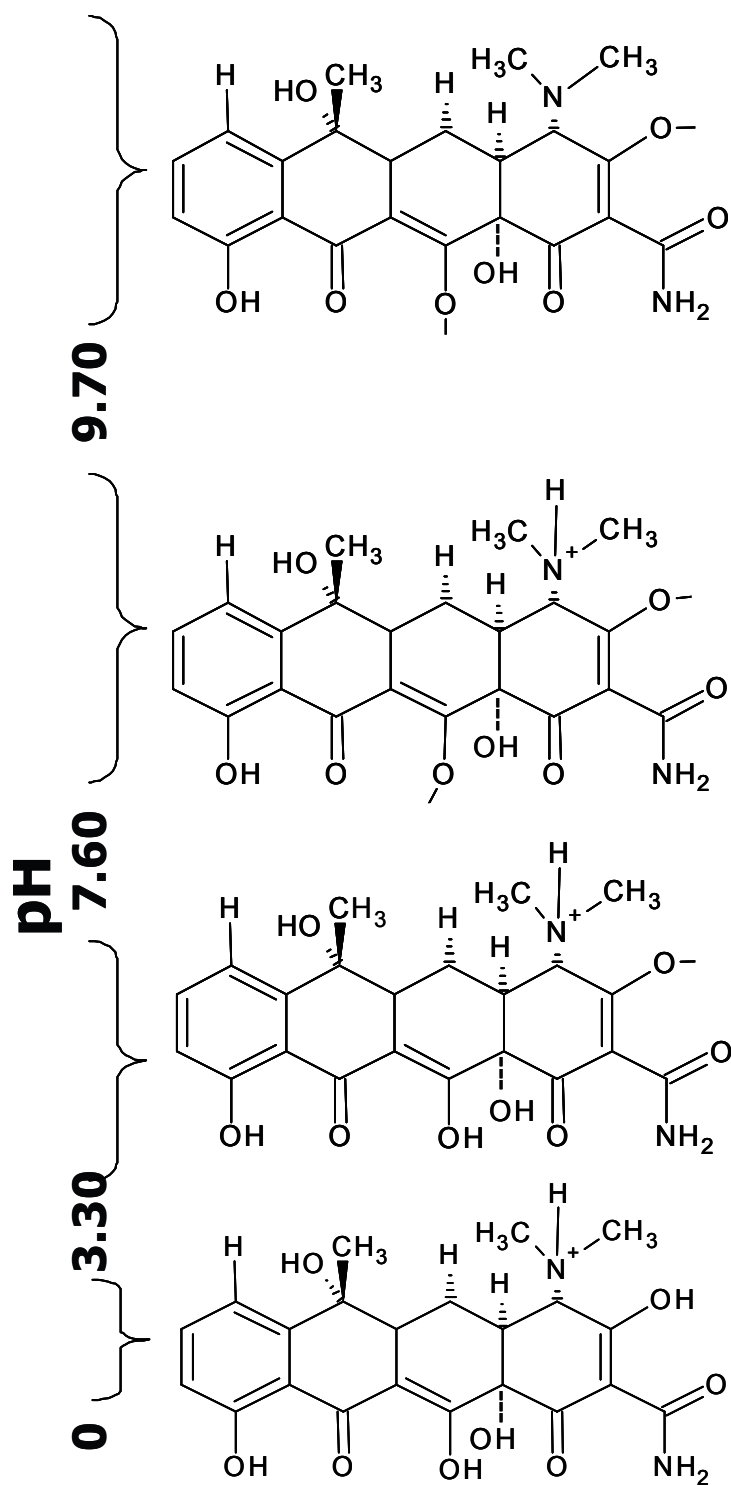


Figure 22. Conjugated Forms for Tetracyclines at different pH range

As shown in figure 18, the TC when placed in contact with crumb rubber at pH 3.80 were quickly adsorbed during the first two hours, followed by a fast release and subsequent slow adsorption process that did not reach equilibrium even after 76 hours. This unusual behavior was observed at all pH including 1.80 where no net adsorption was observed at the end of the contact period.

In search for an explanation to this unusual behavior seen during the first 10 hours, we focused our attention on the capacity of TC to form complexes with metals. Zinc oxide is used as a catalyst in the process of rubber vulcanization⁴¹ and is known to be present in the crumb rubber matrix (0.55 to 2.79 %). Previous works have discussed the Zn availability from the crumb rubber matrix^{42,43,44}. Besides, Alamo. L. (2006)⁴⁵ studied the release of Zn from crumb rubber used in this work at different pH values during a 24 hours period. These results show a release of 1.11 ppm Zn at pH 3.00 from 10.00 g/L of crumb rubber. Alamo's results suggest that the pH was influencing the Zn release from crumb rubber and than TC-Zn complexes may help to explain the unusual adsorption behavior observed during the first hours of the TC/crumb rubber sorption experiments.

Figure 23 summarizes the adsorption behavior of TC as a function of pH. At pH 1.80 no net adsorption was observed, however at pH 3.80, 7.00 and 10.00 a net adsorption of 11.89, 18.63 and 19.45 % was observed respectively. These results suggest that the sorption capacity of crumb rubber was better as increased the pH. This fact, indicated that is obtained a greater concentration decrease at basic pH and therefore a higher adsorption could be observed. Then,

we proceeded to performed adsorption batch test at pH 10.00 and used liquid chromatography to determine possible degradation of TC at high pH.

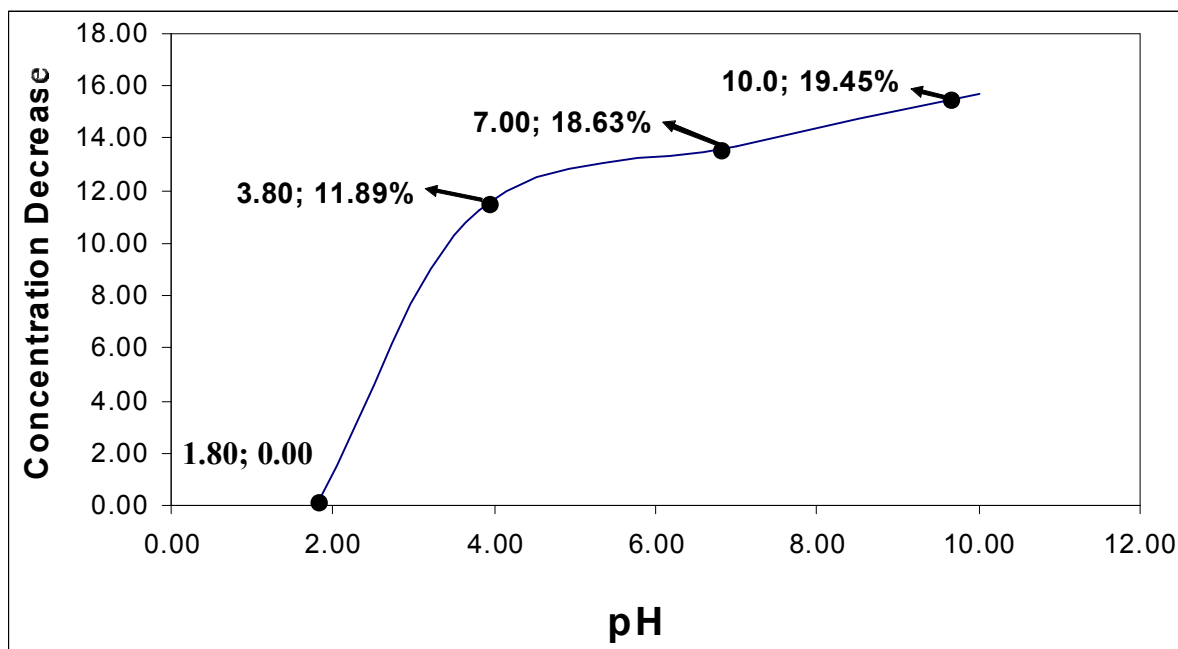


Figure 23. TC Sorption Behavior as function of pH

4.2 Liquid Chromatography with UV-Vis Diode Array Detection Results

In this case, the contact time was 96 hours under room temperature conditions and 10.00 g/L of crumb rubber mesh 14-20 was used for the adsorption test and at pH 10.00.

To evaluate the performance of the filtering device (figure 14b), 380 μ L of a 60.00 ppm TC solution was passed through of the filter. The concentration before and after filtration were 59.81 y 59.56 ppm, respectively. These results suggested no significant sorption of TC by the

filtering device. Therefore, all samples and standards were filtered prior to analysis to eliminate any suspended solids.

It was obtained a UV-Vis spectra with mobile phase to determine how much could be shifted the absorption maxim wavelength (figure 24). The mobile phase contained 50 % acetonitrile, 0.2 % HCOOH, pH 3.20 at a flow rate of 0.50 mL/min.

Figure 24c shows a chromatogram of a 50.00 ppm solution of TC using a diode-array detector. The chromatogram shows two peaks, one small at 1.4 minutes and a large at 1.6 min. The low-intensity peak at 1.4 min may be a reversible degradation product of TC^{30,31}.

Figure 24a shows the UV-Vis spectra of the peak at 1.6 min, which shows two absorption maxims wavelength at 270 and 365 nm. These absorption maxims were shifted with respect to the two absorption maxims wavelength obtained with the Beckman UV-Vis spectrophotometer, which were at 260 and 360 nm (figure 24a). The isoabsorbance plot (figure 24b) verified the two absorption maximum wavelength.

We selected a broad range of λ (300 – 420 nm), which included the centered maxim absorption wavelength to detect possible degradation products. In the literature^{31-34, 46}, there is evidence that the tetracyclines can decompose and absorption maximum wavelength for TC decomposition products can change, which, will not allow detecting the possible TC decomposition products if a very narrow wavelength range was used.

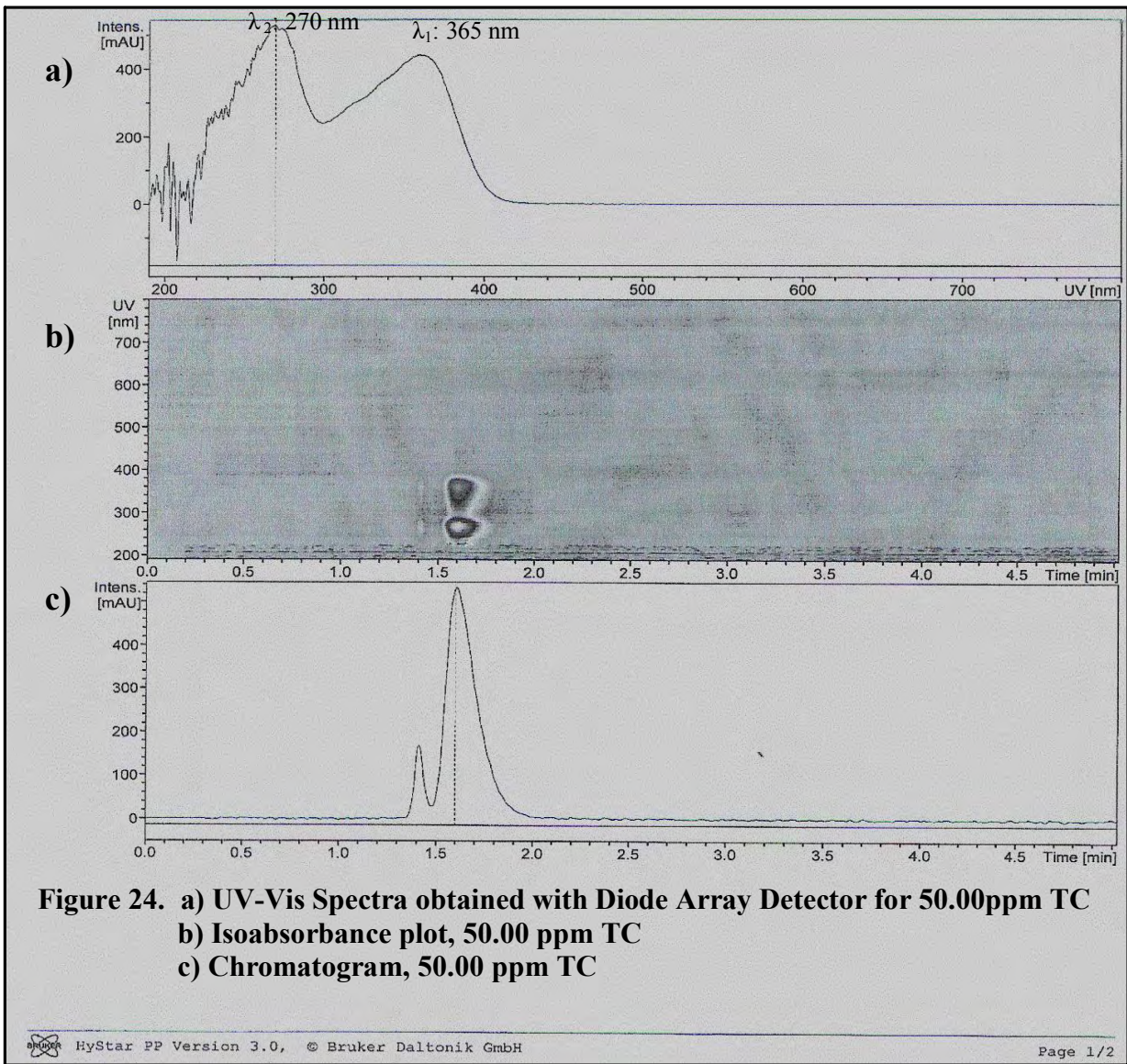


Figure 25 shows a calibration curve using LC with diode array detection. Calibration curves constructed within of the 300 – 420 nm and 244 – 300 nm ranges were used to calculate the LOD and LOQ within each range. The LOD and LOQ in 300 – 420 nm range were 0.10 and 0.34. The LOD and LOQ in 244 – 300 nm range were 0.28 and 0.94.

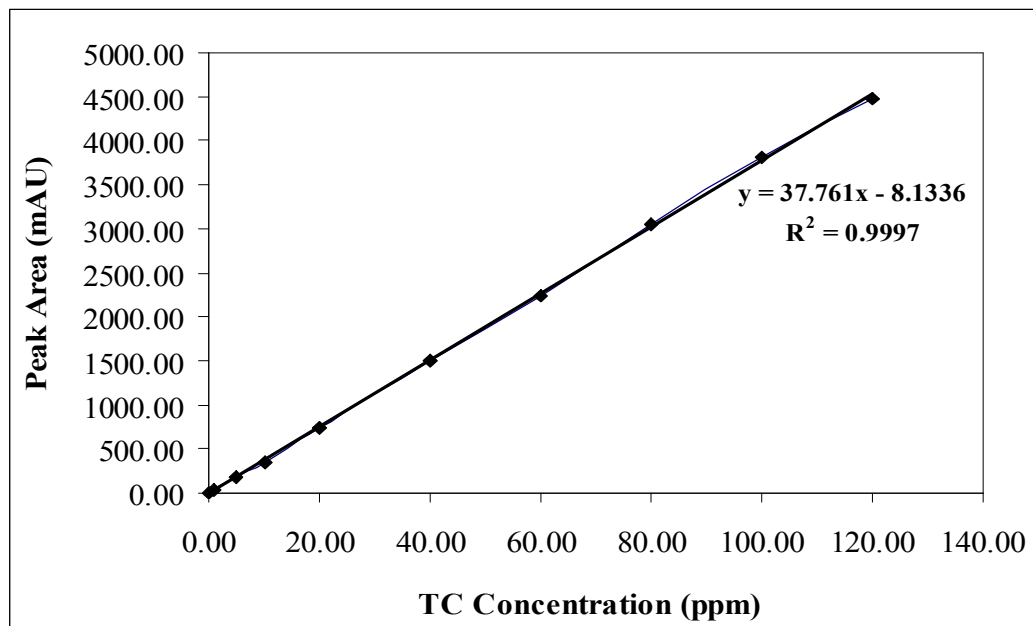


Figure 25. Calibration Curve for TC Using LC Diode-Array Detection

Figure 26 shows the chromatogram for the control test, which correspond to the TC solution with no crumb rubber. At initial time is observed the main peak of TC with elution time of 1.61 min. It is also observed a small peak to the left. This small peak to the left of TC peak was also observed by Wu. Y. et al³⁴ and Anderson C.R. et al³³, which they assigned to epi-tetracycline (e-TC). The e-TC is a product due to reversible TC epimerization^{31,33,34} in the C₄ and it is favored at acid pH and even at pH around 7.00 (figure C1 in appendix c). It is observed another 3 peaks of low intensity in the chromatogram at initial times, which were well separated and more intense at 96 hours. Those peaks showed retention time at 2.74, 3.13 and 4.34 min, respectively. At 96 hours, the small peak attributed to e-TC almost disappeared, which confirms that at basic pH, e-TC is not favored and can be progressively reverted to original TC, as reported in several publications^{31, 33, 34}.

The chromatograms for the sample showed in figure 27 exhibit a similar pattern to the control samples (figure 26), except that the intensities and areas were lower. This suggested that the difference was because the degradation products of TC can be competing for an adsorption sites fraction in the crumb rubber. These samples were analyzed after 12 days and the percents recoveries varied from 62.91 – 130.53. The accuracy was measured as percent relative error and varied from 0.32 to 8.49 %. Table 7 has a summary of the chromatographic data obtained at pH 10.00.

After liquid chromatography measurements with diode-array detection, we concluded that at pH 10.00 was observed a significative degradation as shown in the figure 28. These results were in agreement with previous works. Wu. Y., and Fassihi. R. in 2005³⁴ indicated that stability profiles of metronidazole, tetracycline and famotidine were reached at pH near to 4. Halling-Sorensen. B. et al in 2002³¹ and Anderson. C.R. et al, in 2005³³ coincided that TCs are generally fairly stable in dilute acids. Furthermore, Pena. A.L.S. et al⁴⁷ found that acidic aqueous solutions at pH 4.0 were successful in tetracycline extraction without resulting in degradation during three days.

With the preliminary results of the adsorption tests for TC with crumb rubber, we can concluded that adsorption of TC was possible at pH values near its different pKa⁴⁸, however, at pH 10.00 there was a significant TC degradation. There is no equilibrium even at 76 hours of exposition. Beside, there is possible formation of TC-Zn complexes that might be influencing the adsorption behavior of TC into the crumb rubber.

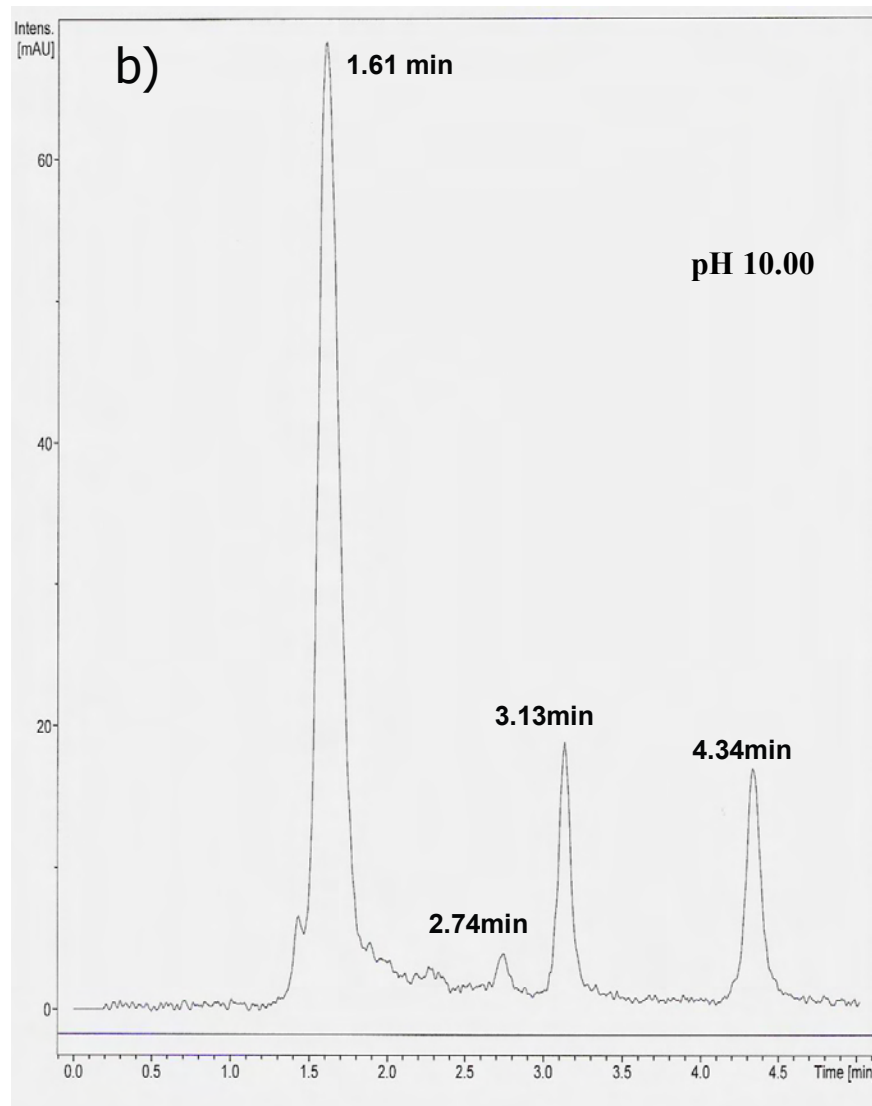
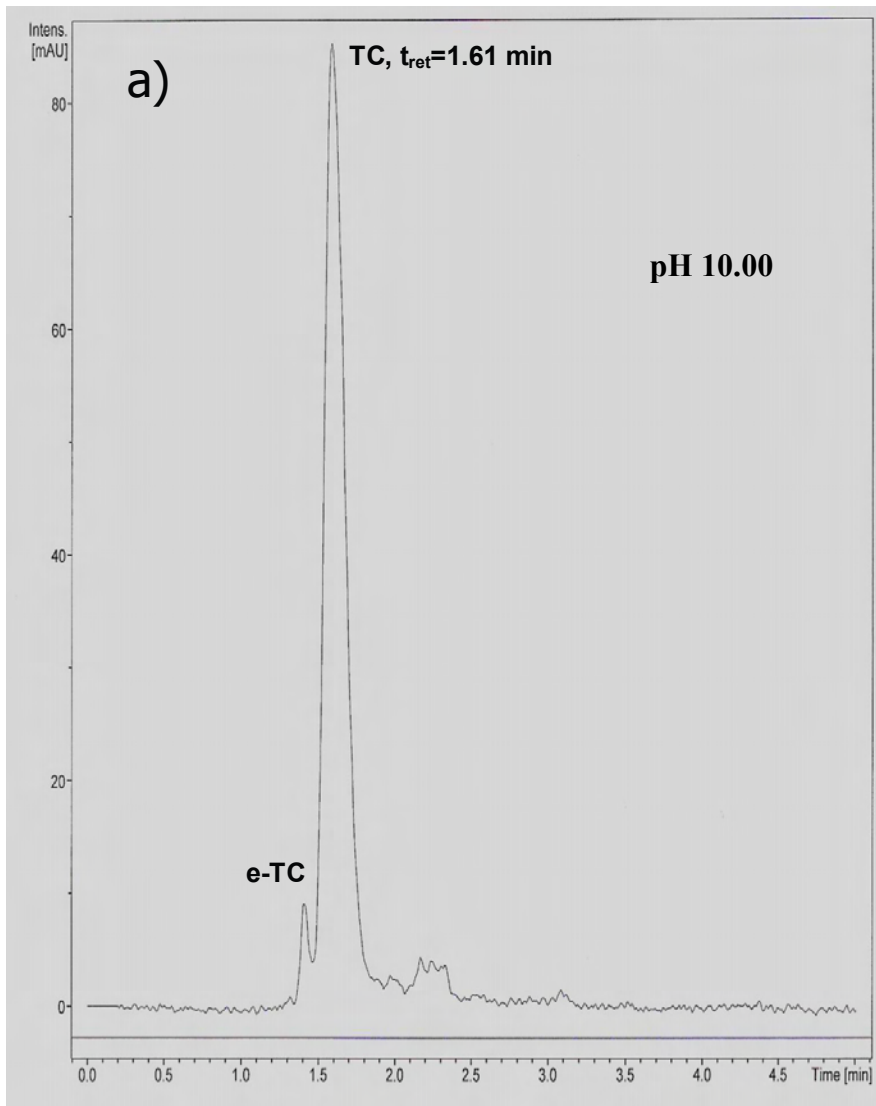


Figure 26. Control Chromatograms

a) $t=0$ seg

b) $t=96$ h

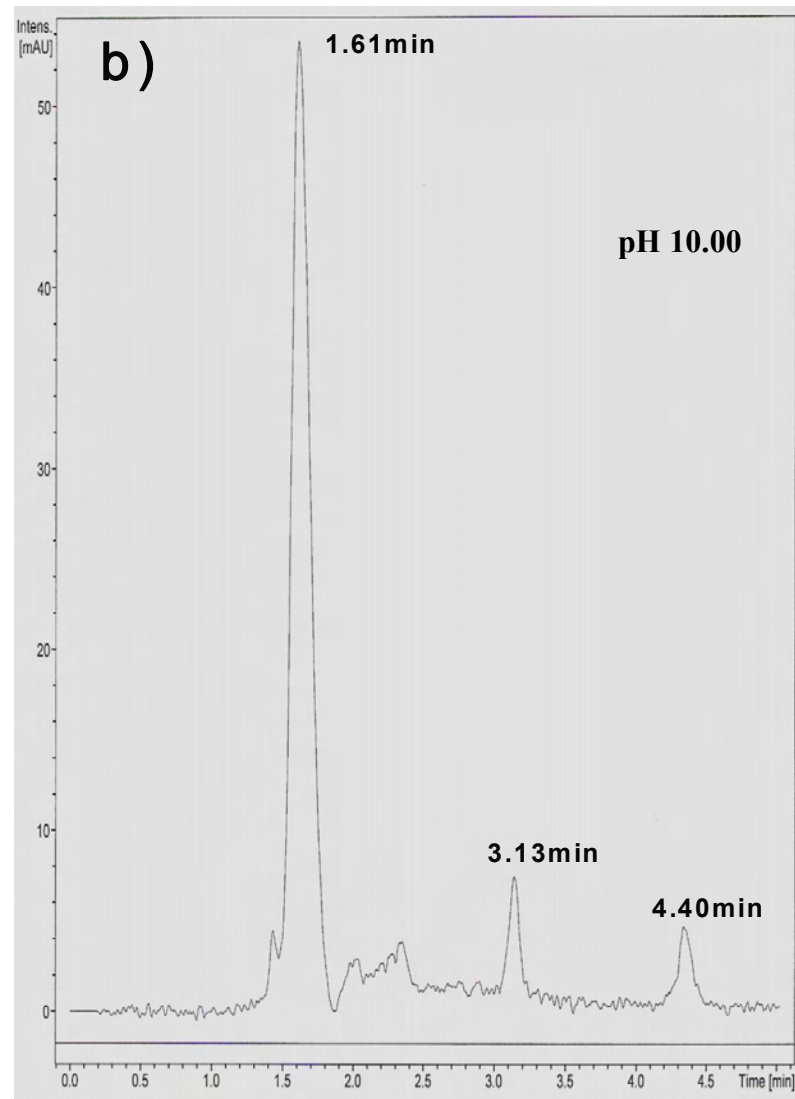
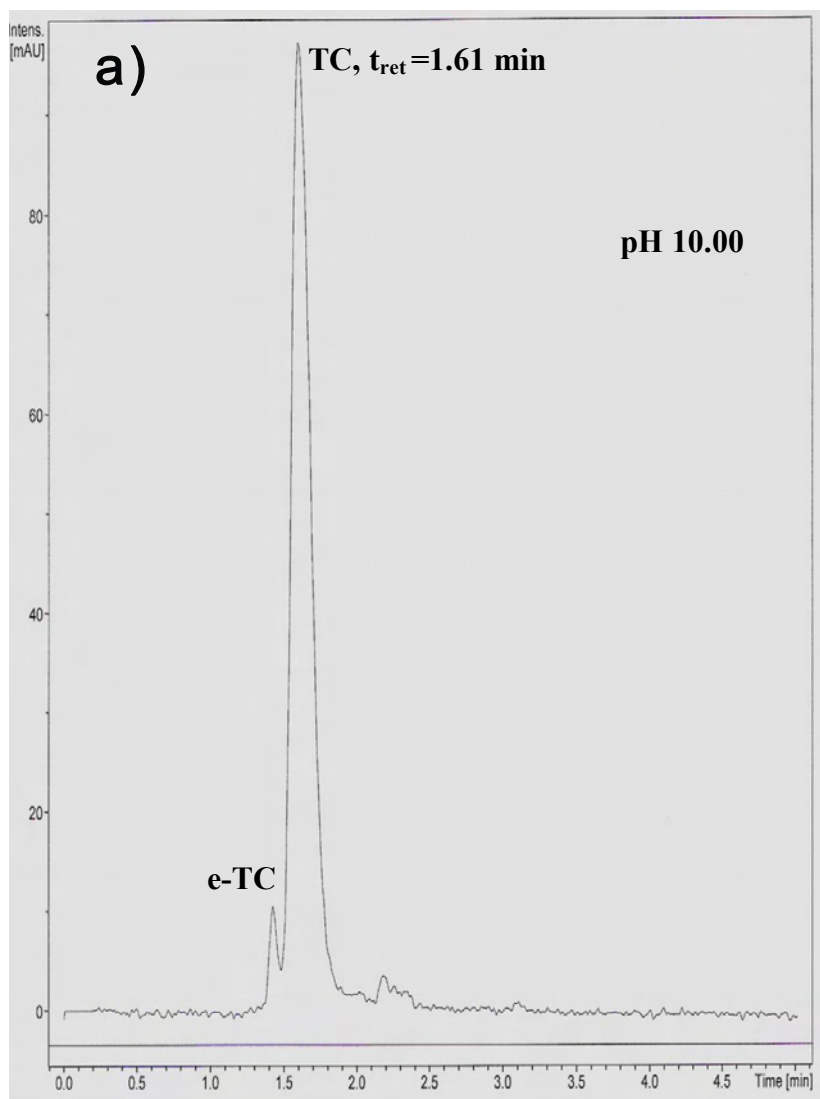


Figure 27. Sample Chromatograms

a) $t=0$ seg

b) $t=96$ h

Table 7. Calculated Concentration for pH 10.00 Batch Experiment with HPLC UV-Vis Diode Array Detection

Time (hours)	Average Measure Concentration (ppm) for Sample with UV-VIS HPLC	s	Average Measure Concentration (ppm) for Control with UV-VIS HPLC	s
0	91.79	±2.27	97.03	±0.95
1	91.79	±2.27	83.67	±6.05
2	82.84	±5.38	89.70	±0.05
4	84.03	±8.30	90.72	±0.83
8	75.50	±4.86	87.72	±5.56
12	79.14	±11.30	92.69	±5.25
24	80.21	±3.65	85.62	±1.24
48	68.28	±3.04	81.01	±1.49
96	60.92	±7.11	71.62	±3.36

s: standard deviation

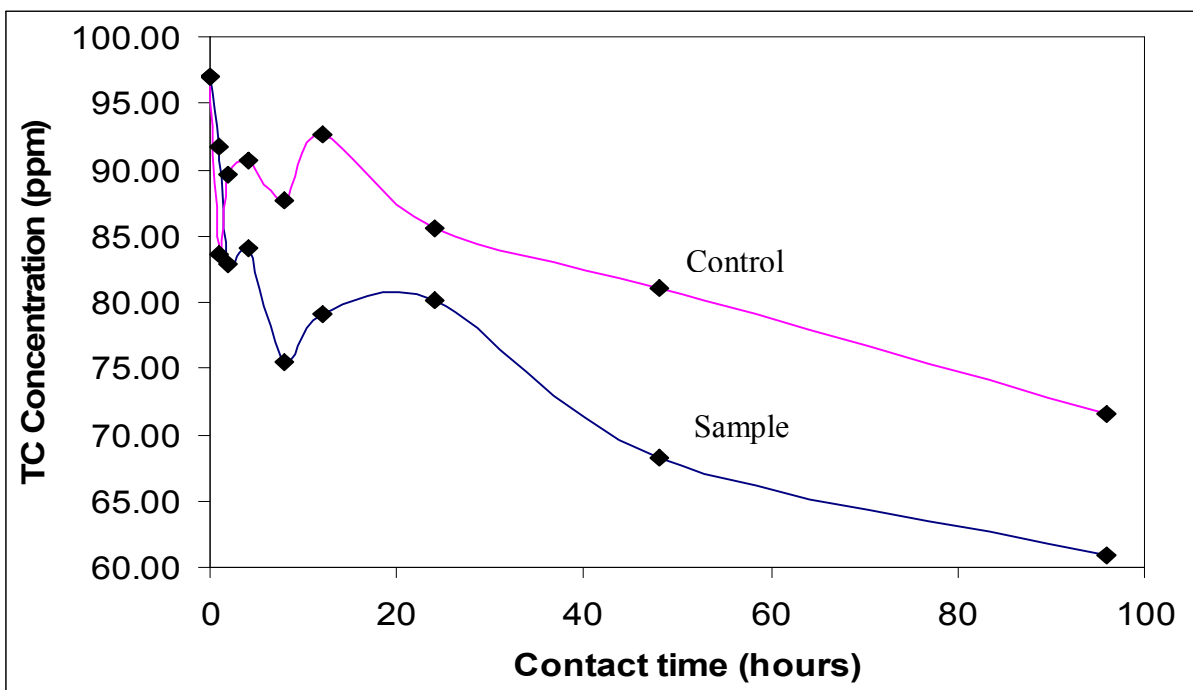


Figure 28. Sorption Behavior for TC Control (pink) and Sample (blue) analyzed With HPLC UV-Vis Diode Array Detection

4.3 Scanning Electron Micrography of the Waste Tire Crumb Rubber Without Additional Treatment

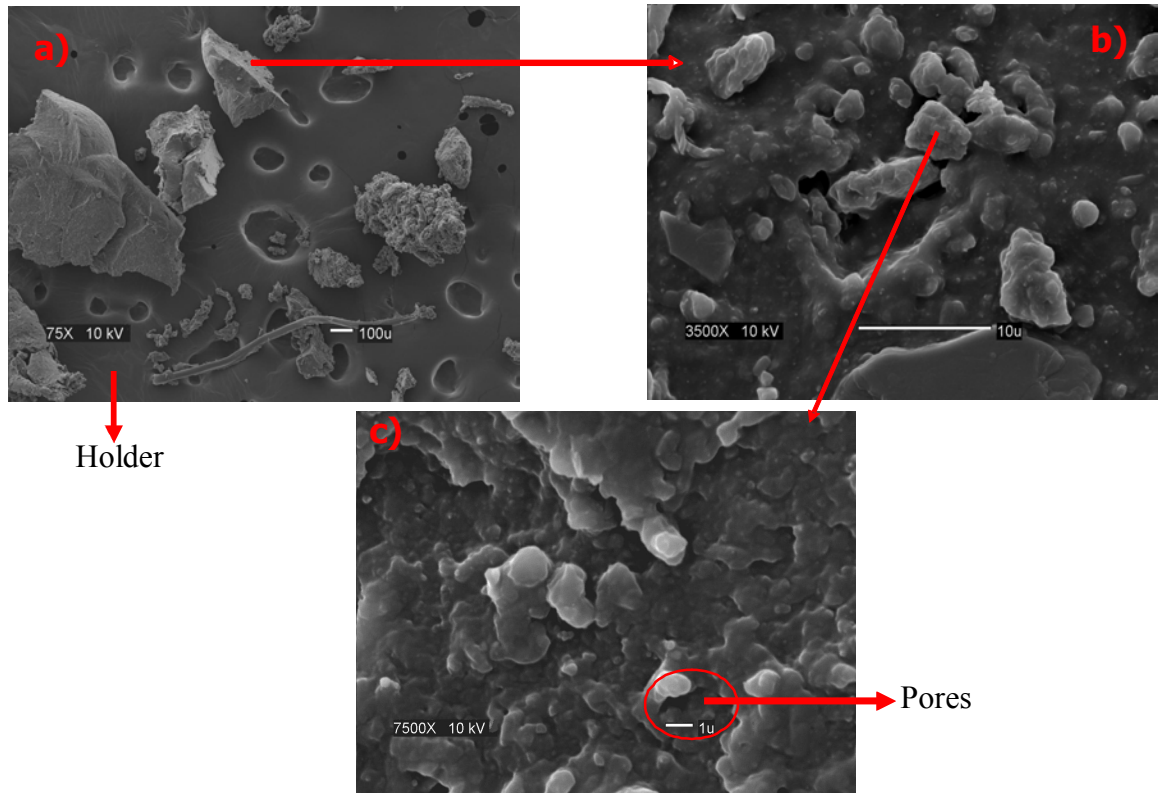


Figure 29. Scanning Electron Microscope (SEM) pictures of Tire Crumb Rubber Mesh 14-20
a) 75X b) 3500X c) 7500X

Figure 29 shows a scanning electron microscopy corresponding to the 14-20 mesh crumb rubber used in our experiments. The micrograph 29a shows a magnification of 75X and shows the irregular morphology of the crumb rubber. The magnification was increased to 7500X (figure 29c) and it is observed an irregular and rough surface with some pores. These pores size fall within the macropores size range. No structures corresponding to mesopores were observed.

4.4 Sorption Tests Analyzed by LC/MS/MS

Based in previous results and the findings of other investigators it was decided to work with pH at 3.80, at which the TC is stable as reported in the literature^{31, 33, 34, 47}.

4.4.1 LC/MS/MS Chromatography

In addition to using the mass spectrometer in a total-ion acquisition mode, MS quantification can be obtained by the following options:

- Selected Ion Monitoring (SIM): the instrument is set to monitor ions of specific m/z values. Each ion in SIM analysis is monitored for longer time than any ion in a total-spectrum analysis.
- Selected Reaction Monitoring (SRM): this mode requires MS/MS and is performed by using the internal or external standard method. The internal standard method is the most convenient because to can reduce errors due to degradation, variability in percent recoveries, adsorptive loss, dilutions and instrumental variability. To quantify with the internal standard, it is used the relative signal ratio of the sample with respect to the internal standard.⁴⁹

In this work we used the SRM mode with the internal standard method. In SRM analysis, precursor ions are selected, isolated and collisionally induced dissociated (CID) to product ions of specific m/z values and their ions current registered.

The product ions of TC protonated molecular ion (445.10 m/z) was monitored for quantification using the Selective Reaction Monitoring (SRM) mode. Then, the product ions at 410.20 and 427.17 m/z were monitored to obtain the extracted ion chromatogram (EIC) for TC. Alternatively, was obtained the EIC of demeclocycline internal standard (DMC IS), monitoring the product ions at 448.06 and 430.03 m/z resulting of the fragmentation on DMC protonated molecular ion peak (465.19 m/z). The figure 30 shows the EIC for TC (first peak) and DMC IS (second peak) which were used for quantification:

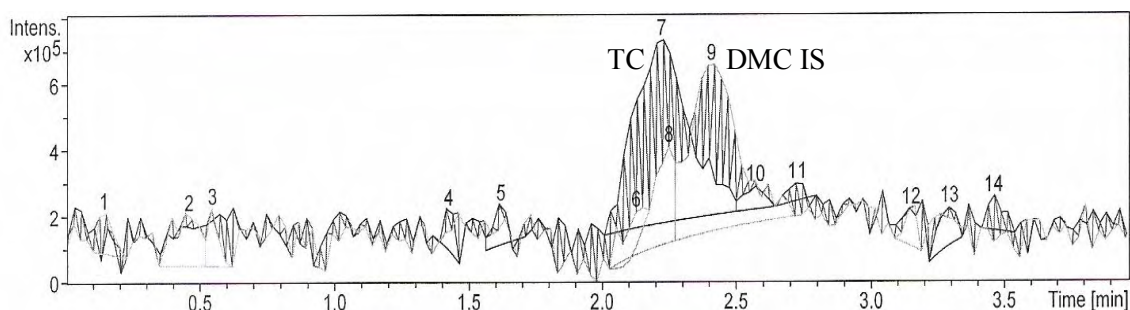


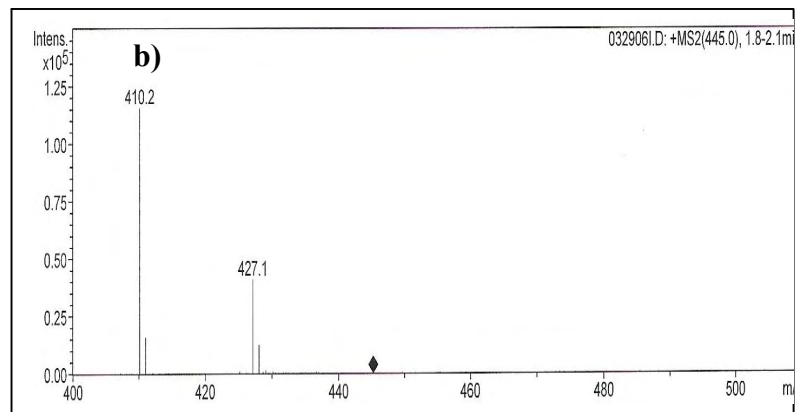
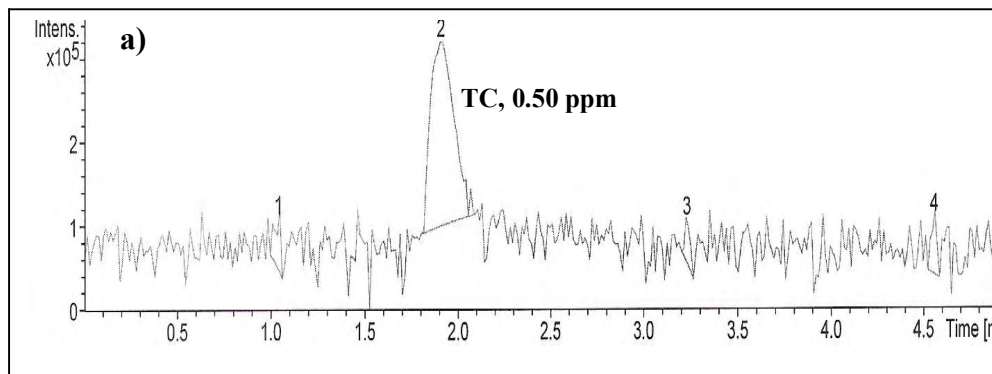
Figure 30. Extracted Ion Chromatogram (EIC) for TC and DMC IS

The TC and DMC are not completely resolved due to similarities in their structure; however this is not impediment for their quantification because the MS can discriminate based on the different mass to charge ratios. The concentration of the DMC IS used was 1.50 ppm and was kept constant throughout the analysis. DMC exhibited a major response than TC in the MS detector.

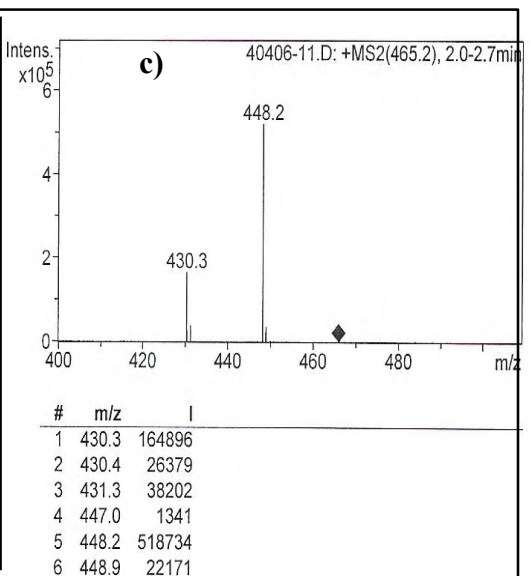
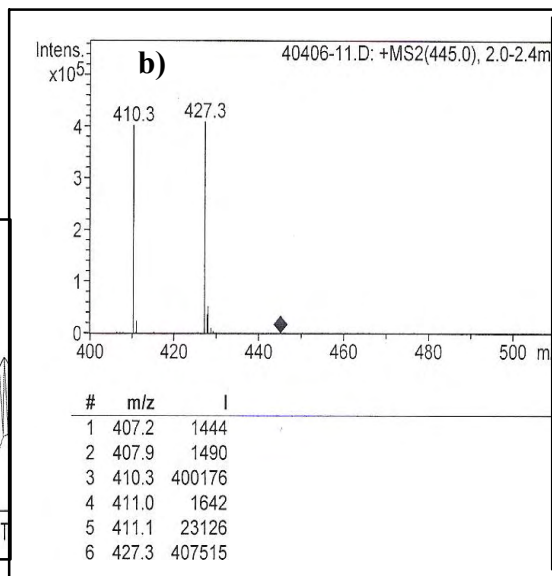
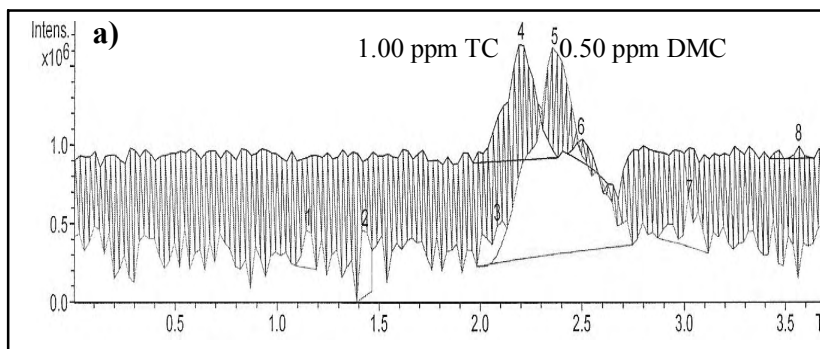
The optimized chromatographic conditions for LC/MS/MS analysis were obtained using a mobile phase consisting of 40 % acetonitrile in water (1.0 % formic acid) at pH 2.43. The rate flow was 0.40 mL/min. Figure 31a shows the chromatogram obtained under optimized chromatographic conditions for 0.50 ppm TC and its MS/MS spectra is shown in figure 31b. Figure 32a shows the chromatogram obtained for the mixture of (1.00 ppm TC and 0.50 ppm DMC). The peak labeled 4 corresponds to TC and its retention time was 2.3 minutes. The peak labeled 5 corresponds to DMC internal standard and its retention time was 2.5 minutes. In the figure 32b and 32c can be observed the MS/MS spectra for the peaks 4 and 5, respectively.

The table 8 summarizes the retention time and areas for TC and DMC peaks. The area measured of 3 repetitions for TC had a relative standard deviation (RSD %) of 0.53 % and for IS DMC a RSD of 2.57 %.

The method LOD was determined as the lower concentration of the analyte that produce an ions current signal with a relationship S/R of 3/1. In this terms, we prepared target analyte solutions with initial concentration of 0.50 ppm and decreased the concentration until the observed signal was 3-fold greater than noise. DMC LOD and LOQ were 0.005 and 0.05 ppm respectively. For TC was estimated the LOD approximately 0.03 ppm and LOQ approximately 0.10 ppm



**Figure 31. a) Chromatogram (EIC) Obtained for TC under Optimized Chromatographic Conditions
b) MS/MS Spectra on 445.10 m/z TC Parent Ion for the Chromatogram in figure 31a**



**Figure 32. a) Chromatogram (EIC) Obtained for (1.00ppm TC + 0.50ppm DMC) Mixture under Optimized Chromatographic Conditions
b) MS/MS Spectra on 445.10 m/z TC Parent Ion for the Chromatogram in figure 32a
c) MS/MS Spectra on 465.20 m/z DMC IS Parent Ion for the Chromatogram in figure 32a**

Table 8. Area, Retention Time and RSD for Chromatographic Mixture (TC+IS) Run

Compound	RT (min)	Average RT	Area (counts*min)	Average Area	S	RSD %
1.00 ppm TC	2.2	2.2	7962844	7934561	41849.2607	0.52743
1.00 ppm TC	2.2		7886487			
1.00 ppm TC	2.2		7954352			
0.50 ppm DMC	2.4	2.4	7957141	7748255.7	198882.331	2.566801
0.50 ppm DMC	2.4		7561173			
0.50 ppm DMC	2.4		7726453			

RT: Retention time (minute)

S: standard deviation

RSD%: Percent relative standard deviation

4.4.2 LC/MS/MS Measurements

A calibration curve was constructed with seven calibration levels from 0.10 to 3.00 ppm using DMC internal standard (IS) at concentration of 1.50 ppm, at a concentration in the medium point of calibration as shown in figure 33. This curve was adjusted to quadratic relations. The points corresponding to the internal standard response varied between $\pm 2 \sigma$ (standard deviation), which is acceptable. The axis Y depicts TC standards areas divided by internal standard areas ratio and the axis X shows TC standards concentrations divided IS concentration ratio.

Samples from 3 adsorption experiments series with two replicas, a control test and a blank at pH 3.80 were analyzed. Selected sorbents were

- a) 14-20 Mesh Crumb Rubber
- b) 30 Mesh Crumb Rubber
- c) Carbon Black

The contact time was 168 hours with a TC initial concentration of 10.00 ppm and the crumb rubber concentration was 10 g/L for all tests.

The outcome of the Alamo' results⁴⁵ demonstrated a release of Zn from crumb rubber mesh 14-20 in deionized water at acids pH. The crumb rubber was washed to eliminate metals that can complexes the TC and affect the adsorption during the first hours. The washing procedure consisted of placing in contact the crumb rubber with 2.50 % HNO₃ in constant agitation during 24 hours followed by three additional rinses with deionized water.

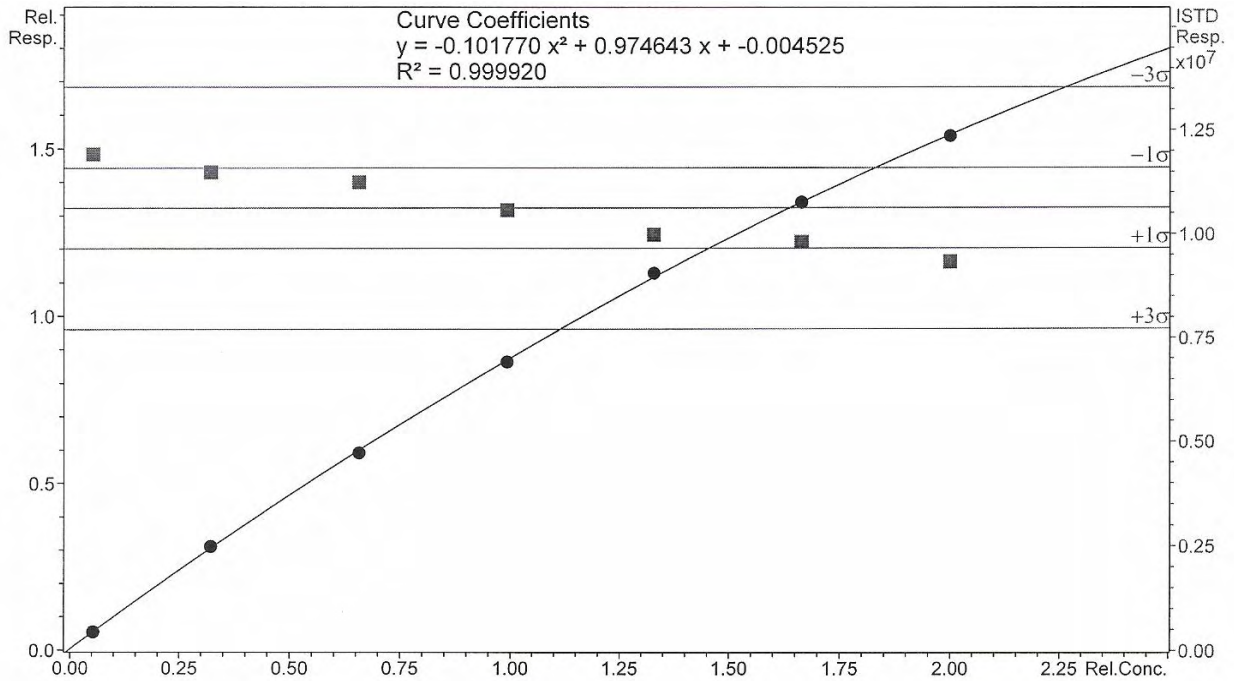


Figure 33. LC/MS/MS Calibration Curve

4.4.2.1 Sorption Test Using Mesh 14-20 Crumb Rubber

The table 9 shows the data obtained for 14-20 mesh crumb rubber without washing. At initial time ($t = 0$ seg), the initial concentration was 9.85 ppm TC and after 7 days the concentration decreased to 4.13 ppm. It is important to mention that we observed significant changes in the pH for the samples (see table C1 in appendix C), which at $t = 0$ seg, the pH was 3.84 and $t = 168$ hours was 5.71. The precision of measurements was determined as percentage relative standard deviation (RSD %) of duplicate analysis and varied between 0.17 – 6.59 %. The reproducibility was determined with two sample replica and expressed as RSD% and results were between 0.53 – 7.53 %.

Table 9. LC/MS/MS Data for Non Washed Mesh 14-20 CR Samples

T (h)	C (ppm)	s	RSD %
0	9.85	0.44	4.42
1	8.04	0.40	3.83
2	9.58	0.33	3.45
4	9.51	0.32	3.36
8	9.43	0.05	0.53
24	8.60	0.65	7.53
48	8.43	0.10	1.14
96	6.43	0.45	7.00
120	5.65	0.13	2.28
168	4.13	0.05	1.21

C: Concentration

T: Contact Time (hours)

CR: Crumb rubber

s: Standard deviation

RSD: Relative standard deviation

The data for the control sample is shown in table C2 in appendix C. It is observed a decrease in TC concentration in the control samples, which may be due to TC degradation. Accordingly, the net TC removal was 48.22 % at 7 days after considering the TC degradation observed in the control samples. Accuracy measurements for the experiments were made with a quality control of 1.50 ppm for 3 repetitions and the percentage recovery was calculated using 4. The accuracy and recovery percentage were 96.60 % and 73.27 %, respectively.

Figures 34 show the variation in TC concentration versus the contact time for the samples (green curve) and control samples (light blue curve) with mesh 14-20 without wash the CR (NWCR).

The results show a similar behavior during the first hours, as those obtained by UV-Vis spectrophotometry, even when the batches concentration were ten times lower than the evaluated

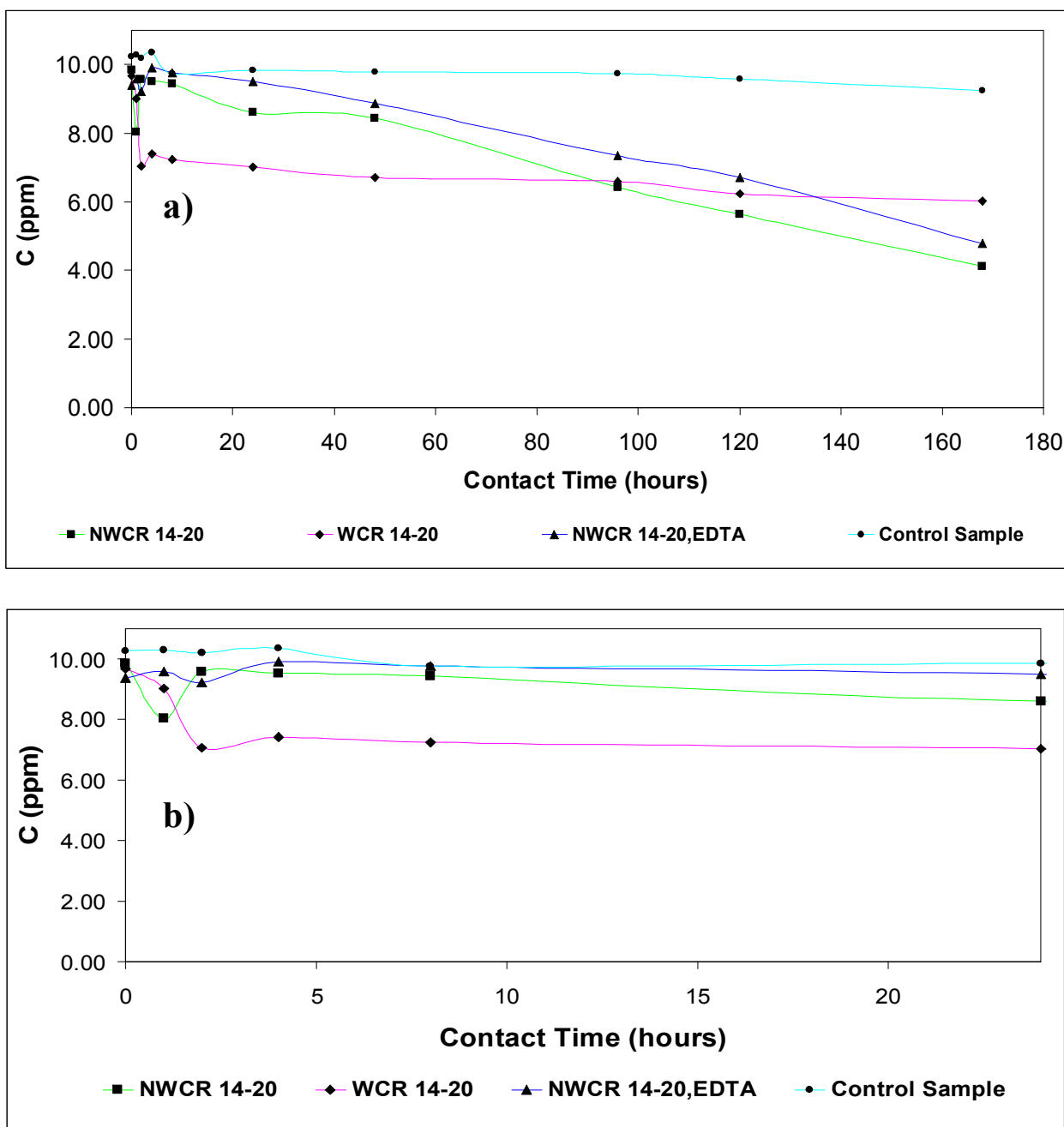


Figure 34. Sorption Behavior for Experiments Using Crumb Rubber Mesh 14-20
a) Scale until 168 hours
b) Scale until 24 hours
LC/MS/MS Measures at pH 3.80

with UV-Vis spectrophotometry. The concentration of samples shows a reduction of approximately 60 % after 168 hours without taking in consideration TC degradation (table 9), however equilibrium was not reached. These results also show the unusual behavior of a clearly quick concentration decrease followed by TC release during the first hours of the experiment, as is shown in figure 34b (green curve) for the first 24 hours (expanded view). Table 10 present the data for 14-20 mesh crumb rubber samples, when washed with HNO₃ 2.5 %, to eliminate superficial metals, mainly Zn. The data for control samples of this experiment is shown in table C3 of the appendix C.

Table 10. LC/MS/MS Data for Mesh 14-20 WCR Samples

T (h)	C (ppm)	s	RSD %
0	9.68	0.29	2.97
1	9.00	0.08	1.97
2	7.05	0.78	12.56
4	7.40	0.29	4.01
8	7.24	0.10	1.41
24	7.02	0.37	5.27
48	6.70	0.34	5.09
96	6.60	0.73	12.09
120	6.24	1.48	23.76
168	6.02	0.30	4.51

WCR: Crumb rubber wash with HNO₃ 2.5 %

When the crumb rubber is washed with acid is observed a net concentration decrease from 9.68 ppm (t=0seg) to 6.02 ppm TC after 168 hours of contact as shown in figure 34a (pink curve).

During the first hours is observed a smaller decrease in concentration when compared to the NWCR mesh 14-20. This drop was observed during the first 2 hours followed by a light increase in concentration. This concentration increase suggests TC release according with the pH changes that has been observed in the samples, which influence the Zn-TC interaction. These results could suggest that most of the superficial Zn has been lixiviated from crumb rubber. We did not observe a significant change of pH in comparison to the non washed crumb rubber as is shown in table C1 of the appendix C. Figure 34a shows that for the WCR, the behavior is more regular in the second stage between 1-2 hours and the final concentration was 6.02 ppm in comparison with non washed crumb rubber (4.13 ppm). In the data for WCR control samples (table C3 in appendix C) was observed a concentration decrease, which implies that the TC net removal was of 37.41 % considering the degradation that occurred. If the zinc is removed by washing the crumb rubber, the behavior in the first hours becomes more regular, however a net increase in adsorption is observed when compared NWCR and WCR (final concentration was 6.02 and 4.13 ppm, respectively), thus suggesting superficial Zn is probably involved in the process. That adsorption percentage difference between NWCR and WCR can be explained with the adsorption of a Zn-TC complex fraction onto crumb rubber surface.

In addition, we performed another NWCR mesh 14-20 adsorption batch test with the addition of 80.00 μ L EDTA 0.002M from initial time. These results are showed in the dark blue curve of the figure 34a and table C4 in appendix C. EDTA is known as a strong complexing agent and could bind to Zn from crumb rubber to inhibit the TC-Zn interaction and allow free TC. As

shown in dark blue curve of the figure 34b (which shows the first 24 hours), the disappearance of the initial drop when compared to the NWCR mesh 14-20 (green curve) suggested the inactivation of the TC-Zn interaction during the first hours, mainly. These results indicated that EDTA complex to the Zn metal, thus inactivating the free Zn ions in solution. The net adsorption was slightly affected by EDTA because the TC net removal in presence of EDTA was 39.25 % when compared with the absence EDTA which was 48.22% suggesting Zn participation on the adsorption process. TC degradation observed in the control samples was taken in consideration on all samples. The measured precision and reproducibility for this experiment varied between 0.10 – 10.39 % and 0.21 – 2.36 %, respectively. Accuracy and percent recovery were 114.67 y 113.98 % calculated as described in page 91.

Inductively Coupled Plasma (ICP) measures by duplicate were performed to samples from our adsorption batch experiment up to 4 hours with non washed CR mesh 14-20 samples and CR blank. The results can be observed in table 11 and showed a progressive increase of Zn in solution until 4 hours. In the blank at 0 seg Zn levels was not detectable. At 1 hour was 0.38 ppm Zn and increased to 0.58 ppm Zn at 4 hours. The CR blank was comparable with the samples. The samples showed a concentration of 0.36 ppm Zn and after 1 hour increased to 0.54 ppm at 4 hours. The ICP results confirmed the availability of Zn in solution during the first few hours of the experiments thus suggesting interaction with TC leading to TC-Zn complex formation responsible for the unusual behavior observed with the non washed crumb rubber. The addition EDTA to sequester and inactivate the Zn or the acid wash of the CR to remove the Zn improves the TC adsorption behavior during the first hours. Figure 35 indicated an

immediate MS signal decrease when Zn⁺² ions at 83.41 ppm are contacted with 8.00 ppm TC solution. MS/MS signal is specific highly and is affected by adduct formation such as complexes, leading to a decrease in the ionization efficiency.

Table 11. ICP Zn Measurements for an Adsorption Batch

CR Blank, pH 3.80	Zn Concentration (ppm)	s	(TC+CR)Sample, pH 3.80	Zn Concentration (ppm)	s
o seg	0.00	0.01	o seg	0.00	0.01
1 hour	0.38	0.02	1 hour	0.36	0.00
2 hours	0.59	0.01	2 hours	0.50	0.01
4 hours	0.58	0.01	4 hours	0.54	0.01

s: standard deviation

The 83.41 ppm Zn was calculated based in 0.6000 g of crumb rubber with an average of 1 % Zn percentage found tire rubber composition. The measures precision and reproducibility for the Zn experiment varied between 0.05 – 7.71 % and 1.65 – 10.49 %, respectively. Accuracy and percent recovery were 97.05 y 76.70 %.

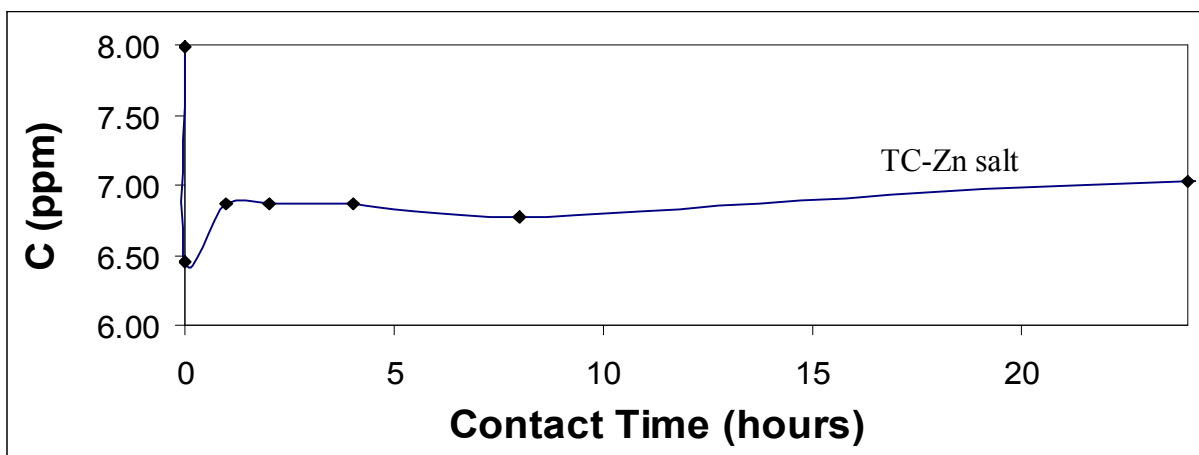
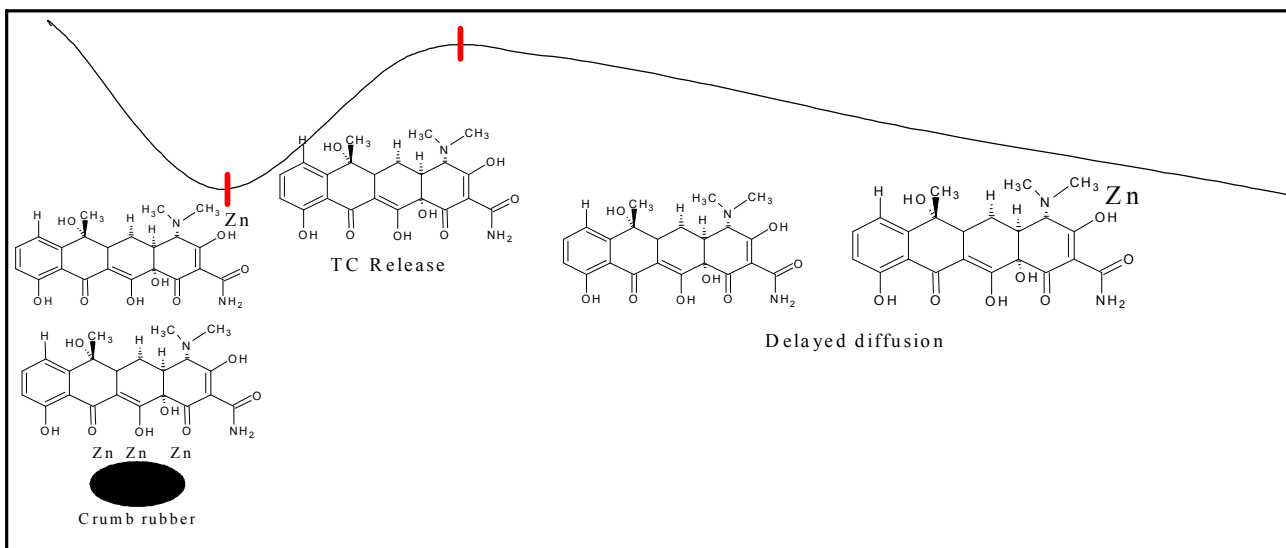


Figure 35. LC/MS Measures for TC in contact with Zn salt – pH 3.80 – 8.00 ppm TC and 83.45 ppm Zn

All this facts support the Zn release, which is in agreement with the findings in our laboratory and other references^{36, 42, 43, 44}. As discussed in the theoretical background, TC can form complexes with several multivalent cations, such as Mg^{+2} , Ca^{+2} , Zn^{+2} , Al^{+3} , Fe^{+2} (28,31,33,34). Kim. Y. et al²¹, suggested a first adsorption in the crumb rubber surface and then diffusion process toward the crumb rubber bulk of organic compounds that they studied, such as: toluene, ethylbenzene, trichloroethylene. In this way, TC adsorption by crumb rubber can be divided in three stages, as is shown in figure 36. In the first stage the concentration decrease is very possible due to TC binding with metals, mainly Zn in CR surface and in solution. A second stage due to TC partial release and third stage, in which, a delayed diffusion occur due probably to the crumb rubber morphology that do not allow the fast access to adsorption site, such as carbon black particles present in the crumb rubber bulk. We are proposing that the interaction between TC and Zn can be through amino-alcohol function. Noyori et al⁵⁰, found this kind of interaction in the alkylation promoted by amino-alcohols with organic-zinc compounds.



0 seg

Figure 36. Proposed Sorption Process of TC by Crumb Rubber

168h

4.4.2.2 Sorption Test Using Mesh 30 Crumb Rubber

The table 12 presents the data of the samples with crumb rubber mesh 30. After 168 hours of contact time, the concentration was as low as 2.65 ppm (see dark blue curve in figure 37a). The removal efficiency was significantly greater than the observed for mesh 14-20. This is related with the crumb rubber smaller size and therefore greater exposure of surface area and carbon black. Mesh 30 crumb rubber particles have an average diameter of 0.60 mm and the mesh 14'20 crumb rubber have an average diameter of 1.20 mm and greater variability in size. In the first hours, this data presents a similar behavior to the non washed 14-20 mesh crumb rubber. It was observed a pH changes from 3.83 at $t = 0$ seg to 6.41 at the end of the experiment as is shown in table C1 in appendix C. The TC net removal was of 59.80 %, after considering the degradation in the control samples. The control samples for this experiment reported the greater concentration decrease that for other experiments, which was due to a delay in its analysis, as is shows in table C5 in appendix C. The reproducibility were between 1.08 – 9.86 % and the precision were between 0.04 – 12.74 %. Accuracy and recovery percent were 99.39 and 80.87 %.

After acid washing, the WCR mesh 30 presented a more regular behavior during the first hours as is shown more clearly in pink curve of the figure 37b (expanded view) and table 13. The concentration decreased for TC was from 9.51 ppm at $t= 0$ seg to 4.37 ppm at 168 hours. The data for control samples is presented in table C6 in appendix C. The measured precision and reproducibility for this experiment varied between 0.24 – 16.85 % and 0.80 – 8.82 %, respectively. Accuracy and percent recovery were 94.00 y 83.53 %.

Table 12. LC/MS/MS Data for Non Washed Mesh 30 CR Samples

T (h)	C (ppm)	s	RSD %
0	9.70	0.26	2.68
1	7.51	0.13	1.74
2	8.33	0.27	3.49
4	7.54	0.07	1.08
8	6.92	0.15	2.13
24	6.05	0.27	4.43
48	5.51	0.23	4.11
96	4.57	0.24	5.18
120	4.47	0.44	9.86
168	2.65	0.09	3.29

Table 13. LC/MS/MS Data for Mesh 30 WCR Samples

T (h)	C	s	RSD %
0	9.51	0.28	2.89
1	5.33	0.18	3.39
2	5.36	0.04	0.80
4	5.86	0.85	3.82
8	5.28	0.15	2.78
24	5.26	0.14	2.79
48	4.91	0.10	2.12
96	4.90	0.21	4.24
120	4.54	0.10	2.15
168	4.37	0.39	8.82

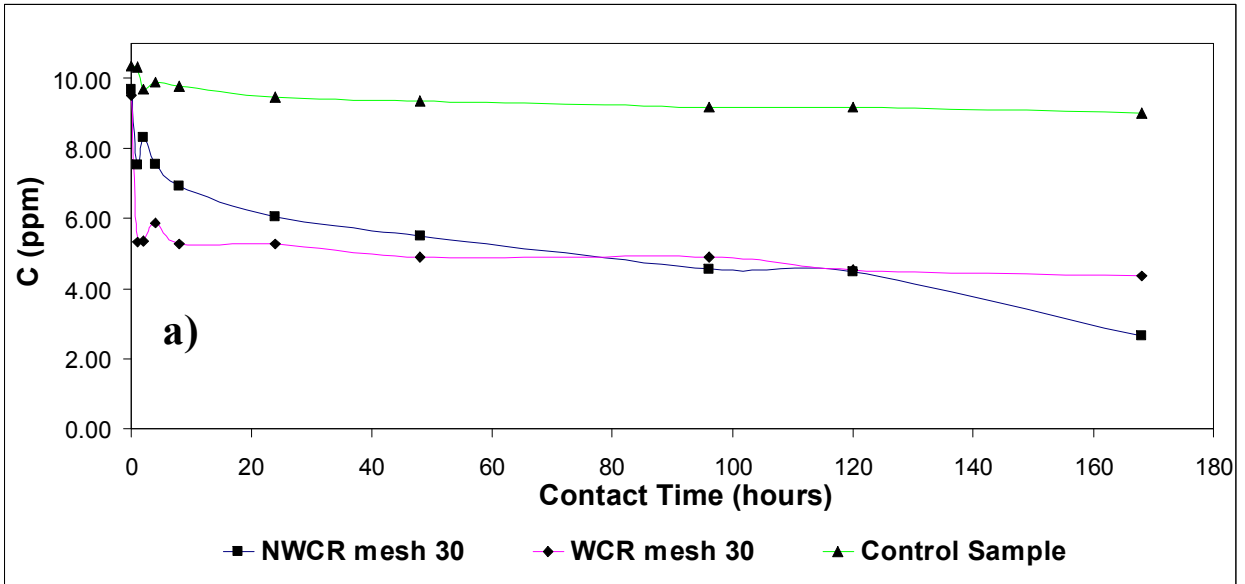


Figure 37a. Sorption Behavior Using Crumb Rubber Mesh 30 until a scale of 168 hours LC/MS/MS Measures at pH 3.80

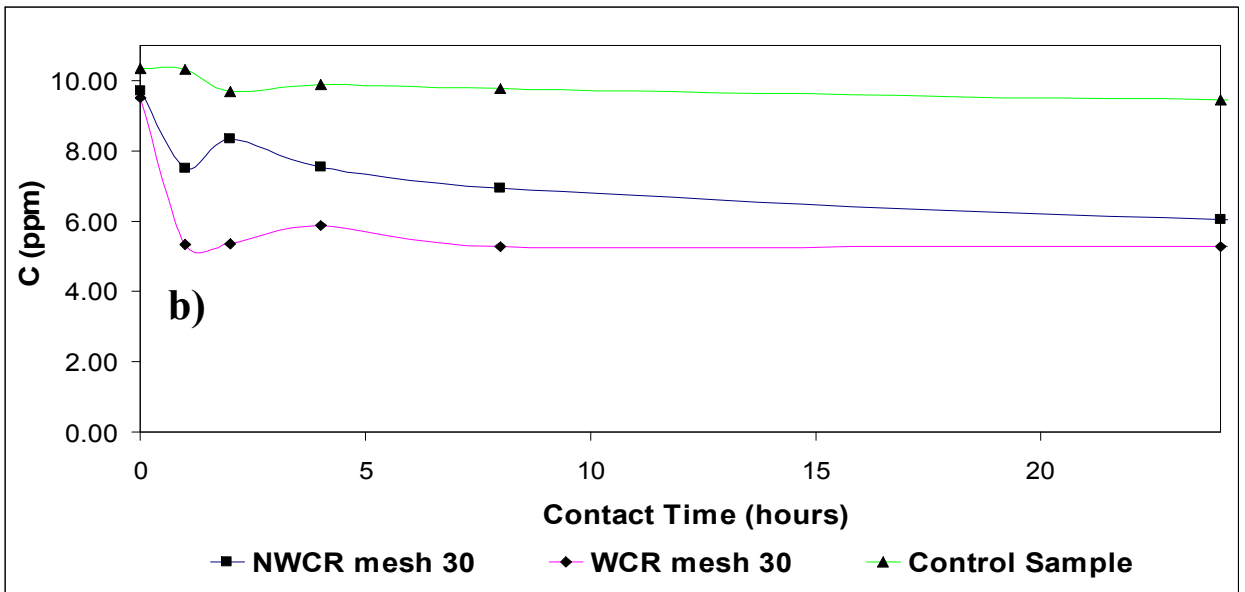
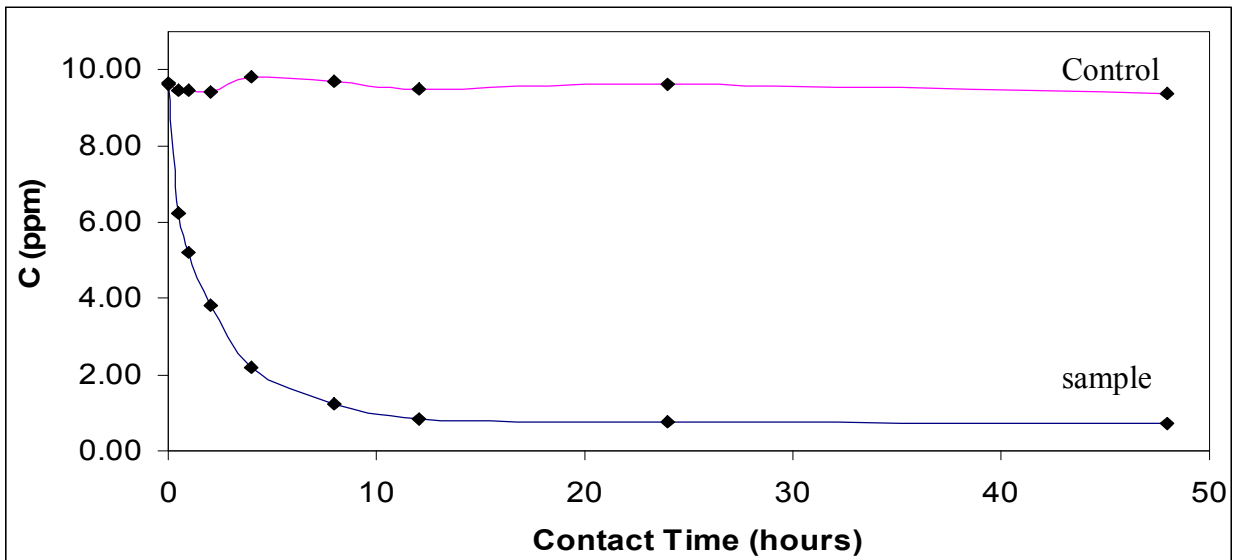


Figure 37b. Sorption Behavior Using Crumb Rubber Mesh 30 until a scale of 24 hours LC/MS/MS Measures at pH 3.80

4.4.2.3 Sorption Test Using Carbon Black

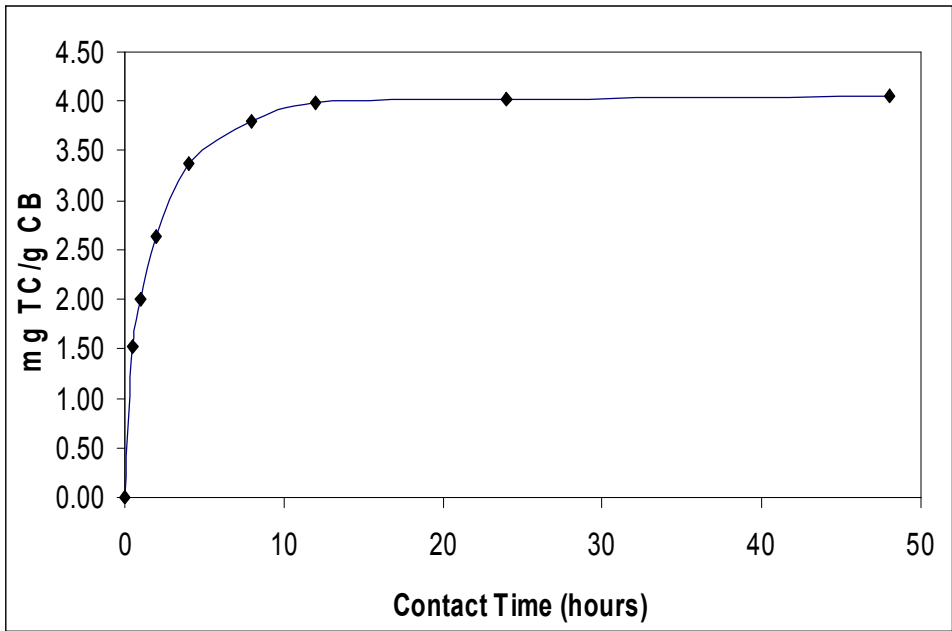
This test with carbon black was performed in order to test the hypothesis that the increase in TC adsorption when using CR mesh 30 was due mainly to increase the exposition of carbon black as a result of the increase in the surface area. As mentioned earlier, the tire rubber has carbon black at a percentage of 10.97 – 45.60 % in its matrix. It was considered an average of 22 % for this test and therefore, was used 0.1320 g carbon black N330 with 10.00 ppm of TC solution. The contact time was 48 hours. As is shows in figure 38, the adsorption equilibrium for carbon black started approximately at 12 hours. After that time the concentration was remained at 0.76 ppm as average. It was not observed significant changes in pH (see table C1 in appendix C). The TC was almost completely removed using the carbon black N330. It was observed a regular behavior of the sorption, what is probably due to the carbon black matrix is lower in metals. The carbon black presented a load capacity and percent removal at 24 hours of 4.02 mg TC/g CB and 92.20 % respectively as can be observed in figures 39 and 40. The control samples data are presented in table C7 of appendix C. The precision and reproducibility measures for this experiment varied between 0.00 – 10.88 % and 0.00 – 12.66 %, respectively. Accuracy and percent recovery were 94.00 y 94.54 %. Table 14 summarizes the data for samples of the CB experiments.



**Figure 38. Sorption Behavior Using Carbon Black N330
LC/MS/MS Measures – pH 3.80 – 10.00 ppm TC**

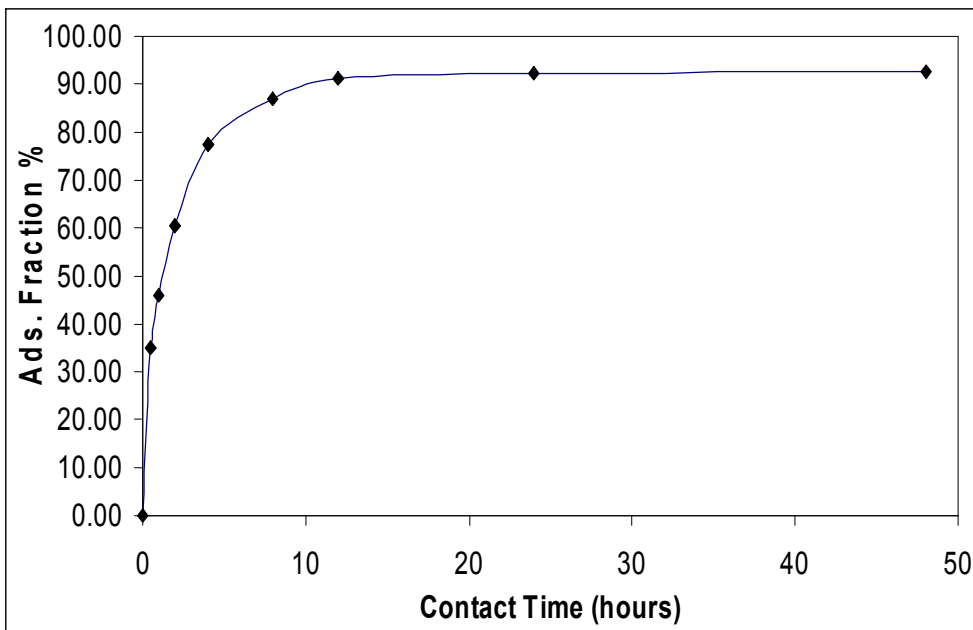
Table 14. LC/MS/MS Data for CB Samples, 1 μ L EDTA 0.002M

T (h)	C	s	RSD %
0	9.60	0.08	1.00
0.5	6.25	0.26	0.85
1	5.20	0.42	4.23
2	3.80	0.14	8.16
4	2.18	0.28	3.72
8	1.25	0.10	12.66
12	0.85	0.06	8.00
24	0.75	0.06	7.70
48	0.70	0.00	0.00



T (h)	mgTC/gCB
0	0.00
0.5	1.52
1	2.00
2	2.64
4	3.37
8	3.80
12	3.98
24	4.02
48	4.05

Figure 39. TC Uptake capacity for CB N330 Experiment, 1.0 μ L EDTA 0.002 M



T (h)	Ads. Fraction
0	0.00
0.5	34.90
1	45.83
2	60.42
4	77.29
8	86.98
12	91.15
24	92.19
48	92.71

Figure 40. TC Adsorption Fraction for CB N330 Experiment, 1.0 μ L EDTA 0.002 M

In another experiment, carbon black was placed in contact with TC in presence of Zn. As for the crumb rubber experiments its was considered an average of 22 % of carbon black and 1 % Zn in according with the tire rubber composition in order to prepare the carbon black and Zn solution. The samples for the LC/MS analysis were divided with EDTA added and with the absence of EDTA. EDTA was added to inhibit the TC-Zn complexation. It was not observed a notable pH change and had a very significant decrease in concentration at 24 h for samples in the absence or presence of EDTA as is shown in figure 41 when compared with the results obtained with CR mesh 14-20 and mesh 30 (figure 34b and 37b). These results are evidence of the sorption affinity of tetracycline to carbon black even in the presence of Zn. These results demonstrate an excellent capacity of carbon black to remove TC from aqueous solutions. The data for control samples with 1 μ L EDTA 0.002 M are presented in table C8 of the appendix C.

Table 15. LC/MS/MS Data for Zn – CB N330 Samples, 1 μ L EDTA 0.002 M

T (h)	C (ppm)	s	RSD %
0	10.01	0.66	0.53
1	4.81	0.01	0.01
2	4.07	0.62	0.77
4	3.00	0.12	4.21
8	1.09	0.04	3.33
24	0.22	0.14	64.19
48	0.15	0.30	62.27
96	0.14	0.05	35.52
120	0.14	0.04	32.34

These carbon black experiment results suggest that the adsorption by the crumb rubber can be improved by increasing the surface area and thus exposing the carbon black as is shown in the mesh 30 CR experiments. It is appropriate to think of the possibility to modify the physical structure of the crumb rubber and thus increase the porosity and eventually, expose more the carbon black and thus increase its adsorption capacity for TC and other organic compounds. This would make the use of the crumb rubber a more attractive adsorbent.

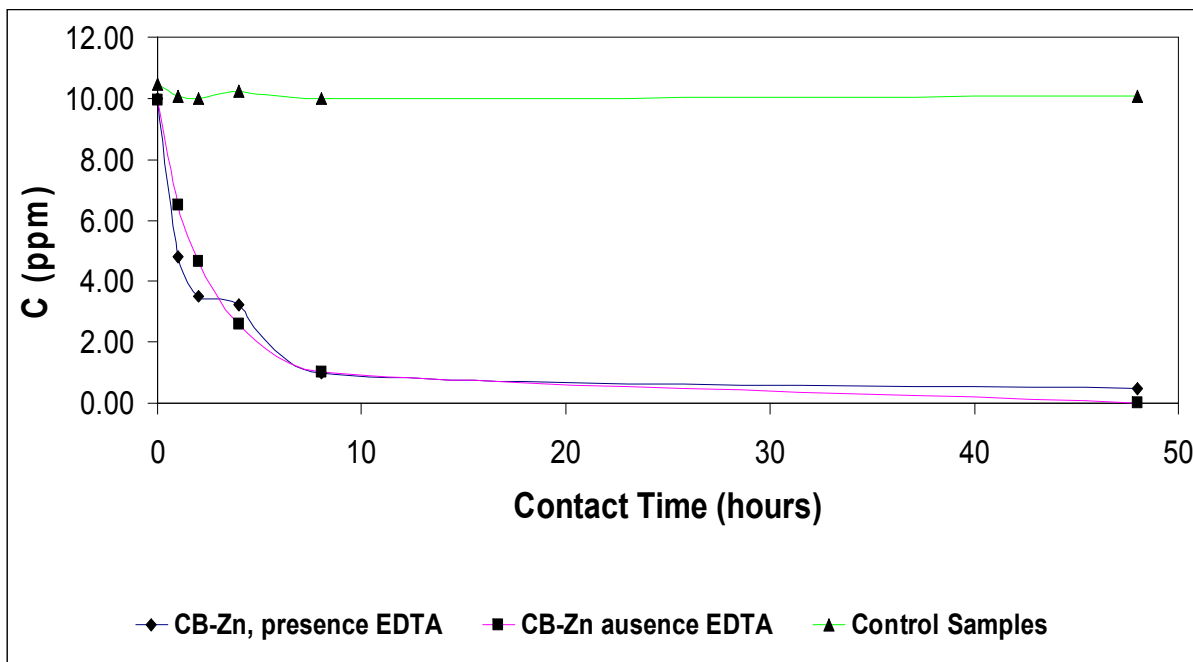


Figure 41. Sorption Behavior for Experiment Using Carbon Black N330 – Zn LC/MS/MS Measures – pH 3.80

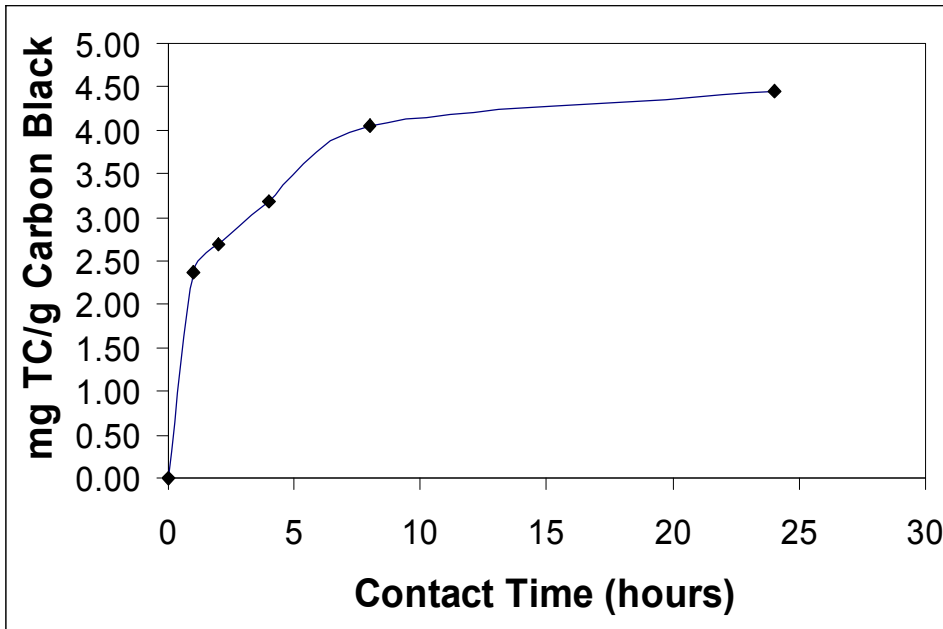


Figure 42. TC Uptake Capacity for Zn-CB N330 Experiment, 1 μ L EDTA 0.002 M

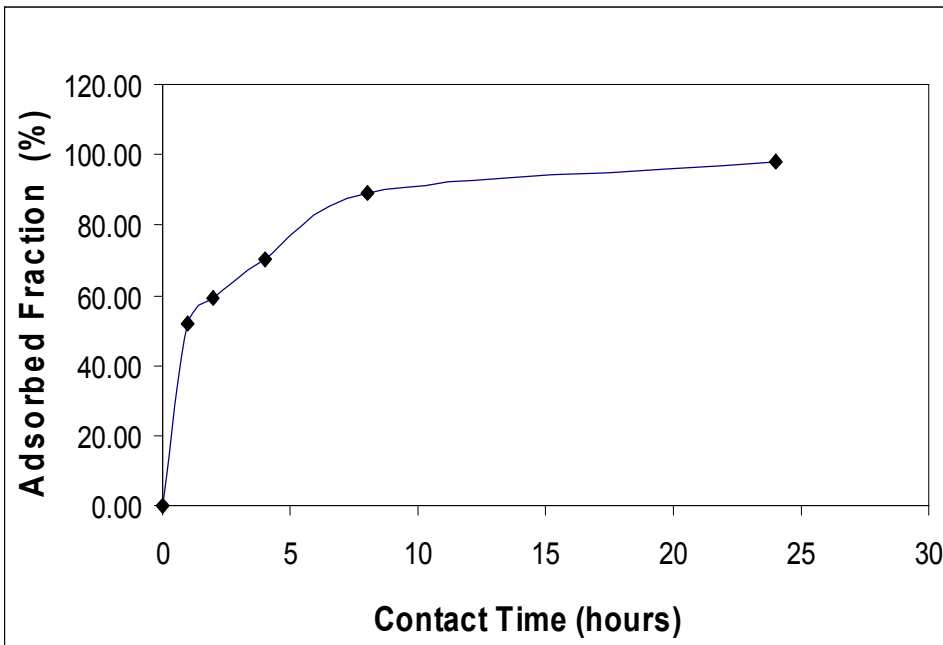


Figure 43. Adsorbed Fraction of TC in Zn-CB N330 Experiment, 1 μ L EDTA 0.002 M

The reproducibility and the precision of the measures for the CB in presence of EDTA varied between 0.01 – 64.19 % and 0.07 – 54.39 %. Accuracy and percent recovery were 103.95 and 85.13 %. It was observed a load capacity and percent removal at 24 h of 4.45 mg TC/g CB and 97.80 % respectively, as it can be observed in the figures 42 and 43. These results demonstrate the excellent capacity for the removal of TC from aqueous solutions using carbon black as a cheaper adsorbent than activated carbon.

In the Zn-CB experiment samples in absence of EDTA (table 16), the concentration at 8 hours was 1.03 ppm. In samples at 24 hours, the readings were below instrument limit of detection. The reproducibility and the precision of the measures varied between 0.00 – 4.88 % and 0.36 – 6.73 %, respectively. Accuracy and percent recovery were 106.00 and 78.33 %. The control samples data are presented in figure C9 of the appendix C. The percent recovery was higher than for the sample without EDTA (table 16, figure 41), which suggests that the percent recovery is improved by adding EDTA and thus avoid interactions of TC with metals. The load capacity at 8 h was 4.08 mg TC/g CB and percent removal at 24 h was 100.00 as is shown in figures 44 and 45 respectively.

These experiments with CB suggest that it plays a significant role in the crumb rubber sorption process and could be improved if the metals are eliminated, the porosity increased or decreased the CR size. Table 17 shows the time delay before the samples analysis and the degradation percentage calculated based on the initial and final concentration of control samples. The mesh

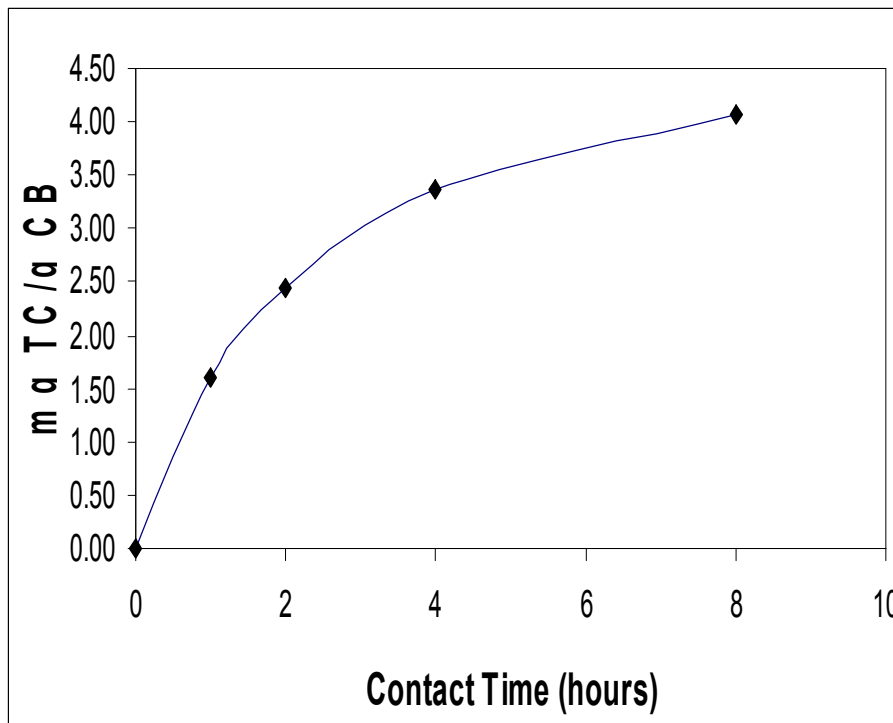
30 CR samples presented a greater degradation and it is explained because the samples were analyzed after 15 days. These results indicate that TC decomposes with time, significantly increasing after 8 days prior to analysis. The carbon black will be an efficient absorbent mainly for these compounds that can degrade even more in weathering conditions. The table 18 summarizes the main data for the sorption experiments.

Table 16. LC/MS/MS Data for Zn - CB N330 Samples without EDTA

T (h)	C (ppm)	s	RSD %
0	9.94	0.38	2.72
1	6.48	0.19	2.92
2	4.63	0.10	2.07
4	2.60	0.10	3.14
8	1.03	0.05	4.88
24	0.00	0.00	0.00
48	0.00	0.00	0.00
96	0.00	0.00	0.00
120	0.00	0.00	0.00

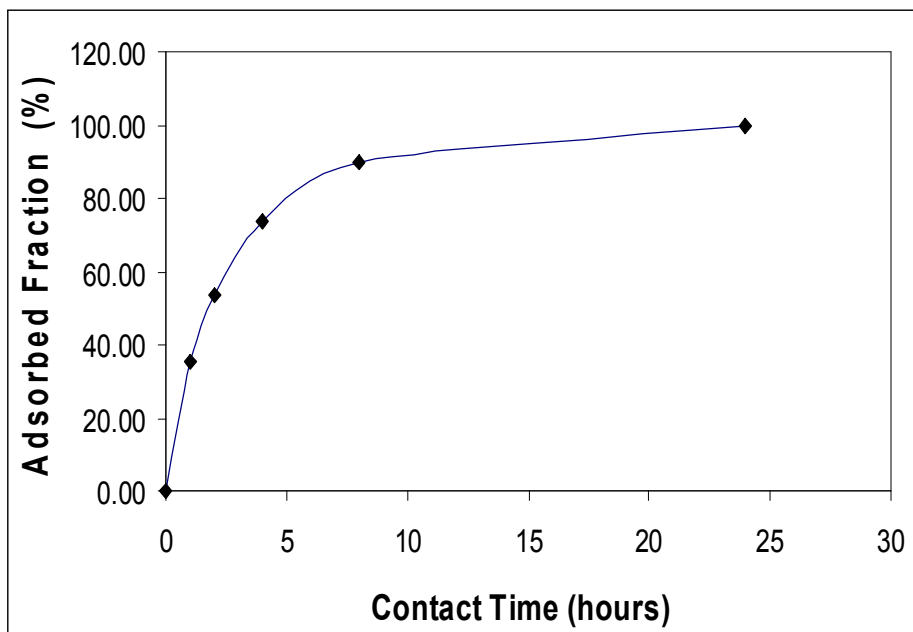
Table 17. Degradation Percent for TC

Experiment Specifications	Analysis of Samples	% Degradation
a) 14'20 Mesh NWCR	13 days	9.85
b) 14'20 Mesh WCR	11 days	0.40
c) 30 Mesh NWCR	15 days	14.43
d) Zn	10 days	3.25
e) Zn-Carbon Black, 1 μ L EDTA 0.002M	9 days	0.79
f) Zn-Carbon Black	8 days	0.23



T (h)	mg TC/g CB
0	0.00
1	1.60
2	2.44
4	3.36
8	4.08

Figure 44. TC Uptake Capacity for Zn-CB N330 Experiment without EDTA



T (h)	Ads. Fraction (%)
0	0.00
1	35.20
2	53.70
4	74.00
8	89.70
24	100.00

Figure 45. Adsorbed Fraction of TC in Zn-CB N330 Experiment without EDTA

Table 18. Summarized Data Obtained by LC/MS/MS for Sorption Experiments

Experiment	Conc. Decrease Range (Sample)		Removal %	Reproducib. % RSD Range	Average Recovery %	Prec. as % RSD	Accuracy
	t _{initial}	t _{final}					
a) Mesh 14-20 NWCR	9.85 ppm	4.13 ppm	48.22, 168h	0.53-7.53	73.27	0.17-6.59	96.60
b) Mesh 14-20 WCR	9.68 ppm	6.02 ppm	37.41, 168h	1.41-23.76	89.40	0.09-8.69	104.20
c) Mesh 14-20NWCR,EDTA	9.38 ppm	4.78 ppm	39.25, 168h	0.21-2.36	113.98	0.10-10.31	114.67
d) Mesh 30 NWCR	9.70 ppm	2.65 ppm	59.80, 168h	1.08-9.86	80.87	0.04-12.74	99.39
e) Mesh 30 WCR	9.51 ppm	4.37 ppm	41.01, 168h	0.80-8.82	83.53	0.24-16.85	94.00
f) Carbon Black, 1 μL EDTA	9.60 ppm	0.70 ppm	89.81, 24h	0.00-12.66	94.54	0.00-10.80	94.00
g) Zn/Carbon Black, 1μLEDTA	10.00 ppm	0.22 ppm	91.06, 24h	0.01-64.19	85.13	0.07-54.39	103.95

NWCR: non washed CR

WCR: washed crumb rubber (CR)

EDTA: disodium ethylenediaminetetracetate

CHAPTER V

CONCLUSIONS

- The varied composition of the crumb rubber matrix influences the TC adsorption
- TC adsorption tends to be better at pHs which approximates its different pKa.
- TC form complexes with zinc present in the crumb rubber.
- The metal (Zn) elimination by acid wash allow that the TC adsorption to become more regular during the first hours.
- Carbon black is a excellent adsorbent to remove TC from aqueous solutions
- The sorption capacities of crumb rubber improves at low TC concentrations
- At strong alkaline pH causes significant decomposition of TC
- The TC adsorption by crumb rubber was better with a smaller size crumb rubber, mesh 30 due to increased exposition of the CB.

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APPENDIX
APPENDIX A
Rubber Properties

Table A1. Rubber Properties

Physical Properties	Metric	English	Comments
Density	<u>0.95 g/cc</u>	0.0343 lb/in ³	Uncompounded
Mechanical Properties			
Hardness, Shore A	30 - 100	30 - 100	Depends on compounding
Hardness, Shore D	30 - 45	30 - 45	Depends on compounding
Tensile Strength, Ultimate	<u>28 MPa</u>	4060 psi	Compounded Tire
Elongation at Break	100 - 800 %	100 - 800 %	
100% Modulus	<u>0.0015 GPa</u>	0.218 ksi	
Shear Modulus	<u>0.0005 GPa</u>	0.0725 ksi	
Electrical Properties			
Electrical Resistivity	<u>1e+016 ohm-cm</u>	1e+016 ohm-cm	
Dielectric Constant	2.9	2.9	1 kHz
Dielectric Constant, Low Frequency	2.9	2.9	1 kHz
Dielectric Strength	<u>50 kV/mm</u>	1270 kV/in	Step by step
Dissipation Factor	0.03	0.03	1 MHz
Dissipation Factor, Low Frequency	0.002	0.002	60-1000 Hz
Thermal Properties			
CTE, linear 20°C	<u>225 μm/m-°C</u>	125 μin/in-°F	
Heat Capacity	<u>0.44 J/g-°C</u>	0.105 BTU/lb-°F	
Thermal Conductivity	<u>0.15 W/m-K</u>	1.04 BTU-in/hr-ft ² -°F	
Maximum Service Temperature, Air	<u>80 °C</u>	176 °F	
Minimum Service Temperature, Air	<u>-55 °C</u>	-67 °F	
Optical Properties			
Refractive Index	1.526	1.526	

APPENDIX B
Adjustment of Critical Parameters for LC/MS/MS Analysis

1. Establishing Critical Parameters

LC-MS quantitative analysis depends on several important parameters, such as, LC separation, the ionization and detection mode of the target or product ions in MS/MS, detection and quantification limits, the ions trap parameters and the sample preparation procedures. These must be optimized before the analysis⁴⁵.

All parameters of the ion trap were optimizing individually and the MS was calibrated with a calibration mixture consisting of solution of reserpine 5 ng/ μ L in isopropanol to proportion 19:1:1 of acetonitrile: reserpine solution: deionized water. The mass range of calibration was between 0 – 1200 m/z, which covered our working range. Figure B1, shows the peaks and their intensities characteristics for reserpine.

In the quantification mode with SRM mode, it was necessary to take the MS and MS/MS spectrum of tetracycline and the IS internal standard (demeclocycline, DMC). Parent ion of TC (M+H) was detected in agreement with the TC molecular weigh in 445.35 m/z (figure B2). The MS/MS spectrum was formed by fragmenting the (M+H) ion. Product ions were observed in 427.10 and 410.20 m/z with the corresponding loss of 18 and 35 m/z, attributed to early loss of water and subsequent loss of ammonium. As shown in figure B3, these peaks were very well selected with good intensities.

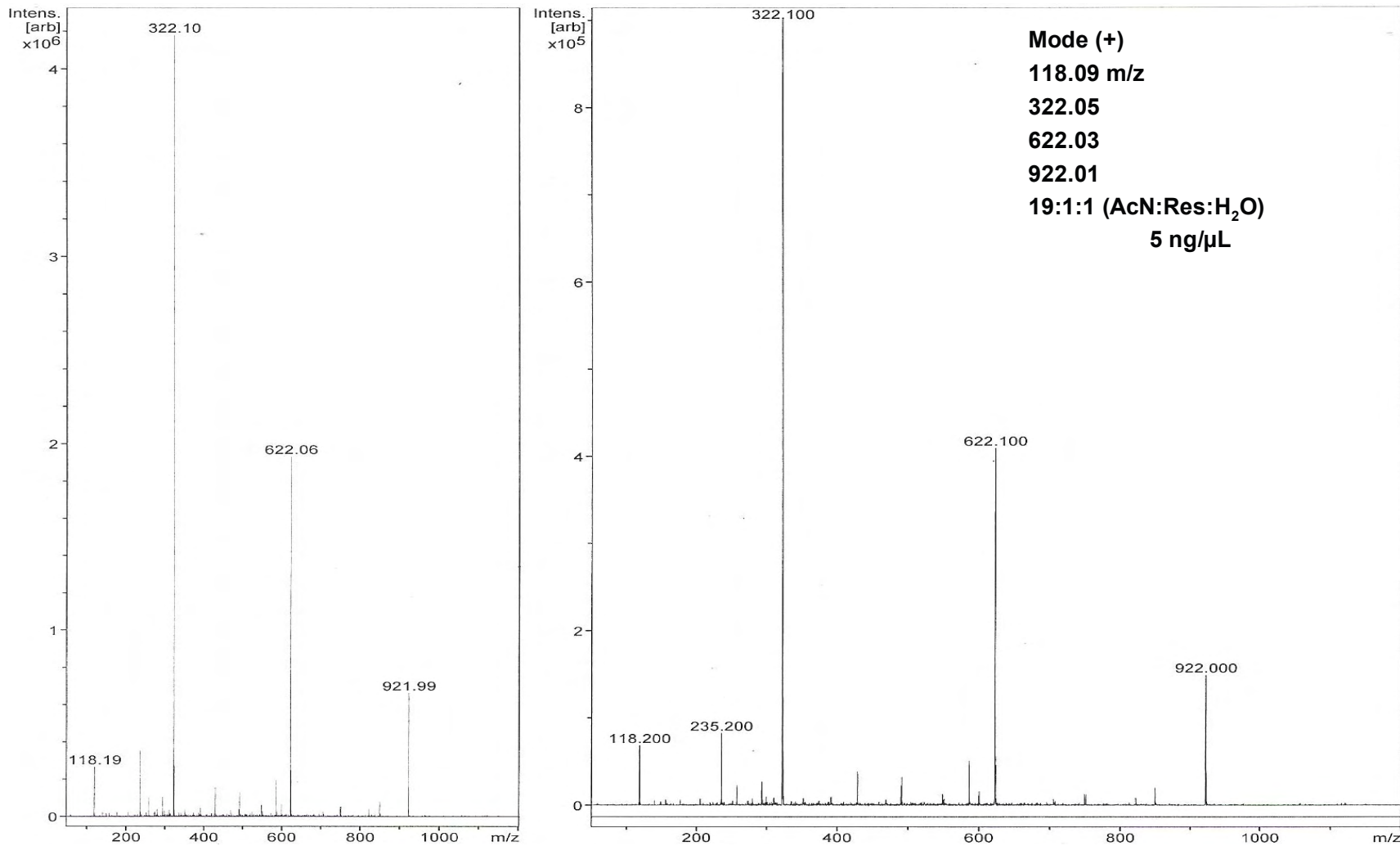


Figure B1. Reserpine Calibration Mixture for the Mass Spectrometer

Figure B4 corresponds to the MS spectrum of DMC. It is showed the (M+H) ion in 465.10 m/z corresponding to protonated molecular weight. DMC MS/MS spectrum showed two product ions in 448.08 and 430.00 m/z (figure B5). These peaks were attributed to first ammonium loss with water subsequent water loss, respectively.

2. Optimization of the Chromatographic Separation

To optimize the parameters for the chromatographic separation, we evaluated several mobile phases with 0.50 ppm TC solutions. The product ions (410.20, 427.17 m/z) of the TC protonated molecular ion were monitored. We obtained the extracted ion chromatogram for these product ions. The following mobile phases, with their respective flow rates, were evaluated:

- a) 50 % Acetonitrile 0.8 % formic acid, pH 2.50; 0.60 mL/min
- b) 20% Acetonitrile, 0.4 % formic acid, pH = 2.75; 0.5 mL/min
- c) 30 % Acetonitrile 0.2 % formic acid, pH 2.60; 0.40 mL/min
- d) 40 % Acetonitrile 1.0 % formic acid, pH 2.43; 0.40 mL/min

The chromatographic run with 50 % acetonitrile 0.8 % HCOOH mobile phase can be observed in figure B6-1. The extracted ion chromatogram (EIC) showed a poor peak definition and the ions corresponding at 410.20 and 427.10 m/z exhibited low intensities.

The evaluation of 20% acetonitrile (0.4 % HCOOH) mobile phase shown in figure B6-2 have a peak with good intensity, however the peak shows tailing.

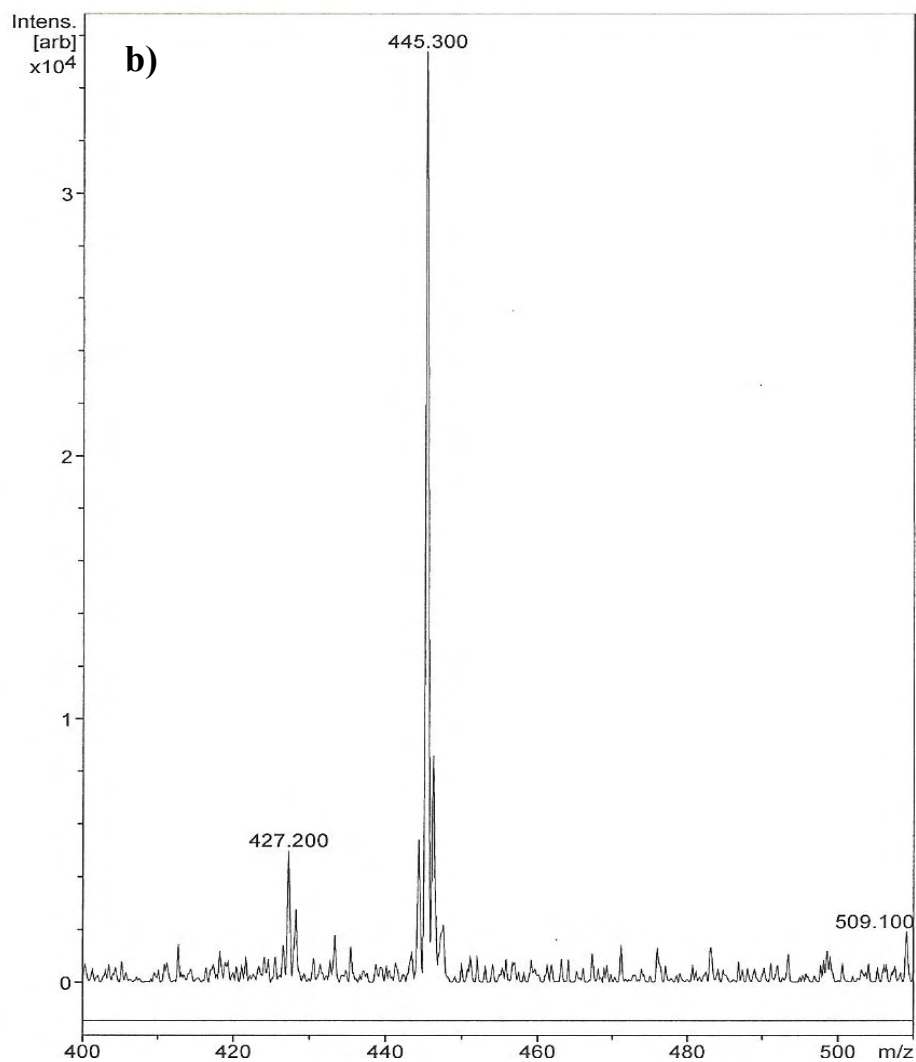
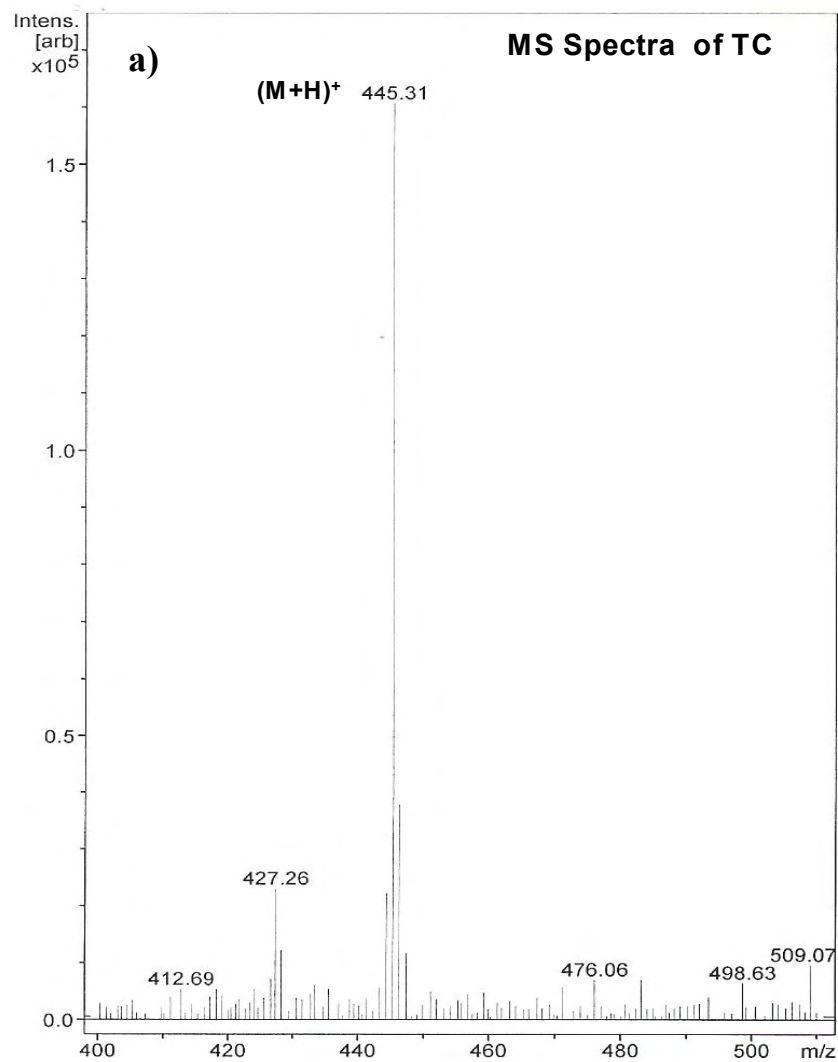


Figure B2. MS Spectra of TC

a) Line MS spectra

b) Profile MS spectra

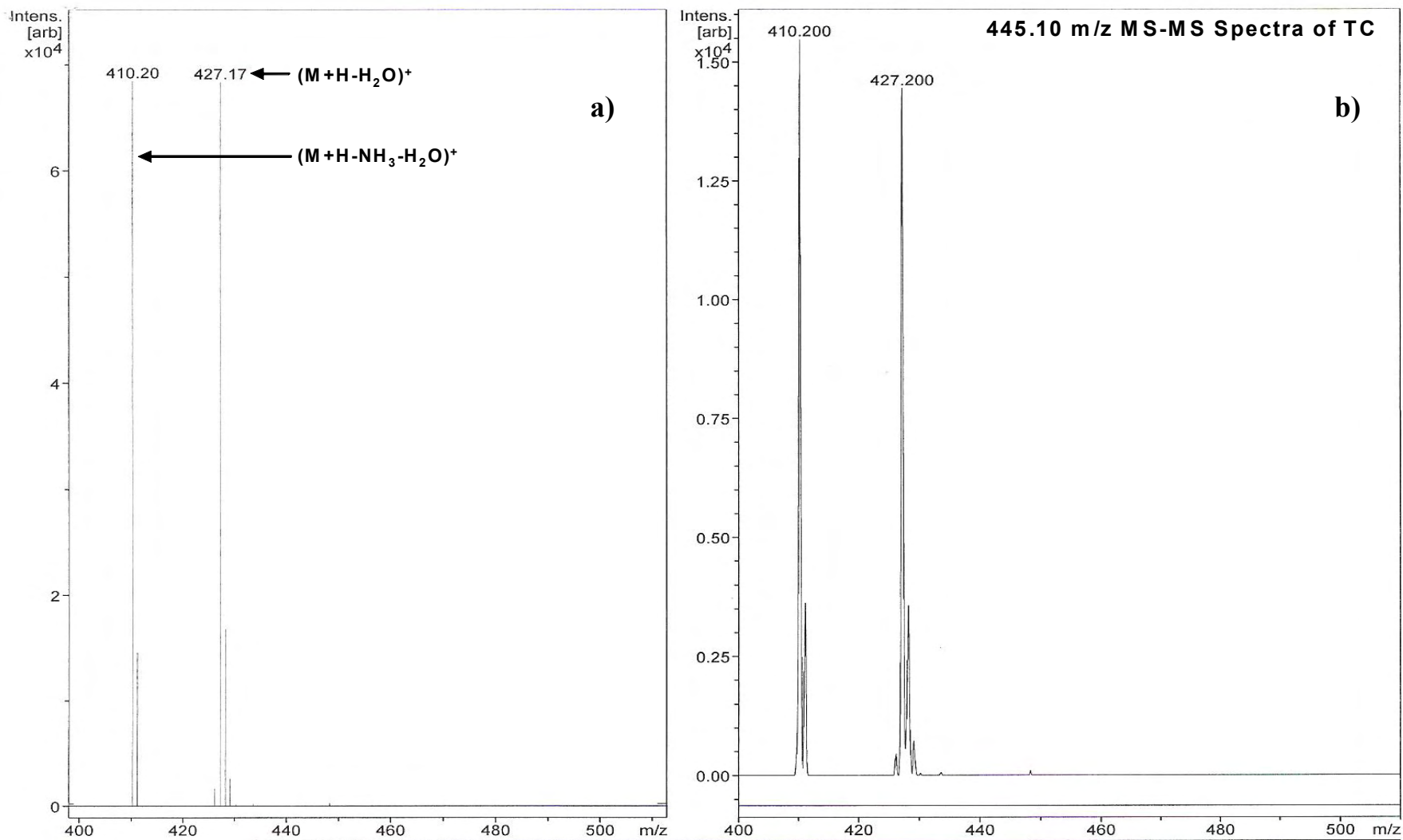


Figure B3. MS/MS Spectra of 445.31 m/z TC Parent Ion
a) Line MS/MS spectra b) Profile MS/MS spectra

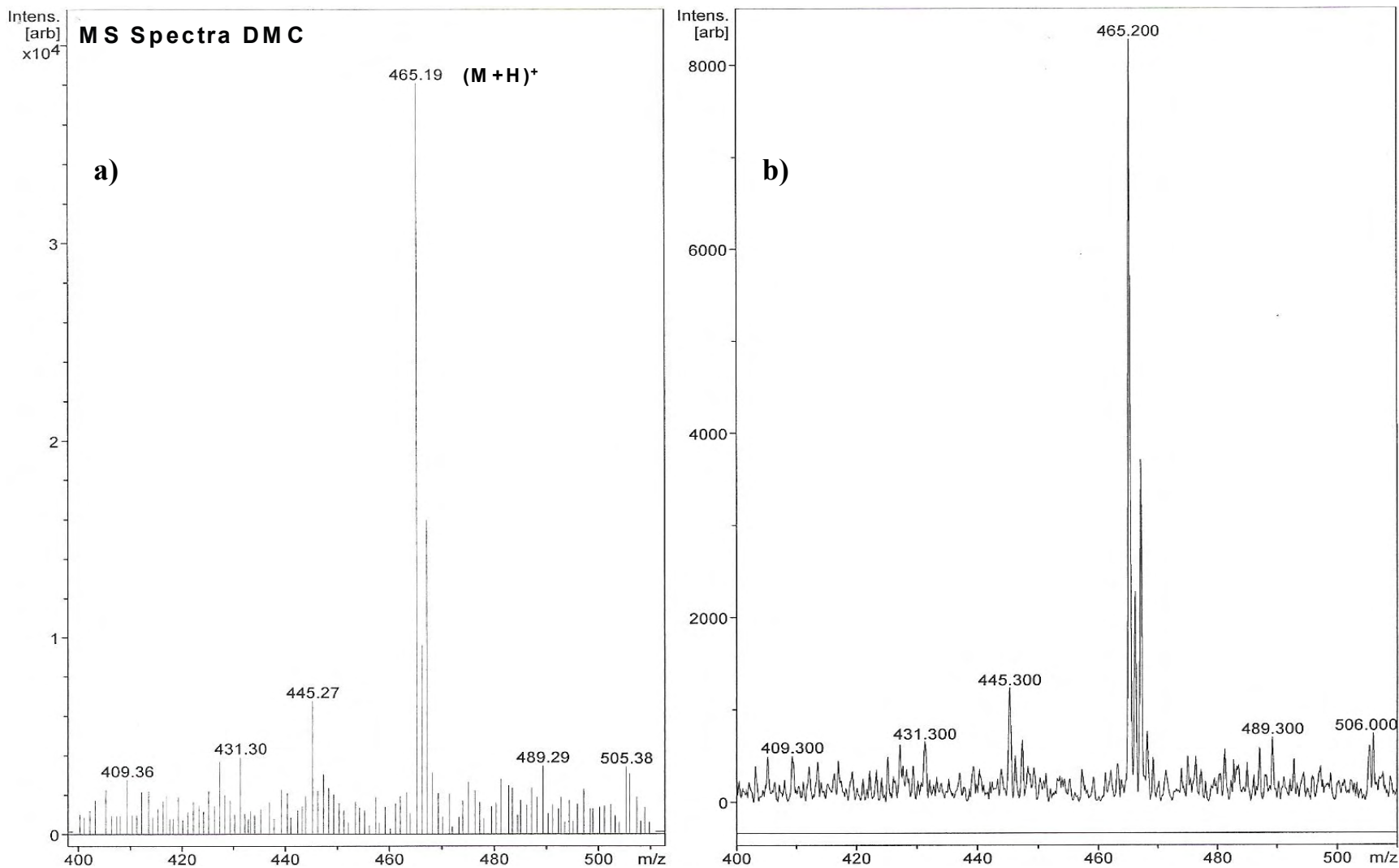


Figure B4. MS Spectra of DMC
a) Line MS spectra b) Profile MS spectra

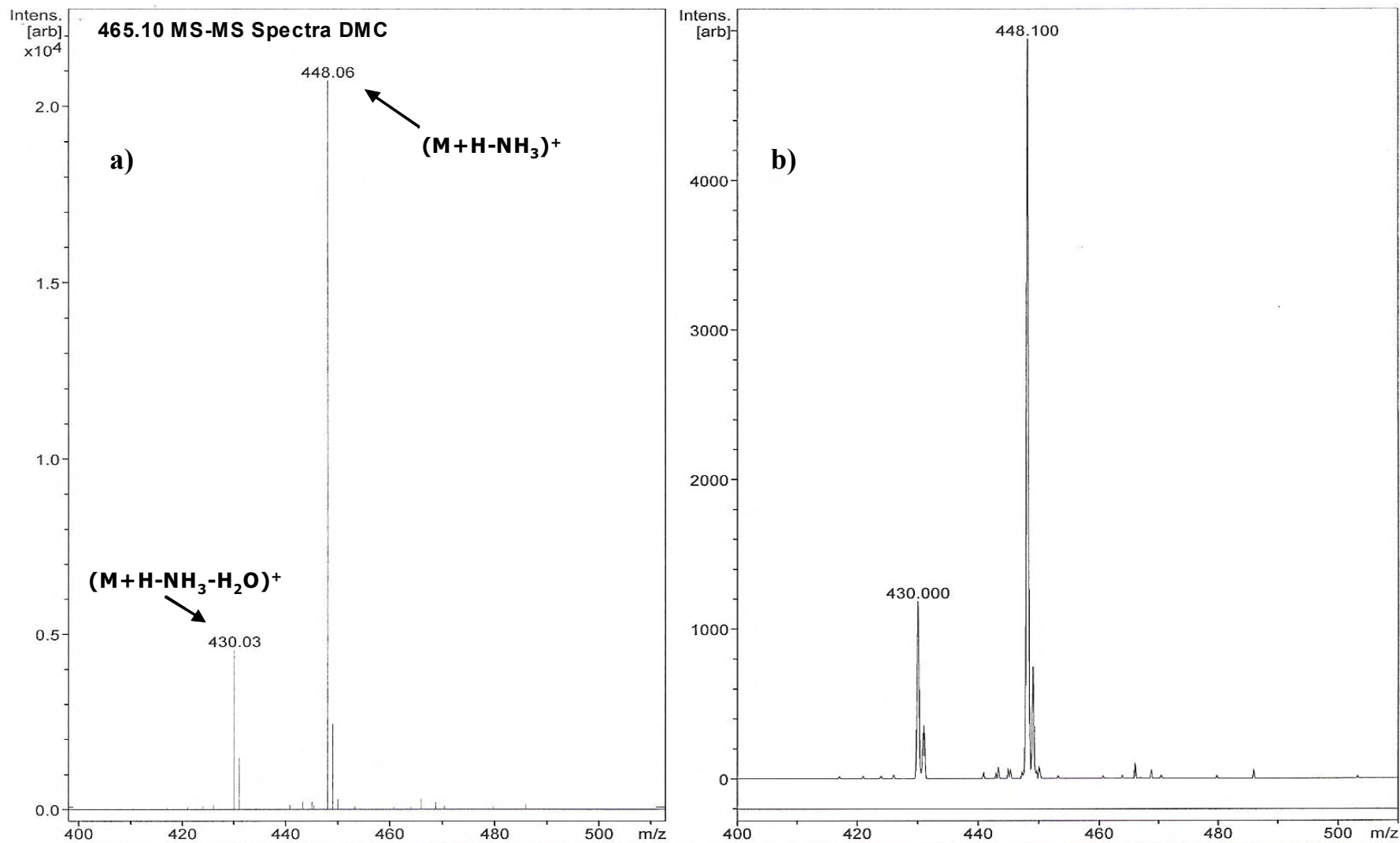
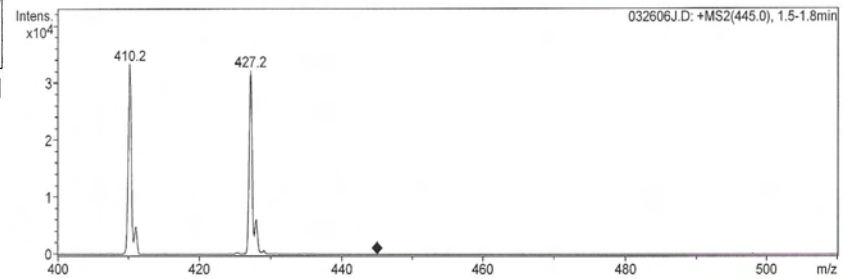
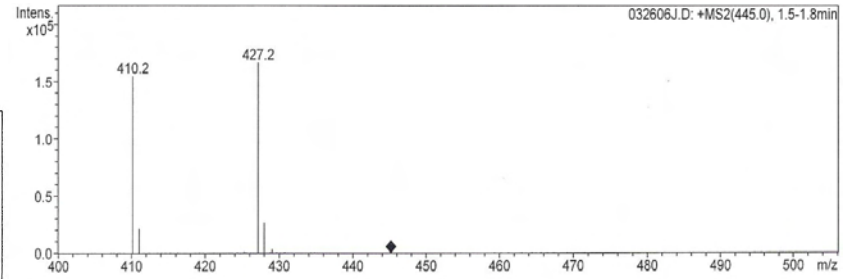
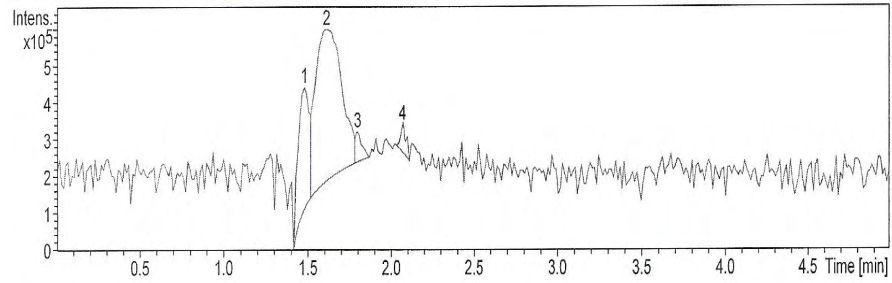


Figure B5. MS/MS spectra of 465.19 m/z DMC parent ion
a) Line MS/MS spectra b) Profile MS/MS spectra

1)



2)

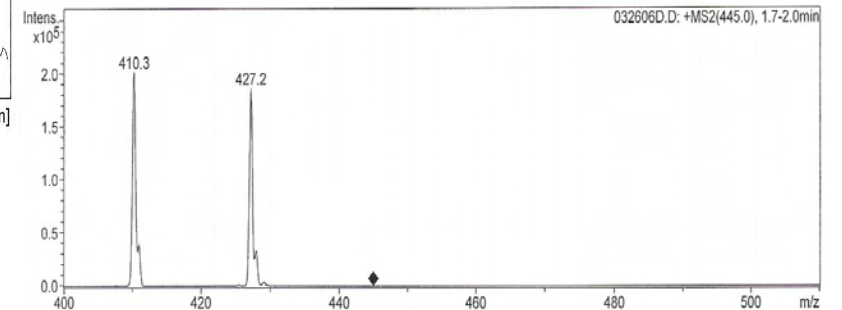
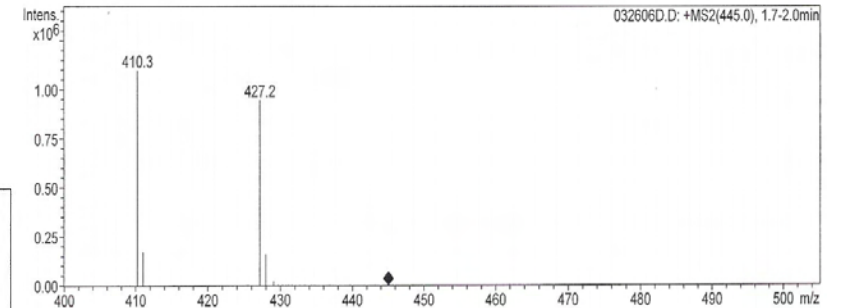
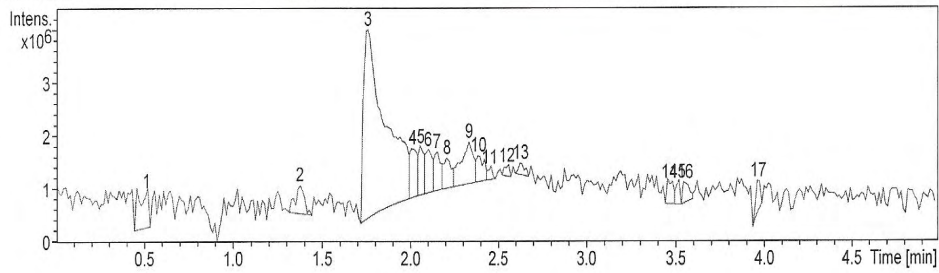
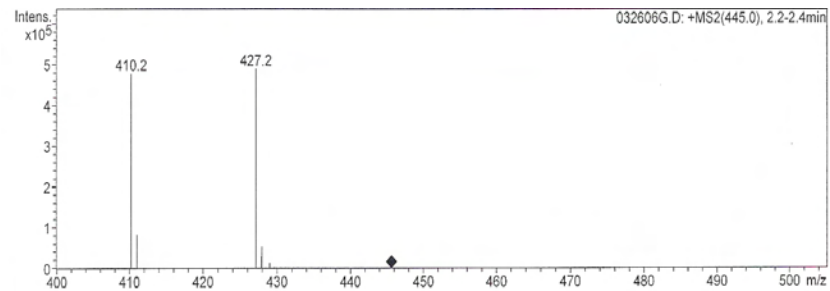
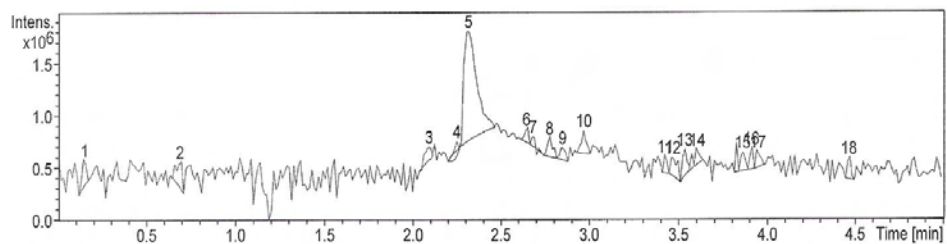


Figure B6. (EIC) Chromatogram obtained for TC:
1) Mobile Phase a 2) Mobile Phase b

1)



2)

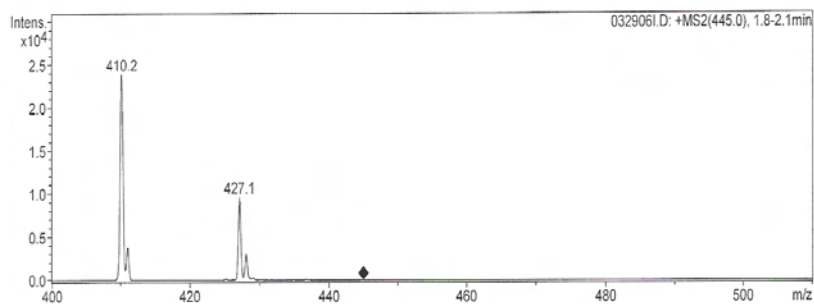
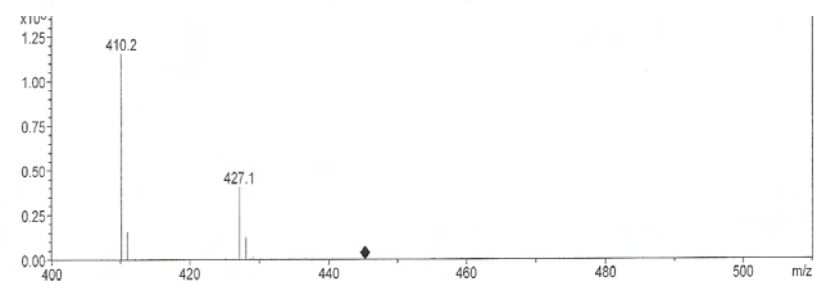
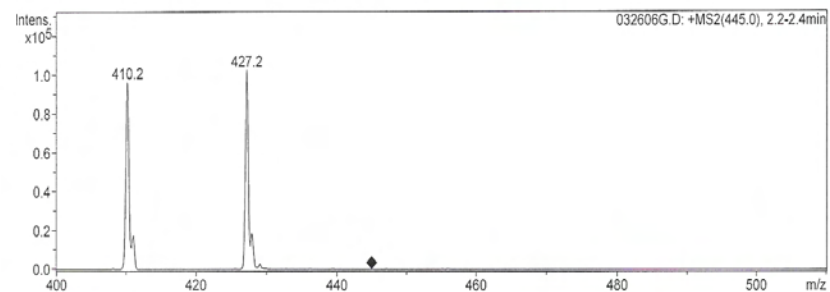
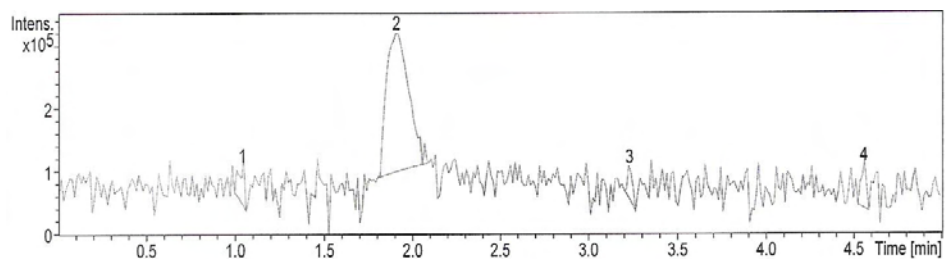


Figure B7. (EIC) Chromatogram Obtained for TC:
1) Mobile Phase c 2) Mobile Phase d

The mobile phase with 30 % acetonitrile (0.2 % HCOOH) shows good peak shape with poor ion intensity for ions product 410.20 and 427.20 m/z as can be seen in figure B7 -1.

The mobile phase using 40 % acetonitrile (1.0 % HCOOH) gave good peak symmetry with better ion intensity as can be shown figure B7-2, therefore it was selected to perform our experiments presented in this work.

APPENDIX C

Table C1. pHs Measurements for Adsorption Test Batches

Batch Name	pH initial	pH final
0.60g NWCR 14-20		
Sample	3.84	5.71, pHf 24h =5.55
Blank	3.82	6.02, pHf 24h =5.65
Control	3.85	3.88, pHf 24h =3.90
0.60 g NWCR 14-20 EDTA		
Sample	3.84	5.86
Blank	3.82	6.81
Control	3.85	3.97
0.60 g WCR 14-20		
Sample	3.80	3.98
Blank	3.83	3.95
Control	3.82	3.84
1.20 g WCR 14-20		
Sample	3.81	4.44
Blank	3.83	4.30
Control	3.82	3.84
0.60 g NWCR mesh 30		
Sample	3.83	6.41
Blank	3.83	6.45
Control	3.84	3.90
0.60 g WCR mesh 30		
Sample	3.81	3.92
Blank	3.83	3.98
Control	3.80	3.82
Carbon Black N330		
Sample	3.80	4.36
Blank	3.82	4.32
Control	3.81	3.84
Zn-Carbon Black N330		
Sample	3.85	3.79
Blank	3.84	3.90
Control	3.82	3.80
Zn Experiment		
Sample	3.81	3.85
Blank	3.82	3.80
Control	3.83	3.82

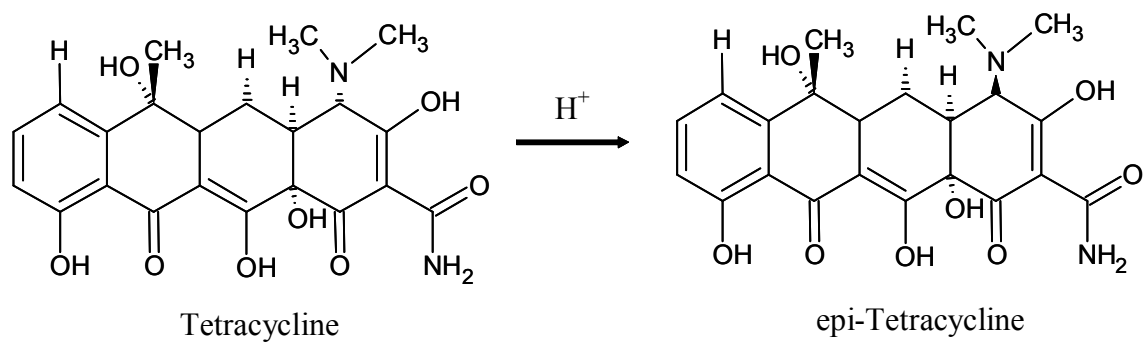


Figure C1. Conversion of TC to epi-Tetracycline

Table C2. LC/MS/MS Data for Non Washed Mesh 14-20 CR Control

T (h)	C (ppm)	s	RSD %
0	10.25	0.21	2.07
1	10.30	0.42	4.12
2	10.20	0.00	0.00
4	10.35	0.21	2.05
8	9.75	0.07	0.72
24	9.85	0.07	0.72
96	9.75	0.64	6.53
120	9.57	0.06	0.67
168	9.24	0.21	2.30

Table C3. LC/MS/MS Data for Mesh 14-20 WCR Control

T (h)	C (ppm)	s	RSD %
0	9.88	0.07	0.73
2	9.88	0.00	0.00
4	10.1	0.11	1.13
8	9.87	0.19	1.89
24	9.87	0.12	1.20
48	9.89	0.05	0.47
96	9.81	0.06	0.61
120	9.81	0.01	0.09
168	9.84	0.11	1.08

Table C4. LC/MS/MS Data for NWCR Mesh 14-20 Control, 80.00 μ L EDTA 0.002M from Initial Time of the Batch Experiment

T (h)	C	s	RSD %
0	10.21	0.33	3.20
1	9.97	0.18	1.79
2	9.86	0.12	1.20
4	9.81	0.58	5.25
8	9.73	0.32	3.39
24	9.66	0.44	4.52
48	10.3	0.21	2.06
96	9.67	0.55	5.66
120	9.43	0.98	10.39
168	9.21	0.48	5.25

Table C5. LC/MS/MS Data for Mesh 30 WCR Control

T (h)	C	s	RSD %
0	10.35	0.22	0.22
1	10.31	0.1	0.1
2	9.68	0.53	0.53
4	9.88	0.25	0.25
8	9.78	0.24	0.26
24	9.46	0.47	0.47
48	9.34	0.44	0.44
96	9.17	0.27	0.27
120	9.18	0.22	0.22
168	9.00	0.21	0.21

Table C6. LC/MS/MS Data for No Washed Mesh 30 CR Control

T (h)	C (ppm)	s	RSD %
0	9.98	0.03	0.34
1	9.96	0.00	0.04
2	9.95	0.08	0.81
4	9.92	0.03	0.30
8	9.48	0.00	0.00
48	8.87	0.39	4.39
96	8.47	0.01	0.17
120	8.87	0.40	4.46
168	8.54	0.05	0.58

Table C7. LC/MS/MS Data for CB N330 Control

T (h)	C	s	RSD %
0	9.65	0.07	0.73
0.5	9.45	0.07	0.74
1	9.45	0.07	0.75
2	9.40	0.00	0.00
4	9.80	0.02	0.00
8	9.70	0.06	0.00
12	9.50	0.14	1.49
24	9.61	0.03	0.35
48	9.37	0.06	0.63

Table C8. LC/MS/MS Data for Zn – CB N330 Control, 1.00 μ L EDTA 0.002M

T (h)	C (ppm)	s	RSD %
0	10.47	0.66	6.28
1	10.06	0.05	0.51
2	9.98	0.76	7.89
4	10.23	0.16	1.47
8	9.99	0.58	5.82
24	10.30	0.24	2.35
48	10.09	0.10	0.97
96	10.04	0.06	0.63
120	9.68	0.19	1.93

Table C9. LC/MS/MS Data for Zn – CB N330 Control without EDTA

T (h)	C (ppm)	s	RSD %
0	10.28	0.28	2.72
1	10.21	0.39	3.35
2	10.75	0.21	1.97
4	11.05	0.07	0.64
8	10.90	0.28	2.59
24	10.70	0.00	0.00
48	10.60	0.57	5.34
96	10.20	0.14	1.40
120	10.05	0.07	0.70