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ANTIBIOTICS IN THE DIEP RIVER AND POTENTIAL ABATEMENT USING GRAPE SLURRY WASTE

By

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DECLARATION

I, Rumbidzai Chitongo, declare that the contents of this thesis represent my own work, and that the thesis has not been submitted before for any degree or academic examination in any other university. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Pharmaceuticals have found extensive application in human health management. They are released into the environment through urine, excreta and inappropriate disposal methods. Residues of pharmaceutical products have been reported to show toxic consequences in some freshwater and marine organisms. Antibiotics are one of the most important groups of common human pharmaceuticals widely in use as prescribed and non-prescribed drugs. Antibiotics and their metabolites have been quantitated in water and found in trace levels. But even at such low concentrations they can maintain high biological activities with potential adverse effects on humans and animals. Unfortunately, many pharmaceutical compounds are resistant to breakdown in the environment, hence they have tendency for environmental magnification, since they are designed to be biologically active. Therefore, there is need to evaluate their environmental levels and their possible abatement methods using simple, cheap and low cost techniques, in order to avert their potential toxic consequences. In this research, a cost effective, robust, selective and rugged method for the analysis of antibiotics in water samples using liquid chromatography was developed, and used for monitoring levels of the selected antibiotics in Diep River. Also, an effective remediation procedure for these contaminants in water was developed using activated carbon produced from grape slurry waste.

The biomass was collected from a wine plant in Stellenbosch, processed to 250 – 350 µm mesh particle sizes followed by pyrolysis using optimized conditions with respect to time, temperature and the N_2 flow. The resulting carbon materials were modified with HCl, H_2SO_4, and KOH and characterized using Fourier Transform Infra-Red (FTIR) spectroscopy, scanning electron microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX) to study surface morphology and mineral characterization.

Results showed that the β-lactam antibiotics, amoxicillin (AMX), ampicillin (AMP) and chloramphenicol (CHLR) occurred at variable concentrations in the Diep River. The levels detected ranged: AMX, < DL – 13.29 ± 0.14 µg/L and < DL – 11.55 ± 0.04 µg/L in winter and spring respectively; AMP, < DL to 1.24 ± 0.24 µg/L and < DL – 5.27 ± 0.06 µg/L in winter and spring respectively; but CHLR did not occur at detectable concentration. The carbonization and activation of the grape slurry facilitated large surface area, enhanced pore size distribution and availability of increased active sites, resulting in improved adsorption efficiencies of the adsorbents. The EDX analysis of the adsorbent modified with HCl (GSAa), adsorbent modified with H_2SO_4 (GSAb), adsorbent modified with KOH (GSB) and the un-carbonised, unmodified adsorbent (GS), indicated high carbon content, which is a characteristic of good adsorbent materials. The FT-IR spectra pattern revealed the presence of prominent absorption bands consisting of the asymmetric C-H and symmetric CH_2 bond

vibrations at 2920.77 cm^{-1}, asymmetric stretching vibrations of C=O in the range 1600 - 1818.44 cm^{-1} and an asymmetric stretching vibrations of the C-C(O)-C or C-O group at 1032.56 cm^{-1}, defining the presence of some functionalities that can enhance their adsorption capacity.

Results from sorption studies showed that the modified grape slurry biomass waste efficiently removed β-lactams from simulated wastewater with the exothermic chemisorption mechanisms majorly influencing the sorption processes.

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DEDICATION

This Project is dedicated to

The Lord Almighty

The Chief Cornerstone of everything

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GLOSSARY

AMP: Ampicillin

AMX: Amoxicillin

C_e: Equilibrium concentration (mg/L)

CHLR: Chloramphenicol

C_o: Initial concentration (mg/L)

C_t: Concentration at a given time, t (mg/L)

DL: Detection Limit

E_a: Activation Energy

EDA: Electron-donor-electron-acceptor

EDC: Endocrine disrupting compound

EDX: Energy dispersive X-ray

EOC: Emerging organic contaminant

STP: Sewage treatment plant

ETP: Effluent treatment plant

GS: Un-carbonized and unmodified adsorbent

GSAa: Adsorbent modified with HCl

GSAb: Adsorbent modified with H2SO4

GSB: Adsorbent modified with KOH

H_2SO_4: Sulphuric acid

H_3PO_4: Phosphoric acid

HCl: Hydrochloric acid

HLB: Hydrophilic-lipophilic balance

ICH: International conference of harmonization

KOH: Potassium hydroxide

LC: Liquid Chromatography

LOD: Limit of detection

LOQ: Limit of quantification

MIC: Minimum inhibitory concentration

NaOH: Sodium hydroxide

PBP: Penicillin-binding protein

PCB: Polychlorinated biphenyls

PFC: Perfluorinated compounds

PPCP: Pharmaceutical personal care product

q_e: Amount of antibiotics adsorbed from solution at equilibrium (mg/g)

q_t: Amount of antibiotics adsorbed from solution at a given time, t (mg/g)

RSD: Relative standard deviation

SD: standard deviation

SEM: Scanning electron microscopy

SPE: Solid phase extraction

UHPLC: Ultra-high-pressure liquid chromatography

UV-DAD: Ultraviolet-visible Diode-array detector

WWTP: Wastewater treatment plant

$ZnCl_2$: Zinc chloride

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human and animal health management relies on the use and application of pharmaceutical products such as medications, mineral supplements, antiseptics, disinfectants and cosmetics for prophylaxis and therapeutic purposes. One of the major classes of pharmaceutical personal care products (PPCPs) applied for human and veterinary health purposes are those used for the treatment and management of a large number of medical conditions. Most of them are specific in action and effectiveness and so are classified into different groups depending on their intended use and mode of action. Common among the classes of pharmaceuticals are antibiotic, analgesic/non-steroidal anti-inflammatory drugs, anthelmintic, steroidal and non-steroidal hormones and many others. However, antibiotics are one of the most important groups of common pharmaceuticals widely in use, prescribed and non-prescribed among the different classes of pharmaceuticals.

The extensive use of antibiotics has led to the release and presence of their residues in different environmental compartments. This is because only a portion (≈ 10%) of prescribed and non-prescribed drugs is metabolized and made use of by humans and animals; with the rest excreted through sweat, urine and excreta (Reverte *et al*., 2003). Both un-metabolized parent form and metabolic derivatives are released through domestic discharge into municipal sewerages and adjoining surface waters. They are redistributed/partitioned between different environmental matrices via several pathways (Lindberg *et al*., 2005).

Although, the widespread occurrence of trace concentrations of residues of pharmaceuticals in aquatic system of some water catchment columns have been reported, little is known about their levels of environmental occurrence, transport, and ultimate fate, aside from their intended use. Pharmaceutical residues have, however, been reported not biodegradable in many environmental matrices, since they are manufactured to be bioactive. In the environment, they may not undergo further breakdown from the metabolic form in which they are released, hence they may show persistent behaviour with possible bio-magnification tendencies (Nebot *et al*., 2007). Antibiotics for example, display pseudo-persistence in their native and metabolite forms. Unfortunately, they are continuously added to the environment through inappropriate disposal of unused or expired products, discharges from domestic sources and industrial manufacturing processes. Despite the human health benefits derived from the use of antibiotics, they are potential environmental contaminants. Lin *et al*. (2009) reported that the occurrence of antibiotics in the environment suggests potential environmental pollution even at low concentrations. At such low concentrations, they can

maintain a high biological activity with potential adverse effects on humans and animals. For example, in some aquatic and marine organisms, residues of some pharmaceutical products have been reported to show toxic consequences such as endocrine disruption, feminization/masculinization of fish, imposex (i.e. the development of both male and female organs on the same species) in gastropods/cephalopods etc. (Nikolaou *et al*., 2007). The bioaccumulation of some pharmaceutical residues beyond minimum inhibitory concentrations (MICs) poses the probability of drug resistance in the environment for some microorganisms. Hence, antibiotics and other endocrine disrupting chemicals (EDCs) have been classified as emerging environmental contaminants (Nikolaou *et al*., 2007).

Unfortunately, the removal of these compounds in full-scale water treatment plants has not been well studied. Most of the existing wastewater treatment procedures to date are not effective in removing antibiotics and other drug residues. This implies that other micro-pollutants may enter the environment, disperse, and persist to a greater extent than anticipated (Kolpin *et al*., 2002). Reports from recent studies show that, the detection of pharmaceutical residues in wastewater treatment plant (WWTP) influents, and the number of pharmaceuticals detected in the final effluents from WWTPs is increasing, thus indicating incomplete removal or degradation of the compounds (Houndt & Howard, 2000). This may not be unconnected with the fact that most wastewater treatment plants were originally not designed to remove antibiotics and other pharmaceutical products, hence their inadvertent and subsequent release into natural water systems (Heidari *et al*., 2013). Nebot *et al*. (2007) reported that final effluents from WWTPs have been identified as a principal route for the introduction of these compounds into the natural aquatic environment.

Antibiotics are also capable of passing through all natural filtrations allowing them to ultimately reach drinking water (Miao *et al.*, 2004). Studies conducted on the occurrence of antibiotics in various water samples including surface water, ground water and drinking water revealed their presence and wide distribution (Petrovic *et al.*, 2005). The presence of antibiotics in sources of drinking water is of concern, especially where antibiotics go through drinking water treatment plants resulting in contamination of table water. There are indications that pharmaceutical residues may have adverse health effects on organisms exposed to chronic low-levels over time although not all the health consequences are fully known.

The general lack of data on the occurrence of antibiotics and other pharmaceutical products in water and aquatic eco-systems is because only few analytical methods capable of detecting these compounds at their low levels existed, until the recent advancement in analytical method development. The development of sensitive and selective analytical

techniques for the recovery, separation, detection and identification of trace and ultra-trace levels of these substances in biological and environmental matrices cannot be over-emphasized (Kolpin *et al.*, 2002). This is necessary to provide a robust assessment of the presence and potential risk of pharmaceuticals to wildlife and humans (Nebot *et al.*, 2007). Potential concern about the environmental presence of antibiotics is the development of antibiotic-resistant bacteria and the potential increased toxicity of chemical mixtures. Their potential fatal effects even at trace levels underscore the need for pragmatism in the rational management of pharmaceutical residues in water systems and other environmental matrices. There is also the need to develop a simple cost effective approach to remediate pharmaceutical contaminated media in order to mitigate their potential deleterious consequences.

1.2 Statement of Research Problem

There has been a significant increase on the dependence and consumption of prescribed and non-prescribed pharmaceutical products worldwide resulting in corresponding increase in production. Substantial proportion of these products are however discharged into the environment via human and animal wastes (urine and faeces), domestic and industrial discharges, wastewater release, improper disposal of unused and expired medications, and from the use of contaminated materials such as farm litter, solid wastes, sludge and sediment as manure (Baker & Kasprzyk-Hordern, 2011). Residues of many pharmaceuticals pass through the water treatment process unresolved, or may even be exacerbated in effluents from wastewater treatment facilities into receiving water bodies, since many wastewater treatment facilities were not designed for the removal of such contaminants and some other pollutants of organic origin (Heberer, 2002). Consequently pharmaceuticals may further be redistributed via heterotrophic transfer, in food webs.

Among the various classes of drugs largely produced, and in use for broad application either on prescription or over the counter are the antibiotics. These include beta lactams, tetracycline, sulfonamides, microlides, fluoroquinones, etc. They are used as therapeutic or prophylactic agents to fight various bacterial, viral, and fungal infections and diseases. Modes of administration range from ingestion, intravenous, and external application. In recent times, the occurrence and widespread presence of residues of human pharmaceutical products in the environment has become a concern due to their resistance to breakdown in unintended environments, and their potential toxic consequences even at trace concentrations (Diaz-Cruz & Barcelo, 2005). This is because many of the produced antibiotics and other classes of drugs were designed to be active, in specific terms or in broad spectrum in biological systems. Also, there may be gradual ecological accumulation and build-up of pharmaceutical products' residues in the environment if the rate with which they degrade or dissipate in the environment surpasses their environment loading rate. Thus,

pollution from pharmaceuticals is a complex phenomenon, ascribable to thousands of different molecules belonging to various therapeutic classes, with different physico-chemical properties, chemical structures, environmental behaviour and persistence (Carballa *et al.*, 2004)

However, the presence of antibiotics in aquatic environments is not desirable because of the potential likelihood of causing toxic effects on organisms and ecosystem alteration. Renew & Huang (2004) reported that the continuous occurrence of sub-lethal levels of residues of antibiotics in aquatic environments may perturb microbial ecology and increase the proliferation of antibiotic-resistant pathogens, and this may also pose a threat to human health. Challenges such as these are complex and cannot be solved by a simple event, but rather through continuous and multi-facet processes that will ensure that pollution levels are assessed and controlled.

1.3 Justification for Research

The quality of aquatic system is of great importance. This is because the health and well-being of water and other aquatic resources is essential for the existence and maintenance of ecosystems, as well as human health and other organisms that depend on water and water resources. Contaminating aquatic systems limit the availability of water resources for human use, resulting in health problems, environmental degradation, hunger, poverty and reduction of biodiversity. Among these contaminants are β-Lactam antibiotics. It is acknowledged that antibiotics represent the largest group of compounds that are carcinogenic and teratogenic and also pose high toxicities to aquatic organisms (Larsson *et al.*, 2007; Le-Minh *et al.*, 2012). Aquatic organisms, especially fish are part of the human diet and thus, leave humans exposed to the toxic effects of β-Lactam antibiotics. Hence, assessment and abatement studies of antibiotics in the environment are highly essential. Data from this study will provide information towards better understanding of β-Lactam antibiotics and also help aquatic management authorities in decision making.

1.4 Research Objectives

The overall objective of this research is to assess the levels of β-lactam antibiotics in surface water (in the Diep River) and their possible abatement using carbonized grape slurry waste.

The specific objectives are:

1. To assess the levels of selected β-lactam antibiotics in the Diep River.
2. To explore the potential removal of β-lactam antibiotics using grape slurry waste (charred).
3. To determine possible mechanisms of removal of β-lactam antibiotics from simulated wastewaters.

1.5 Scope of study (Delimitations)

Organic pollutants especially the endocrine disrupting group of contaminants consisting of pharmaceuticals, pesticides, herbicides, detergents, heavy metals and steroid hormones are present in surface water and effluents. This study will focus on the evaluation of the levels of beta-lactam antibiotics namely amoxicillin, ampicillin and chloramphenicol, in selected surface water catchment sampling stations along the Diep River. The study will also attempt to prepare and characterize adsorbents using grape slurry for the removal of antibiotics from simulated wastewater.

CHAPTER TWO

2.0 LITERATURE REVIEW

Pharmaceutical compounds are designed to be activity specific, and produce desired biological effects upon administration to organisms. They are designed to interfere with specific biological systems such as receptors or enzymes (Comerton *et al*., 2009). Thus, they comprise a broad spectrum of enzyme substances that contain acidic and basic functional groups in their structures in order to achieve a prophylaxis or therapeutic effect.

Much of the administered pharmaceuticals are however not completely metabolized during the biochemical conversion processes in recipient organisms (human and animals), and these are released in wastes deposition. Unfortunately, residues of these substances have been reported to be detected in surface water, wastewaters and soil samples resulting in undesirable effects. Therefore, there is a growing concern about their impacts on non-target organisms, in aquatic and terrestrial environments. Volumes of pharmaceutical products are manufactured daily to meet the health demands of humans, as well as the economic demands of agriculture and livestock farming practices (Le-Minh *et al.,* 2010; Watkinson *et al.,* 2007). These drugs are produced to meet different medical needs and are classified into several groups depending on their target effects or specific modes of action. These include antibiotics, anthelmintic, non-steroidal anti-inflammatory drugs/analgesics, etc.

2.1 Antibiotics

The term antibiotic refers to any substance or agent that is capable of inducing negative biological activity against living organisms. Traditionally, antibiotics refer to those substances or compounds (biological or chemical) that eradicate and/or inhibit the metabolism, growth, or life cycles of microorganisms (Homem & Santos, 2011). This was extended to cover specific groups of both pathogenic and non-pathogenic microorganisms.

Most of the different types of bacterial or microbial infections (specific or broad terms) that are known in humans, animals and plants dates as back as the 1930s (Manzetti & Ghisi, 2014). These infections include those caused by pathogenic and non-pathogenic microorganisms such as bacteria (e.g. pneumonia, meningitis), fungi (e.g. candidiasis) and viruses (e.g. influenza).

Substances belonging to the antibiotic pharmaceutical class may therefore be listed based on the specific type of organism they impact biological action on (Kummerer, 2009). These include any substance that has antifungal, antibacterial, antiviral, antitumor or anti-parasitic activity (Kummerer, 2009). Generally, ‘antibiotics’ refer to chemotherapeutic agents that have capacity to alter or inhibit the activity and growth of microorganisms such as fungi, bacteria, protozoa, etc. in biological systems. Some substances of the biological/biochemical origin in

nature also possess the ability to induce antibiotic effects (e.g. bacitracin, penicillin, etc.). They may however be semi-synthetic or completely synthetic.

2.1.1 Structure, properties and biological activity of antibiotics

Antibiotics are compounds having structures largely encompassed by cyclic components, represented by benzene rings, piperazine units, hexahydropyrimidines, as well as sulphonamides, quinolone and morpholine groups (Renew & Huang, 2004; Manzetti & Ghisi, 2014).

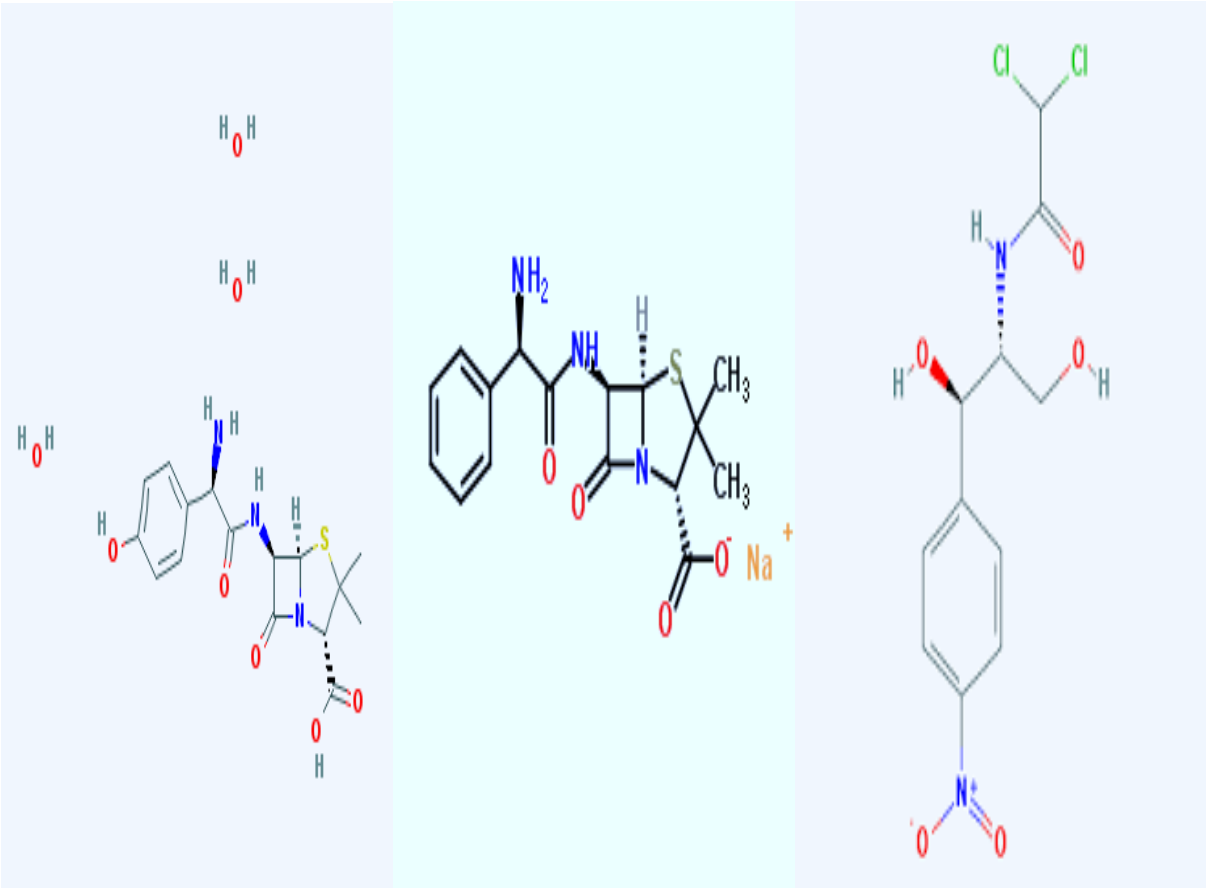

Figure 2.1: Structures of Amoxicillin, Ampicillin and Chloramphenicol

According to Comerton *et al.* (2009), most antibiotics are non-volatile and at neutral pH they exist in ionized form. Compounds of antibiotics have meta-stable properties due to their complex structures. After administration in humans or animals, they yield activated metabolites, conjugates and hydroxylated forms leading to a range of diverse active chemical compounds (Kennedy *et al.,* 1989; Manzetti & Ghisi, 2014). Some of the active chemical compounds released instigate the desired prophylaxis or therapeutic effects, while excess of it are continuously released into the environment with possible reactive properties but most importantly unknown consequences.

2.2 Classification of Antibiotics

Antibiotics are complex molecules which possess different functionalities within the same molecule. They consists of a diverse group of chemical compounds that can be classified into different groups, based on a variety of criteria such as their spectrum, chemical structure, or their mechanism of action (Homem & Santos, 2011). These include varying subgroups such as β-lactams, quinolones, tetracyclines, macrolides, sulphonamides and many others (Kummerer, 2009).

Early classification of antibiotics was based on the type of action they exert on microorganism such as bacteria; which could be either bactericidal or bacteriostatic. Bactericidal antibiotics annihilate the harmful bacteria while the bacteriostatic compounds tend to slow down microbial growth, thus giving the host body a chance to combat microorganisms with natural immune system (Kummerer, 2009; Christian *et al.,* 2003). Bacteriostatic antibiotics' action was reported to show minor side effects compared with bactericidal antibiotics (Heberer, 2002; Le-Minh *et al.,* 2012). The action of both bactericidal and bacteriostatic antibiotics takes advantage of the differences in the structures of the cells of bacteria and that of its host (Le-Minh *et al.,* 2012).

A second basis for the classification of antibiotics is on basis of their chemical structure. This is because antibiotics with same chemical structure will generally show similar patterns of antimicrobial (antibacterial) activity, effectiveness, allergic potential, as well as similar toxicity (Homem & Santos, 2011). This classification basis is incidentally the most common basis for the classification of antibiotics with few examples including sulphonamides, tetracyclines, macrolides, β-lactam antibiotics, etc.

The third basis for the classification of antibiotics is reliant on their spectrum, defined by the number of the organisms that are affected by the same drug (Heberer, 2002). The spectrum of antibiotics' effectiveness is therefore grouped into either, (i) narrow spectrum or (ii) broad spectrum drugs. Broad spectrum antibiotics are capable of affecting a variety of microorganism types e.g. bacteria, and fungi, and can be administered where the specific microorganism to be eliminated is not clearly known (Xu *et al.,* 2007; Homem & Santos, 2011). Narrow spectrum antibiotics are usually administered where the specific types of the microorganism to be eliminated are known, since they are engineered to be effective on specific microorganisms, and less effective on others (Homem & Santos, 2011).

Antibiotics can also be classified based on the route of administration of the drugs. The common route of administration is oral, that is, ingestion through the mouth. There are other routes of administration that are, however, more effective. These include intravenous administration involving direct injection or via intravenous fluids/drips, and through topical

applications such as with eye drops, ear drops, and throat drops, or ointments (Xu *et al.,* 2007; Homen & Santos, 2011).

2.3 Therapeutic, prophylaxis and other use of antibiotics

Antibiotics are an important pharmaceutical group that is largely prescribed in medical care, because of their effectiveness in fighting infections that were assumed untreatable before their discovery (Le-Minh *et al.,* 2012). They are considered as the most developed and successful class of drugs that has been applied to facilitate human and animal health. This category of pharmaceuticals act on their target bacterial or micro-organisms by either destroying or slowing down their growth (Kummerer, 2009; Fent *et al.,* 2006; Gracia-Lor *et al.,* 2012). Antibiotics can be administered on humans and animals on two application bases: (i) use of antibacterial drugs for disease prevention - prophylaxis and (ii) use of antibacterial drugs for treatment - therapeutic.

The extensive use of antibiotics is triggered by human development, developments in science and technology, human population explosion etc. Early applications of antibiotics were as ecto-parasiticides with biocidal capacity; and were and still are applied externally on skin or animal fur to eliminate fungi, bacteria and parasitic insects such as tick in domestic and farm animals. Oral ingestion or intravenous use of antibiotics followed the discovery of parasitic microorganisms. Thus antibiotic pharmaceuticals are used extensively in human and veterinary medicine to treat hundreds of ailments and diseases, ranging from relatively mild conditions such as acne to potentially life-threatening conditions such as pneumonia. Zwiener *et al.* (2000) reported that this class of drugs has generally helped in the revolution of 20th century medicine, treating categories of microbes such as bacteria, and fungi based infections and diseases. A combination of antibiotics with vaccination has led to the possibility of nearing the eradication of diseases such as tuberculosis in the developed countries. Some of the detailed prophylaxis and therapeutic use of antibiotics are presented in Table 2.1 below:

Table2.1: Prophylaxis and Therapeutic Applications of Antibiotics

Type of Antibiotic	Treatment applied for	Notes
Penicillin	Skin infection, ear infection, dental infection, Gonorrhea, urinary tract infection, respiratory tract infection (Florey, 1944).	Can be combined with beta-lactamase inhibitors which protect penicillin from harmful bacterial enzymes that might destroy it before its effective function
Cephalosporin	Skin infection, staph infection, Gonorrhea, otitis, bronchitis, step-throat, tonsillitis, pneumonia (Herrell, 1945).	Mostly used in surgical prophylaxis
Sulfonamides	Mainly used to treat kidney infection (Callahan, 1953)	Can fight various types of infection. However, have a very negative side effect of high probability of damaging the kidneys. Can also cause increased skin sensitivity to the sunlight.
Fluoroquinolones	Pneumonia, bronchitis, urinary tract infection, skin infection, sinusitis (Thiele-Bruhn & Beck, 2005)	Mild side effects like an upset stomach. However there is possibility of fatal side effects like permanent nerve damage causing peripheral neuropathy. Drugs also affect the growth of bones
Macrolides	Genital infection, respiratory tract infection, soft tissue infection, gastrointestinal infection (Thiele-Bruhn & Beck, 2005)	Usually given to people who are sensitive to penicillin. Side effects involve gastrointestinal discomfort.

2.4 Classes of Antibiotics

Antibiotics also known as antimicrobial drugs, has been reported to demonstrate an excellent potent and safe profile to humans and animals upon administration. This class of pharmaceuticals are derived from two main origins; (a) those obtained from natural sources such as beta-lactam antibiotics e.g. penicillins and cephalosporins; or protein synthesis inhibitors, such as aminoglycosides, macrolides, tetracyclines, polypeptides, etc. and (b) the synthetic agents. Synthetic antibiotic drugs are however produced in such a way that they are capable of exerting selective toxicity to target microorganisms.

The activities and effectiveness of antibiotic drugs against any specific bacteria or microbial is dependent on the following factors/properties:

(a) affinity of the drug for the target
(b) permeability of the drug (how well it can get to its target)
(c) stability to bacterial enzymes that inactivate or destroy the drug

It has been realized that β-lactam antibiotics, including the sub-groups of penicillins, cephalosporins, and as a marginal fraction carbapenems and others, make up the largest share of human use antibiotics in most countries. The β-lactam antibiotics have been studied and their statistics of total antibiotic use falls within the range of 50 – 70% (Renew & Huang, 2004).

2.4.1 Beta-Lactams (β-Lactams)

Beta-lactam **(**β-lactam) antibiotics refer to a class of broad-spectrum antibiotics that consists of a β-lactam ring as part of their core structure. A β-lactam ring is a four-membered lactam. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl (Malcolm, 2011). The simplest β-lactam possible is 2-azetidinone, (Figure 2.2).

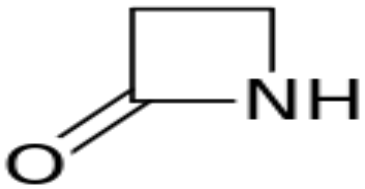

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Figure 2.2: Structure of the β-lactam ring (2-azetidione)

The mode of action of beta-lactam antibiotics, and the non-enzymatic resistance mechanisms to their activity, is intimately linked to the structure and biosynthesis of the bacterial cell wall (Glinka et al., 2005). The majority of the β-lactam antibiotics operate by inhibiting the cell wall biosynthesis in the bacterial organism. The bacteriostatic effect of beta-lactam antibiotics is related to their various interactions and concomitant inhibition of essential enzymes (transpeptidases, carboxypeptidases) involved in the terminal stages of peptidoglycan biosynthesis. These cytoplasmic membrane-associated target enzymes bind the antibiotics covalently, and hence are known as penicillin-binding proteins (PBPs). The bactericidal effect of these antibiotics is due to a second step following on from the inhibition of cell division and growth, in which the activation of an autolytic system causes cell death.

Some of the β-lactam antibiotics include derivatives of penicillin (penams), cephalosporins (cephems), carbapenems and monobactams (Glinka et al., 2005; Malcolm, 2011) (Figure 2.3).

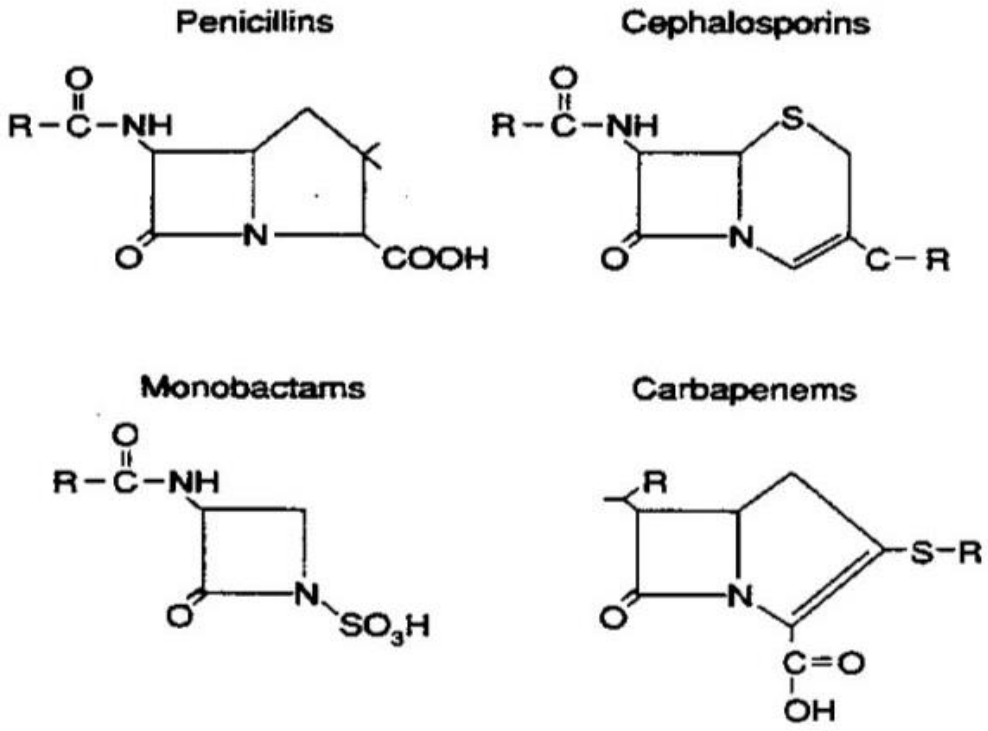

Fig 2.3: Structures of Beta-lactam antibiotics

2.5 Sources of Antibiotics in the Environment

In the previous years, there have been a widespread use of antibiotics in human medicine (approximated annual consumption of 100 000 – 200 000 tons) resulting in an increase in water contamination by these organic compounds (Xu *et al.,* 2007; Homem & Santos, 2011). The intense application of antibiotics in human medicine has become a common therapeutic practice responsible for both high consumption and the gradual accumulation of antibiotics in the environment (Manzetti & Ghisi, 2014).

Due to the extensive and long term usage of antibiotics, pharmaceuticals, personal care products (PCPs), and illicit drugs in human therapy; residues of these organic contaminants has been found in various environmental matrices (Kummerer, 2009). Human pharmaceuticals, including their precursor compounds and transformation products, are discharged into the environment either intentionally or unintentionally during manufacturing processes and through consumption, together with disposal of used and unwanted drugs (Gulkowska *et al.,* 2008). Probable route of human contribution is via ingestion, followed by excretion and disposal into domestic wastewater, entering the sewer network and reaching the wastewater treatment plants. After human administration of pharmaceutical products, the parent compounds and/or conjugates are excreted partially (Stuart *et al.,* 2012). Thus, human antibiotics' main route of discharge is through excretion (urine and faeces) where they enter the sewer network exposing them to waste water treatment plants (WWTPs) (Homem & Santos, 2011).

The other major route for exposure of these drugs is through the sewer system which leads to the sewage wastewater reaching the surface and natural water systems. The general public disposes unused or expired medicines more often through the sewage system. Improper disposal of unused and expired drugs, such as direct discharge into the sewage network, or deposition in landfills, waste effluents from manufacturer or accidental spills during manufacturing or distribution are considered significant sources of contamination (Baker & Kasprzyk-Hordern, 2011). Some other significant contamination points include industrial effluents (manufacturing plants, hospitals, food processing plants), municipal sewage treatment plants and combined sewage-storm-water overflows, resource extraction (mining), waste disposal sites (landfill sites, industrial impoundments, farm waste lagoons) and buried septic tanks (Lapworth *et al.,* 2012; Mompelat *et al.,* 2009; Homem & Santos, 2011).

2.6 Occurrence of Antibiotics in the Environment

A diverse array of synthetic organic compounds is used by society in vast quantities for a range of purposes (Bendz *et al.,* 2005; Lapworth *et al.,* 2012). Through several researches that have been carried out, today the occurrence of EOCs has been better characterized in wastewater and surface water environments compared to groundwater (Nikolau *et al.,* 2007; Lapworth *et al.,* 2012). Pharmaceutical compounds and other related products have been reported to be reaching the environment through various means. In the recent past years, the application of antibiotics in human medicine has enormous and widespread negative effects, consequently the possibility of increased water and land contamination. Besse & Garric (2007) reported that a vast range of drugs including antibiotics have been discovered in effluents and surface waters of several countries. In general, a large diversity of compounds is also thought to be found in wastewaters and surface waters according to Lapworth *et al.* (2012). However, this conclusion could be mainly due to the capability of various analytical methods and limited studies on groundwater sources rather than the actual environmental occurrence. Of great concern however, is the dearth of ability to evaluate the levels of these drugs due to their very low concentrations in different sinks and thus the inability to assess the environmental risk associated with their occurrence levels.

However, the prevalence of chemical compounds with commercial or therapeutic uses, and their variably harmful effects on human and environmental health has given rise to new classes of potential deleterious contaminants. Among them, antibiotics have emerged as one major class of contaminants, considering their widespread usage and their known biological effects. Antibiotics have been detected in terrestrial matrices, bio-solids, soil, hospital effluents, municipal wastewater, surface water, ground water and sea water. The reported

concentrations of antibiotics in water range from sub-nanogram or low nanogram per litre levels (in ground and river waters) to high microgram per litre levels (in hospital effluents), with intermediate concentrations in sewage treatment plants (STPs) effluents (Lindberg *et al.,* 2005). Decontamination of wastewaters is very critical for purification and avoidance of the release of antibiotics to the environment since wastewaters are the major contamination port of antibiotics to the environment especially in urban regions and metropolises (Bendz *et al.,* 2005; Manzetti & Ghisi, 2014). The exposure of pharmaceuticals and their metabolites into the water systems is gaining accelerated attention. It is thus mandatory to monitor pharmaceutical compounds in surface and/or ground waters.

Pharmaceuticals have been found to exist in the environment either as parent compounds or as metabolites/conjugates (Nikolau *et al.,* 2007). When pharmaceutical pollutants are released to the environment, the fate and biological potency of these pollutants can be predicted or assessed based on their special biological and physicochemical characteristics. These characteristics of pharmaceuticals completely differentiate them from other variable industrial chemical compounds. Some of the unique characteristics of pharmaceuticals include polymorphism, their introduction into the environment after human metabolism, their chemically complex structure, and their ability to be ionized and have multiple ionization sites spread throughout the molecule. These compounds are mainly disposed into the sewage system and they are polar, multifunctional and incompletely removed by STPs (Pal *et al.,* 2014). STPs are designed to clean industrial and urban wastewater but unfortunately these plants are not designed to eliminate quantitatively other pollutants such as pharmaceuticals. Several studies have showed that organic micro pollutants, pharmaceuticals included, are available in the effluent of STPs, surface water and ground waters (Zorita *et al.,* 2009). Nevertheless, regardless the long history of antibiotic usage, information regarding the production and use patterns of antibiotics in the whole world is severely limited. However, the accumulation and persistence of antibiotics in the environment poses a threat to either aquatic or terrestrial ecosystems even at the trace levels in which they are detected (Homem & Santos, 2011). This bioaccumulation and pseudo-persistence of these compounds can be better explained by the extensive and indiscriminate use of these compounds in human and veterinary medicine together with their continual introduction into the environmental matrices.

2.7 Health and Environmental Impact of Antibiotics and other EDCs

Pharmaceutical compounds that have been released into the environment lately have been acknowledged to constitute a major health risk for humans as well as aquatic ecosystems (Bendz *et al.,* 2005). By considering the specific mode of action of pharmaceuticals, it can therefore be observed that these compounds are purposely designed to exert an effect on humans, mammals or other vertebrates. The negative effect of pharmaceutical residues is

more important in respect of human health and environment than those of pesticides which are manufactured to kill weeds, fungi and invertebrate varmints (Cleuvers, 2002). The modes of action and interaction of pharmaceuticals' active ingredients in the environment are not clearly understood. Hence, it is not easy to affirm statements about their potential environmental effects. Antibiotics have found an extensive use in human and animal health, but despite all that, it is surprising how these chemicals have received little attention as pollutants in the aquatic environment compared to other chemicals. The current scientific consensus is that low levels of pharmaceuticals in the environment do not pose an appreciable risk to human health (Cunningham *et al.,* 2006).

The possible fatal negative effects that could arise due to the presence of these drugs in aquatic systems remain unknown and, consequently in the recent years they have managed to draw more attention as potential environmental pollutants. Pharmaceutical pollution is interesting in the fact that it does not result primarily from manufacturing but rather from continual and widespread usage, excretion accompanied with improper disposal of both human and veterinary medicines. Generally, pharmaceuticals could be found in any environment that can be inhabited by man and this makes them potentially ubiquitous pollutants. Currently all the data and research conducted so far has provided little evidence that can possibly suggest that pharmaceuticals in the environment are in quantities sufficient enough to cause significant harm. Their use is however expected to increase abruptly with the completion of the human genome project coupled with the rising age of human population.

In order to perform the risk assessment of antibiotics, it is important to question their natural background concentrations in the environment (Kummerer, 2009). The consumption of antibiotics by humans in total, per capita, and the individual share of each compound differ from country to country. Same applies for the prescription rates together with the intake of drugs without prescription which also varies markedly amongst countries (Molstad *et al.,* 2002; Kummerer, 2009). According to various authors who have worked on analysing pharmaceuticals, various aquatic compartments including hospital sewage, municipal sewage, ground water and surface water are all sources of pharmaceutical residues but at very low concentrations in the ng/L to a few µg/L range (Kolpin *et al.,* 2002; Hernando *et al.,* 2006; Duong *et al.,* 2008). Environmental change affected by human activity in coastal regions is well known mainly due to increased human population density. Coastal water is thus considered the ultimate sink for sewage and other by-products of human activities (Bendz *et al.,* 2005). Discrete locations whose inputs into the aquatic systems can be defined in a spatially discrete manner are the point sources of pollution making the spatial plume of pollution generally more constrained.

2.8 Human and Environmental Exposure and Toxicity

The majority of the research output so far conducted, indicate strongly that pharmaceuticals are not completely eliminated in most if not all sewage treatment plants. Pharmaceutical drugs have been detected in ground waters, surface waters and even in marine systems. Pharmaceuticals have thus emerged in particular as a major class of contaminants, considering their widespread usage and unknown biological effects.

Human pharmaceuticals have recently attracted attention of the international scientific community because they form an important group of potential endocrine disrupting compounds (EDCs). Continual introduction of these drugs by sewage effluent might cause them to be “pseudo persistent”, with an additional elevated threat (unknown consequences) for aquatic organisms that may be subjected to continuous exposure.

The presence of different pharmaceutical chemicals contributes to the increasing multiple chemical cocktail that different components of the ecosystem (biotic and abiotic) are exposed to. Despite the relatively low environmental concentrations of the individual drug residues in water and soils, antibiotics possessing a common mechanism of action could have significant additive or synergistic adverse effects (Gulkowska *et al.,* 2008). Comprehensive review on the eco-toxicological effects of antibiotics and some other pharmaceuticals is lacking, hence very little is known about eco-toxicological effects of pharmaceuticals on aquatic organisms (Fent *et al.,* 2006). The possible human and ecological adverse effects upon exposure to antibiotics are also not well known, because dose concentrations of antibiotic compounds do not seem to cause any toxic effects on human health (Larsson *et al.,* 2007).

Although the presence of pharmaceuticals in the environment are believed to only pose a low risk of acute toxicity, there is a huge concern on the sub-acute and chronic exposure of aquatic organisms to antibiotics (Miao *et al.,* 2004). Exposure to low dose concentration of antibiotics in the aquatic environment over a long period of time may impose a risk for microorganisms such as microphytes, algae, mollusks and other benthic organisms. To date, the toxic effects due to long-term exposure to a combination of low concentration range of emerging contaminants are not well understood.

In general, antibiotic contaminants in aquatic environments may result in the perturbation of microbial ecology (Renew & Huang, 2004). It is imperative to attain a better understanding of the occurrence and fate of antibiotics in natural and engineered water systems in order to be able to assess the risks associated with these compounds.

2.9 Effects of Exposure and Toxicity of Pharmaceuticals

The rate at which pharmaceuticals are produced and consumed globally has been observed to steadily increase higher than the rate of global population growth (Yoon *et al*., 2006). A very high quantity of drugs has been disposed into the environment after use in the form of human and animal secretions and also as unused waste. The rate at which pharmaceuticals are spreading, their persistence in the environment and their ability to accumulate in the environment has also changed. The high biological activity of these drugs even at trace levels implies that they can still cause significant damages in the biosphere. A good example of their negative effect is the male fish feminization which has been observed and ruled out to be the effect of anthropogenic pollution in various aquatic systems in different parts of the world (Cleuvers, 2002; Zorita *et al*., 2009). It is discoveries like these that have caused pharmaceuticals to be rendered particularly dangerous pollutants for the environment. The effects of pharmaceuticals particularly antibiotics to the environment could be harmful to human health. It is just unfortunate to realize that the frequency of these compounds in the environment is very high.

Concern has grown over the years pertaining to the occurrence, fate and adverse effects of pharmaceutical residues in the aquatic environment. Antibiotics are a class of drugs that are widely and frequently used in large quantities almost similar to pesticides. In some countries antibiotics are even sold over the counter without the requirement of a prescription (Cunningham *et al.,* 2006; Celiz *et al.,* 2009; Manzetti & Ghisi, 2014). Despite their abundant use, pharmaceuticals do not undergo any scrutiny; they are not tested for low-dosage against long-term exposure. Therefore full extent and consequences associated with the presence of these chemicals in the environment is still a mystery. The discoveries of their concentration levels in the environment through research have implicated a relaxation of thought that pharmaceuticals are unlikely to cause any detrimental effect on the environment. This relaxation is only based on tests performed with individual compounds associated with short term exposure.

Making accurate risk assessments for aquatic environments has become very difficult. This is due to lack of validated analytical methods, non-uniform monitoring of data and definite information about the fate and effects of these compounds and/or their metabolites together with transformation by-products in the aquatic environment. Fortunately it is now known that some pharmaceutical residues can persist in the environment and can find their way back to humans either through drinking water or the food chain. Research has also enlightened us on the development of adverse effects in aquatic organisms even at environmental concentrations once believed to be infinitesimal and harmless. It is thus accepted that there is very limited knowledge with regards to the effects of human exposure to low-dose mixtures

of pharmaceuticals or low-dose pharmaceuticals mixed with other low-dose synthetic pollutants.

2.10 Environmental Fate of Pharmaceuticals

Pharmaceuticals are natural and synthetic chemicals belonging to a wide group of different chemical families, thus they may behave or react differently in the environment. Since majority of these compounds are highly polar and are non-volatile, these properties help antibiotics to stay longer in the different environmental matrices (Hernando *et al.,* 2006; Homem & Santos, 2011). There exist multi-mixed array of several different natural and synthesized chemicals occurring at the same time in the environment, with many different interactions. These interactions may occur as a result of the multiple exposures of biotic and abiotic features of the chemicals (Gobel *et al.,* 2004). Since antibiotics and other pharmaceuticals were specifically designed to be bioactive, they may represent a different kind of interaction and risk when present in different abiotic environments. Their breakdown in the environment have been reported to be slow, hence they may persist, accumulate and/or be redistributed in the environment.

The prevalence in the commercial or therapeutic uses of pharmaceuticals in recent years has given rise to new classes of potential aqueous contaminants. Due to the vast array of possible compounds being classified under 'emerging organic contaminants' (EOC), a lot of studies have been conducted and they selected EOCs according to priority lists established taking into account consumption, predicted environmental concentrations and eco-toxicological, pharmacological and physicochemical data (Fent *et al.,* 2006; Celiz *et al.,* 2009; Lapworth *et al.,* 2012). The chemical properties, functional groups and reactive atoms in the structure of antibiotics determine the fate of these compounds. Different types of antibiotics are thus metabolized differently in biological species especially humans, causing it to produce different types of excess metabolites most of which are released in excretion (urine/faeces) (Manzetti & Ghisi, 2014). The excreted human antibiotics undergo several treatment processes in the wastewater facilities before they can enter surface waters. After they are exposed to surface water they undergo different transport and fate processes in the environment (Huang *et al.,* 2001).

Millions of pharmacologically active substances are consumed and used annually all over the world. It is however surprising that only little is known regarding the ultimate fate and degradation of the majority of these drugs after their intended use. Approximately 90% of the administered drug may be excreted un-metabolized and these metabolites have the potential of being converted back to the active compound again (Manzetti & Ghisi, 2014). Antibiotics used in human medicines are partially metabolized after administration, with a significant

portion of the active ingredient excreted either in same form as the parent compound, derivative of the parent compound or in conjugated forms that can easily be converted back to the parent antibiotic (Heberer, 2002).

Even though antibiotics have been detected in different aquatic environmental matrices, still little is known concerning their distribution in the environment, their mobility and persistence in natural and engineered water systems (Snow *et al.,* 2007; Mompelat *et al.,* 2009). Despite the shortage of information, studies that have been conducted on the transformation and sorption of antibiotics indicate that these processes affect significantly the fate of most classes of antibiotics.

2.11 Antibiotic Resistance

The presence of antibiotics in the environment has introduced a new threat of great concern which is antibiotic resistance. Antibiotic resistance has brought about a crisis that compromise the ability of antibiotics in treating infection thereby also threatening most areas of medicine, surgery included. However, increase in the proliferation of antibiotic-resistant pathogens may pose threats to human health (Renew & Huang, 2004). Even though they are present at vestigial levels, antibiotics have the potential of causing resistance in bacterial populations, and therefore may make them ineffective in the treatment of several diseases caused by similar strains of bacteria (Xu *et al.,* 2006).

Over the past many years, research has exclusively concentrated on conventional 'priority' pollutants. Pharmaceuticals are a group of chemical compounds with a greater probability of causing harm to the environment, but, they have so far received less attention as possible environmental pollutants. Antibiotics are regarded as "pseudo-persistent" contaminants because of their continual introduction into the environment (Gulkowska *et al.,* 2008). As already mentioned, antibiotics block the vital processes in bacteria thereby killing the bacteria or stopping the bacteria from multiplying. This way the work of antibiotics helps the body's natural immune system to then fight the bacterial infection. Antibiotics have the capability of treating and curing various infections making them very important to our health care. It is however very unfortunate that certain bacterium has developed the capacity to resist these drugs. In contemporary times, antibiotics have been largely overused especially for prophylactic and therapeutic purposes to the extent that a number of micro-organisms have developed resistance.

Drug resistance has led to the need for higher dosage of older products to treat infections; this may result in toxicity to the human system (Batt & Aga, 2005). Thus, some antibiotics selected to mitigate or eliminate mutant bacteria are now resisted at the concentrations that were previously effective. These antibiotic resistant bacteria are not found on any list of

antibiotics and they cannot be killed by any common antibiotics present today. This poses a threat and difficulty in the treatment of certain illnesses. The drastic decline in the synthesis of new antibiotics has exacerbated this problem. Most antibiotics that have been developed and synthesized only focus on gram-positive bacteria whereas the antibiotics that can fight against gram-negative bacteria are crucial (Lissemore *et al.,* 2006; Homem & Santos, 2011). The huge threat that our environment and world is faced with due to antibiotic resistance has been recognized up to global levels now.

2.12 Regulation of EOCs in Ground Water

It is a crucial requirement within the framework of various national regulations to monitor the anthropogenic micro-organic pollutants in river basins. Threshold values (standards) need to be established for pollutants that put the groundwater bodies at risk of failing to achieve any of their environmental objectives (Lapworth *et al.,* 2012). The overall aim for undertaking this crucial measure and process is so as to ensure protection and improvement of the quality of the water resources (Lapworth *et al.,* 2012).

It is fair to accept that the spatial and temporal variability of most of the EOCs in the environment is poorly understood up to now and it is a topic of growing concern for both research and regulatory perspectives. In the case of many EOCs, the lack of knowledge on toxicity, impact, behaviour, and limited monitoring data means that threshold values cannot be set as yet (Lapworth *et al.,* 2012; Homem & Santos, 2011). However, in the near future, if these contaminants (EOCs) are found to impose a risk of pollution of groundwater and have the potential of compromising the environment objectives, then standards (threshold values) will be required.

According to Homem & Santos (2011), legal limits have been established so far for antibiotics in food (4 – 1500 µg / kg for milk and 25 – 6000 µg / kg for the other food stuffs of animal origin (Stuart *et al.,* 2012), but, there is no legislation applied to environmental matrices yet. Regardless, the absence of regulatory drinking water, environmental quality standards or groundwater threshold values for much of the micro-organic contaminants does not mean that they do not pose a threat to human health or aquatic ecosystems. In most cases it is rather because their toxicity and environmental occurrence are as yet poorly understood (Lapworth *et al.,* 2012). This then implies that in the coming decades there is going to be an increase in the number of compounds that will be regulated through drinking water standards and/or environmental quality standards.

Regulatory frameworks exist to manage the potential sources of pollution and require monitoring of a number of ‘priority’ organic contaminants in the aquatic environment (Molstad *et al.,* 2002; Le-Minh *et al.,* 2010; Lapworth *et al.,* 2012). According to Lapworth *et al.* (2012),

there is a paucity of data concerning the occurrence and fate of EOCs compared to other anthropogenic contaminants in the aquatic environment because these compounds have not been studied extensively.

2.13 Water Management

Substantial effort has been applied to try and improve the quality of waterways in the recent decades. This effort has been greatly influenced by public concern regarding some visible signs of environmental degradation, (e.g. algal outbreaks associated with nutrient over-enriched) (Schwarzenbach *et al.,* 2006).

Unlike most of the other considered environmental (aquatic) pollutants, antibiotics possess a direct biological action on microbes. In most of the cases, these compounds act as persistent pollutants because they are continually emitted into the environment regardless of some of the compounds having the capability of being highly degraded. These antibiotics that are released into the aquatic environment need to be seriously monitored and dealt with because they contaminate raw, treated and recycled water that is used for drinking, irrigation and recreation (Le-Minh *et al.,* 2010).

The antibiotic residues also have a potential to accelerate widespread bacterial resistance to antibiotics as well as imposing a negative effect in important ecosystem bacteria (through death or inhibition). Antibiotics are predominantly water soluble which favours their entry into the aquatic environment so easily through sewage systems as a result of their consumption and excretion by humans and effluent from farms. Recycling of municipal water for agricultural, industrial and non-potable municipal use is a growing development for water resources management practices in most parts of the world today (Le-Minh *et al.,* 2010).

In an increasing number of countries, treated effluents are intentionally being used to aid with the supplementation of drinking water supplies. In the past decades there has been an emerging interest regarding the occurrence of the so called ‘micro-organic contaminants’ in the aquatic environments, their potential toxicity and environmental fate (Lapworth *et al.,* 2012; Kummerer, 2009; Schwarzenbach *et al.,* 2006).

A threat is imposed on to the aquatic environment by the presence of organic micro-pollutants. These micro pollutants have effects including acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem and losses of habitats and biodiversity, as well as threats to human health (Sanchez-Avila *et al.,* 2012; Jiang *et al.,* 2014). There is a growing concern related to the contamination of ground water resources by micro-organics and the extent of the fatal effects due to this contamination is poorly understood compared to other fresh water resources (Lapworth *et al.,* 2012).It is very important for the health of all

groundwater-dependent ecosystems which include rivers and lakes because in many parts of the world they are the important sources of drinking water.

2.13.1 Wastewater Treatment

The sharp rise in the chemical pollution of surface and ground waters, with largely unknown long term effects on aquatic life and on human health underscore the need for remediation and restoration (Schwarzenbach *et al.,* 2006; Li *et al.,* 2002). The satisfactory disposal of wastewater, either by surface, subsurface methods or dilution, is dependent on its treatment prior to disposal. Adequate treatment is necessary to prevent contamination of receiving waters to a degree which might interfere with their best or intended use, be it for water supply, recreation, or any other required purpose (Ademiluyi *et al.,* 2009).

Unit operations and processes used in functional water works, and/or wastewater treatments plants may vary, although the ultimate objectives remains water purification viz-a-viz the minimization or elimination of contaminants' load in water, so as to ensure the preservation of aquatic quality and life. Water containing organic residues need to be treated in order to remove or minimize their levels in water and wastewater, as well as from other environmental matrices. This is because of their potential to impose acute health risks on humanity (Ali *et al.,* 2012).

Organic micro pollutants such as PPCPs, phenols, phthalates, PCBs, pesticides and PFCs have been reported to be detected in wastewaters, effluents, drinking water and other environmental matrices (Abegglen, 2009). Effluents disposed from industries are more likely to contain residues of these organic chemicals in quantitative amounts. These contaminants may however persist with a potential to build up over time. Their occurrence is further accentuated by the fact that water treatment processes in wastewater/effluent treatment plants and water works for domestic water were not designed with the capability of eliminating or minimizing the levels of these substances, but rather to reduce input of solids, nutrients and organic micro molecules/substrates such as carbohydrates, protein and fatty acids. In spite of this, the continuous daily disposal of organic micro-pollutants and other EDCs into water bodies result in sharp deterioration of water quality.

Wastewater treatment involves the application of known technologies to improve the quality of wastewater. In general terms, wastewater treatment in facilities such as WWTPs/ETPs etc. involves the collection of wastewater in a central, segregated location and passing it through various treatment processes. Since large volumes may be involved, treatment processes are usually carried out on continuously flowing wastewaters (continuous flow or open systems) rather than as batch or a series of periodic treatment processes in which treatment is carried

out on parcels or batches of wastewaters (Gaspard *et al.,* 2006). However, in the continuous flow process, some operations, such as vacuum filtration which involves storage of sludge, chemical addition, filtration and the removal or disposal of the treated sludge, are routinely handled as periodic batch operations.

Wastewater treatment can also be categorized by the nature of the treatment process operation in use, e.g. physical (sedimentation (clarification), screening, aeration, filtration, flotation and skimming, degasification, equalization), chemical (chlorination, ozonation, neutralization, coagulation, adsorption, ion-exchange) or biological (*Aerobic:* activated sludge treatment methods, trickling filtration, oxidation ponds, lagoons aerobic digestion or *anaerobic:* anaerobic digestion, septic tanks, lagoons). A complete water treatment system may consist of the application of a number of physical, chemical and biological processes.

Thus the entire treatment process which may probably combine physical, chemical and biological methods is summarized in five steps: preliminary treatment, secondary treatment, disinfection, sludge treatment and tertiary treatment. The extent of treatment is sometimes indicated by use of the terms primary, secondary and tertiary treatment

2.13.2 Wastewater Treatment Technologies

An exponential increase in the population and social civilization has been observed over the past several decades. The resulting change in affluent lifestyles, uses of resources and continued advances in developments of industries and technology has been accompanied by a sharp modernization and metropolitan growth (Zorita *et al*., 2009). There has been an improved awareness regarding the occurrences of industrial activities that has increased the intensity of deteriorations on several ecosystems which in turn seriously threaten the health of humans and the environment. As a response to this threat, some stringent rules and regulations that concern the emission of contaminants from industrial waste streams by various regulatory agencies has been promulgated and enforced (Le-Minh *et al*., 2010). A simultaneous development through research for the invention of a wide range of improved treatment technologies accompanied with varying levels of successes has accelerated a dramatic progress in the scientific community.

Technologies applied in wastewater treatment are numerous, with each defining a specific objective in the combination of unit operation processes applied in the continuous flow operation. The choice of combination of technologies is a function of the nature and characteristics of the water quality intended for treatment. Abegglen *et al.* (2009) concluded that one probable way to minimize the input of organic micro-pollutants to surface waters is

to integrate an additional treatment step at WWTPs. Therefore, there is need to develop simple, efficient, available and affordable technology for the removal of such substances.

A variety of technologies including precipitation, ion exchange, membrane filtration and reverse osmosis have been used for the treatment of pollutants from wastewaters (Zwiener & Frimmel, 2000). Somehow, most if not all of these methods require expensive equipment and/or continuous need of chemicals making them disadvantageous (Xu *et al.,* 2007; Lin *et al.,* 2009). In addition, the above mentioned methods fail to meet the requirements of the Environmental Protection Agency (Xu *et al.,* 2007).

Amongst these techniques, adsorption has grown to be the most promising option for effective elimination of organic micro-pollutants from water. Adsorption is a process of surface phenomenon and this process is observed to be the most efficient; most promising and widely applied fundamental approach in wastewater treatment processes. Possibility of water re-use in terms of initial cost, simplicity of design, insensibility to toxic substances and ease of operation makes the adsorption process uniquely superior to other techniques (Manzetti & Ghisi, 2014).

2.13.3 Remediation Process: Adsorption

Adsorption can simply be described as a process that involves preferential partitioning of substances from gaseous or liquid phases on to the surface of a solid substrate (Kummerer *et al.,* 2000). Most adsorption phenomena can be applied in most natural physical, biological and chemical systems. During the adsorption process, the adsorbent material attracts and holds the particles of another substance to its surface (Batt *et al.,* 2006; Yoon *et al.,* 2006). However, the adsorbed particles could be either atoms or molecules of the particular substance of interest. For the process to be effective, the adsorbent material must be of a very small size, since smaller sizes offer greater surface areas for adsorption. Usually finely divided solids such as clay soil are very good adsorbents.

Residual or unbalanced forces that are found at the surface of solid or liquid phases are the ones responsible for the adsorption process to occur (Homem & Santos, 2011). These forces have the ability to attract and retain the molecular species with which it comes in contact with at the surface. Essentially, adsorption can be considered a surface phenomenon since the process essentially occurs at the surface of the substance involved. There are two components involved with adsorption, which are the adsorbent and the adsorbate (Manzetti & Stenersen, 2010). The adsorption process takes place at the surface of the adsorbent, whilst the adsorbate is the substance that is being adsorbed on to the surface of the adsorbent:

Adsorbate + *Adsorbent* = *Adsorption*.

For adsorption process to commence, some forces of attraction should exist between the adsorbate and the adsorbent (Yoon *et al.,* 2006; Manzetti & Stenersen, 2010). These attraction forces could be due to Van der Waals forces of attraction which are weak forces or due to chemical bond(s) which are strong forces. Adsorption can thus be classified into two types depending on the types of forces of attraction that will exist between the adsorbate and the adsorbent.

An adsorption process could either be physical adsorption (physisorption) or chemical adsorption (chemisorption). Since physical adsorption is a result of weak Van der Waals forces and electrostatic forces, the process results with the formation of multilayers of adsorbate on adsorbent associated with a low enthalpy of adsorption, ΔH, according to Putra *et al.* (2009). Physical adsorption usually occurs at low temperatures mostly lower than the boiling point of the adsorbate (Le-Minh *et al.,* 2010).
Chemical adsorption is adsorption facilitated by chemical forces of attraction (chemical bond) between the adsorbate and the adsorbent. Chemisorption results with the formation of a unilayer of adsorbate on the adsorbent and enthalpy of adsorption associated with the process is high (Le-Minh *et al.,* 2010). The chemisorption process can occur at any temperature.

The process of adsorption is dependent on several factors which are temperature, pressure, surface area and the activation of the adsorbent. Temperature effects on adsorption are profound, and measurements are usually done at a constant temperature. Graphs of the data are called 'isotherms'. The phenomenon of adsorption has become a very useful tool recently for the separation and purification processes. The employment of solids such as activated carbon and synthetic resins for adsorption is widely incorporated in industrial application for purification of waters and wastewaters (Zorita *et al.,* 2009). Most adsorbents that have been observed to be more effective are typically non-polar adsorbents. This means that they have more affinity with hydrocarbons or oil and organic compounds. Adsorption is widely applied in industry for air conditioning, water purification, prolonging of neurological exposure to specific drugs in pharmaceuticals, etc. (Batt *et al.,* 2006; Yoon *et al.,* 2006; Kummerer *et al.,* 2000; Manzetti & Ghisi, 2014).

2.14 Carbonization

Activated carbons have found extensive application in controlling and treatment of water from organic chemicals because of their high porosity (Li *et al.,* 2002; Gaspard *et al.,* 2006). It was observed that water treated with activated carbons has good quality with less or very low concentrations of dissolved organic micro-pollutants (Balasubramani & Sivarajasekar, 2014). Usually activated carbons are the most common adsorbent for the adsorption process since

they are more versatile and effective. Activated carbons are principally applied for the removal of species by adsorption from both liquid and gaseous phases.

Several notable trends in the development of activated carbon, an adsorbent that has large porous surface area, controllable pore structure, thermo-stability and low acid/base reactivity has been evident, in terms of its versatility for the removal of various types of inorganic and organic pollutants that are dissolved in aqueous media. Using activated carbon for water treatment in a bid to improve the quality and health of water is a very increasing option for researchers (Ozkaya, 2006; Subha & Namasivayam, 2008).

Activated carbons are generally micro porous but in addition to that they also contain meso and macro pores (Mestre *et al.,* 2007). These pores are important in the facilitation of access of the adsorbate molecules to the interior of carbon particles (Mestre *et al*., 2007). Carbon adsorbents are mainly characterized by a porous carbon structure that contains small amounts of different hetero atoms like oxygen and hydrogen. A variable amount of mineral matter (ash content) is also associated with some activated carbons. This ash content is dependent on the nature of the raw material used as a precursor.

Carbons may have different chemical properties. This is as a result of the presence or absence of surface groups, formed by heteroatoms that may bond themselves to the carbon atoms at the edges of the basal planes (Subha & Namasivayam, 2008). Chemical and physical properties of these carbons play a vital role in the behaviour of carbon adsorbents. High specific porosity and high surface area makes activated carbons extreme versatile adsorbents for major industrial significance (Subha & Namasivayam, 2008; Dada *et al.,* 2012).

It is however known that the higher the quality of activated carbon, the greater is the cost for its production and purchase but activated carbon is still the best adsorbent on the market. However, regardless of its prolific applications in adsorption processes, there is a huge barrier to the application of activated carbon by industries which is its high cost coupled with difficulties in regenerating the compound (Dada *et al.,* 2012). Both processes involved for the regeneration of used carbon, i.e. chemical and thermal regeneration, are expensive and on a large scale impractical. These processes rather produce additional effluent and they result in a considerable loss of the adsorbent. It is a result of these problems that has got many scientists to look and try to use cheap, readily available and efficient alternative materials to produce activated carbon (Gong *et al.,* 2005). It was upon the realization of this complication that there has been a growing exploitation in evaluating the feasibility and suitability of natural, renewable low-cost materials for adsorption. Some bioorganic fruit waste has been

applied in generating carbonized adsorbents such as olive stone almond shells and palm fruit carbons (Linares-Solano *et al.,* 1980; Lopez-Garzon *et al.,* 1984; Caturala *et al.,* 1988; Nasser *et al.,* 1996; Berrios *et al.,* 2012). Some agricultural waste products like rice husks, maize cobs, sugarcane bagasse, tea leaves, have also been tried for remediation of various contaminants (Tannin & Gurgey, 1988; Nawar & Doma, 1989; Sun & Xu, 1997; Zhang & Chuang, 2001; Gong *et al.,* 2005; Zvinowanda *et al.,* 2008; Ademiluyi *et al.,* 2009, Han *et al.,* 2011). The application of all these cheap, natural, organic wastes is to try and replace the expensive activated carbon but still be able to effectively reduce and control water pollution (Gong *et al.,* 2005; Ramesh *et al.,* 2005; Hameed *et al.,* 2008). Remediation processes has and are still being exerted on water pollution in all parts of the world.

A variable number of precursors are subjected to a number of different activation processes such as chemical or physical activation in an aim to attain carbon that has high adsorption capacity for a particular application. The internal porosity of activated carbons is responsible for their high adsorptive capacities (Shaibu *et al.,* 2014). Other properties including surface area, pore volume and pore size distribution also account for the advantage that is associated with activated carbons (Hameed *et al.,* 2008; Zvinowanda *et al.,* 2008; Ali *et al.,* 2012). The type of raw material used however, practically dictates the porosity of the activated carbon even though the activation method choice is also another parameter that can influence the final pore size distribution (Hameed *et al.,* 2008).

Two methods are applied for the activation of activated carbon, either physical or chemical activation. With the physical activation method, the carbonaceous precursor is carbonized first and then the resulting char is activated in the presence of some mildly oxidizing gases like carbon dioxide or steam (Zvinowanda *et al.,* 2008; Ali *et al.,* 2012; Ayanda *et al.,* 2013; Mohammad *et al.,* 2014). Chemical activation involves carbonization at a relatively low temperature (e.g. 400 – 700 ˚C) in the presence of a dehydrating agent (e.g. $ZnCl_2$, KOH, and H_3PO_4). During chemical activation both the carbonization and the activation step proceed simultaneously (Ayanda *et al.,* 2013).

2.15 Recovery and Detection of Antibiotics

Emerging chemical/biological contaminants have been released into the environment for quite some time. The analytical instrumentations available then could not achieve lower detection limits of as low as (parts per billion) ppb levels until recent modern analytical instruments became available (Nebot *et al.,* 2007). A wide range of wastewater treatment practices are employed worldwide to remove these compounds. However, their presence has often been reported in WWTP effluents at nanogram per litre (ng/L) concentrations.

Several studies made in the WWTPs have reported that the treatment plants are capable of eliminating around 60% of antibiotics (Batt *et al.,* 2006).

For most of the emerging environmental contaminants, there is hardly environmental survey data, and they are not regulated in the environment. Another reason for this is lack of analytical methods for proper risk assessment and for monitoring of waste, surface and drinking water quality (Simon & John, 2005). The analysis of these typically polar contaminants in environmental matrices (water, wastewater, soils, and sediments) is particularly challenging because low detection limits are required, the samples have a complex nature and there is difficulty in separating these compounds from interferences (Nikolaou *et al.,* 2007).

With the developments in analytical techniques, the analysis of polar compounds and compounds that have high molecular weight (>900 amu) can now be achieved by using liquid chromatographic methods. Several numbers of liquid chromatography (LC) methods have been proposed for the analysis of human pharmaceuticals in natural and wastewaters (Nebot *et al.,* 2007).
Due to the usually low concentrations and complexity of the matrices in which the target compounds are present in the aquatic environments, it is a difficult task to analyse antibiotics in the environment. These compounds are generally in complex water matrices and are present at trace levels making their analysis difficult. A very sensitive analytical method that is suitable for monitoring such analytes in the environment at such low levels needs to be developed.

New extraction and clean-up techniques, coupled with improvements in instrumental technologies provide the needed sensitivity and specificity for accurate measurement. Selective and quantitative extraction is paramount for accurate and sensitive detection in environmental samples (Vieno *et al.,* 2007). Usually the most common approach for the analysis of antibiotics in aquatic environment involves a pre-concentration step using the solid-phase-extraction (SPE) method and a liquid chromatographic separation method.
The SPE method is used for the enrichment and clean-up of aqueous samples and extraction from aqueous matrices. Such methods allow for the separation and qualitative and quantitative detection of the antibiotics or any pharmaceutical compounds with very low detection limits (Lin *et al.,* 2009).

The identification of pharmaceutical residues in environment is of special interest because knowledge of these compounds is a requirement to take measures in order to regulate and minimize their environmental impacts. Whether or not a chemical or other contaminant

becomes an emerging contaminant depends on the availability of analytical methods for its detection in the environment (Homem & Santos, 2011). The analysis of these typically polar contaminants in environmental matrices (water, wastewater, soils and sediments) is particularly challenging because of the low detection limits required, the complex nature of the sample, and difficulty in separating these compounds from interferences (Nebot *et al.,* 2007; Homem & Santos, 2011). Methods for analysis of natural and synthetic human pharmaceuticals continue to be developed and have been the subject of numerous investigations over the past years.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Chemical Reagents

All the chemicals used in this study are listed in Table 3.1 alongside with their supplier and grade.

Table 3.1: Chemical reagents used

Chemical	Supplier	Percentage Purity / Grade
Hydrochloric Acid	Sigma Aldrich	≥ 99 %
Sulphuric Acid	Merck Chemicals	≥ 99 %
Potassium Hydroxide	Sigma Aldrich	≥ 85 %
Sodium Hydroxide	Sigma Aldrich	≥ 90 %
Methanol	Sigma Aldrich	≥ 99.9 %
Amoxicillin	Duchefa Biochemie	> 95 %
Ampicillin	MMelford Biolaboratories Ltd.	Grade A
Chloramphenicol	Duchefa Biochemie	> 98 %
Milli-Q water	CPUT- Chemistry Laboratory	Grade A

3.2 Method Development

A robust and selective method that could be used to detect and quantify the antibiotics of interest (β-lactam antibiotics) was developed on an ultra-high-pressure liquid chromatography (UHPLC) coupled to the ultraviolet-visible-diode-array detectors (UV-DAD). Validation tests for the credibility of the method were performed and calibrations curves for all the analytes plotted. Several analytical parameters including mobile phase composition, mobile phase flow rate, wavelength and pump pressure were tested and optimized for the best resolution and detection of analytes in all analyses performed.

3.2.1 Preparation of Stock Solutions

Variable standard stock solutions for the analytes under study, i.e. β-lactam antibiotics, were prepared by accurately weighing 0.0500 g of each standard and dissolving it in 500 ml of Milli-Q water to give a molarity of 100 mg/L for each standard solution. All solutions were prepared in Milli-Q water obtained from the Millipore RiOs ™ instrument in the CPUT Chemistry laboratory. The working standard solutions for calibration of the instrument, which were in the range 5 – 50 mg/L were prepared by appropriate dilution of the standard stock solutions. Likewise, preparation of hydrochloric acid and sulphuric acid stock solutions were made by diluting the required calculated volume of the respective concentrated reagent into a 1 litre volumetric flask. For solid standards like potassium hydroxide and sodium hydroxide,

the respective calculated masses that would give the required molarity in a litre of Milli-Q water was weighed and dissolved to make a final volume of 1 litre. Standard solutions for antibiotics were prepared every 48 h to ensure precise results before possible degradation of the analytes.

3.2.3 Solid Phase Extraction and Clean-up

Solid-phase extraction (SPE) is a method applied for the pre-concentration of liquid analyte samples. The same method was applied for recovery studies that were conducted in order to assess the efficiency of the method and the SPE cartridges used. Recovery studies were performed by running variable standard solutions of the antibiotics through Oasis hydrophilic-lipophilic balance (HLB) cartridges. The HLB cartridges were first conditioned by running 6 ml of absolute methanol (MeOH) followed by 3 ml of Milli-Q water. After conditioning of the cartridges, 30 ml of the sample standard solutions were run through the cartridges at a flow rate of 5 ml/min. The retained standard analyte samples were eluted twice using 6 ml of MeOH. The extract was then further concentrated to dryness under a gentle stream of cold air and re-dissolved with 2 ml mixture of MeOH-water (50:50, v/v) (Reverte *et al.,* 2003; Abuin *et al.,* 2006; Peng *et al.,* 2008). Prior to analysis, all samples were filtered through 0.45 µm nylon fibre filters. The percentage of the recovered standard was determined by the result obtained from the HPLC analysis. The same recovery procedure was applied on the water samples from the river before analysis.

3.3 Sample Monitoring

Table 3.2: Description of sampling locations

Sampling point	Latitude Coordinate	Easting Coordinate	Site description
P1	-33.801359 S	18.535580 E	Upstream flow from Veld/farms
P2	-33.834589 S	18.522147 E	Stream flow at Table view
P3	-33.837625 S,	18.519621 E	Stream flow beyond Potsdam Wastewater treatment plant point of discharge
P4	-33.847279 S	18.512020 E	Industrial discharge
P5	-33.881853 S,	18.489755 E	Downstream (proximity to ocean; marine water intrusion: Biome)

Water was sampled along the drainage stretch of the Diep River, delimited between -33.801359 S and 18.535580 E and -33.881853 S and 18.489755 E into 2.5 L amber coloured glass bottles taken off the mainstream transect line. Five sampling points (P1 – P5) were identified, with their geo-reference coordinates (Table 3.2), with the sampling head stream at point P1.

3.3.1 Monitoring of Environmental Pollutants

Water samples were collected from the Diep River twice a month and levels of the antibiotics (AMX, AMP and CHLR) assessed. Surface water samples were collected from five different sampling points labelled as P1 to P5 respectively into clean 2.5 L amber coloured glass containers that were filled to the bottle neck. Each bottle was well labelled corresponding to the site from which the water was collected and rinsed with the site water prior to collection. The conductivity, pH, TDS and temperature of the water samples were recorded on site using a HANNA (HI 991300) pH Meter. All collected water samples were kept in an ice chest during transportation to the laboratory for analysis (Nikolaou *et al.,* 2007).

In the laboratory, the samples were filtered immediately on arrival using qualitative Whatman (150 mm) filter paper through vacuum filtration and kept in clean amber bottles in a refrigerator until extraction. The filtration process was done so as to remove any suspended matter in the water samples. The storage procedure employed could preserve the samples for a fortnight (Nikolaou *et al.,* 2007); however, effort was put to ensure sample preparation and analysis was done within 48 h after sample collection (Gulkowska *et al.,* 2008). All water samples were concentrated using the SPE method.

A mobile phase consisting of 40% methanol/water was prepared and degased before each analysis on the HPLC instrument. The instrument would first be purged after being switched on and the mobile phase allowed running through for approximately 30 min prior to sample analysis to rinse the pump cable as well as the separation column. The UV-detector was set to a wavelength of 210 nm (for AMX and AMP) and 254 nm (for CHLR) whilst the DAD-detector was set to a scan range wavelength of 210 – 290 nm. All samples were analysed following the procedures of the developed validated method and each test sample was performed in triplicate.

3.4 Preparation of Adsorbents

Grape slurry waste was collected from uncontaminated wine plants in Stellenbosch farms, South Africa. The biomass (grape slurry) was washed thoroughly with tap water several times until all dirt and inorganic impurities were removed. Distilled water was then used to rinse the biomass until it was clean, and subsequently the grape slurry was dried in the sun until it was dry. The dried biomass sample was ground, sieved into different particle sizes using mesh screens and stored in well labelled airtight containers at room temperature awaiting further experimentation (Li *et al.,* 2002; Gaspard *et al.,* 2006).

The carbonization procedure as described by Gaspard *et al.,* (2006) and Balasubramani & Sivarajasekar (2014) was optimized for temperature, nitrogen flow rate and the time of

carbonization. The biomass was carbonized successfully in an optimized inert environment at a temperature of 650 ^{o}C for 120 min and a nitrogen flow rate of 1 ml/min. Three portions approximately 60 g each, of the carbonized sample were taken and impregnated with solutions of KOH, HCl and H_2SO_4 (activator to precursor ration of 2:1 w/w).

The carbonized samples were soaked in the respective activating reagents for 48 h. After 48 h the activated biomass was then filtered and rinsed with distilled water to remove any excess reagent until the pH was near neutral. After sufficient rinsing of the active sorbent samples, they were oven dried at 100 ^{o}C for 24 h. The pre-treated charred biomass was stored in well labelled air-tight bottles at room temperature (Li *et al.,* 2002; Gaspard *et al.,* 2006).The carbonized activated materials were labelled GSAa, GSAb and GSB for activation using HCl, H_2SO_4 and KOH respectively and the un-carbonized, un-activated biomass was labelled GS.

3.5 Characterization of Adsorbents

The prepared adsorbents were characterized using physical methods as well as instrumental methods. The physical characterization method included determination of the adsorbents' moisture content, ash content and pH. The instrumental characterization methods involved analysis of the adsorbent using scanning electron microscope (SEM), chemical elemental analysis using the energy dispersive X-ray (EDX) and Fourier-transform infra-red spectroscopy (FTIR).

3.5.1 pH Determination of Adsorbents

A similar procedure to one described by Ademiluyi et al. (2009) was applied to determine the pH of each of the adsorbents. 0.1 g of each sample was dissolved in 50 ml of distilled water and the solutions were brought to the boil in conical flasks for 5 min. Samples were allowed to cool to room temperature and then filtered. The pH of the filtrates was determined using a CRISON pH-Meter (GLP 21+, USA) in the laboratory.

3.5.2 Moisture Content of Adsorbents

Initial masses of the ceramic crucibles were measured accurately and recorded using an analytical balance (FA2004 Electronic Balance, Germany). The moisture content of each adsorbent was determined by weighing approximately 0.1 g of each sample into a pre-weighed crucible and placing it in an oven at 110 ^{o}C for 3 h (Ayanda *et al.,* 2012). Samples were placed in a desiccator, cooled to room temperature and masses recorded accurately. The procedure was repeated until a constant mass was obtained and samples were analysed in triplicates. The percentage moisture content was calculated using equation 3.1

$$\% \text{ moisture} = \frac{M1-M2}{M1} \times 100 \quad [3.1]$$

where M_1 is the mass (g) of the original sample and M_2 is the mass (g) of the final dried sample (g)

3.5.3 Ash Content of Adsorbents

An approximate mass of 0.1 g of each sample was weighed into platinum crucibles and placed in a furnace. The furnace was set to a temperature of 500 ^{o}C. Samples were heated at 500 ^{o}C for 4 h, cooled in a desiccator and re-weighed. Experiments were carried out in triplicates. The percentage ash content was calculated by difference in the masses using equation 3.2 (Fernando *et al.,* 2009; Chowdury *et al.,* 2013)

$$\% \text{ ash content} = \frac{W2}{W1} \times 100 \quad [3.2]$$

where W_1 is the mass (g) of original sample weighed before ashing and W_2 is the mass (g) of residue (g) after ashing.

3.5.4 SEM Analysis

The scanning electron microscope instrument (Zeiss EVO® MA15, Scanning Electron Micro analyser) from the University of the Western Cape operated at 10 – 25 kV was used to study the morphologies of GSAa, GSAb, GSB and GS respectively, both before and after adsorption experiments. In the sample preparation for SEM analysis, a thin layer of adhesives serving as carbon glue was attached onto a stub, and very little amount of the samples to be analysed were spread on the stub materials and subsequently viewed on the instrument to obtain micrographs.

3.5.5 EDX Analysis

Elemental analysis together with the phase compositions of adsorbents were conducted using the same SEM analysis instrument described in section 3.5.4 (Zeiss EVO® MA15 Scanning Electron Micro analyser). Qualitative analysis was achieved by the application of secondary electron images onto gold coated samples, whilst quantitative analysis was performed via energy dispersive X-ray (EDX) spectroscopy using an Oxford Instruments® X-Max 20mm^2 detector and Oxford INCA software. The gold coating on samples to be analysed was employed to improve conductivity as well as achieving a flat surface of the sample. As a quality assurance protocol, pure cobalt was used periodically to correct any slight or major detector drifts.

3.5.6 FTIR Analysis

The active functional groups on the adsorbents (GSAa, GSAb, GSB and GS respectively) were analysed by studying the infra-red spectra of the respective adsorbents using an FTIR spectrophotometer (Perkin-Elmer Spectrum, UK).

3.6 Adsorption Experiments

Sorption capacities of the adsorbents GSAa, GSAb, GSB and GS towards the antibiotics (AMX, AMP and CHLR) from solutions were studied through batch sorption experiments. Sorption efficiencies of the adsorbents were assessed using simulated wastewaters. The adsorbates were prepared in Milli-Q water by spiking with 10 ml of 30 µg/L AMX and 40 µg/L of AMP and CHLR respectively.

3.6.1 Batch Sorption Experiment

Batch sorption experiments on β-lactam antibiotics were conducted in 100 ml Erlenmeyer flasks containing 25 ml of AMX (30 µg/L), AMP (40 µg/L) and CHLR (40 µg/L). Required masses of GSAa, GSAb, GSB and GS were added to the respective solutions. The flasks were allowed to equilibrate on a mechanical shaker (Labotec OrbiShake, Germany) at 120 rpm for the required set time. The resulting aqueous solutions after equilibration were filtered and processed through SPE cartridges. Residual concentrations of the antibiotics in solution after the experimental processes were measured using the HPLC-UV-DAD. The quantities of the adsorbates removed by the respective adsorbents (q, mg/g) as well as percentage sorption capacity (% A) were calculated using equations 3.3 – 3.5

$$q_e = \frac{(Co - Ce) \times V}{m} \quad [3.3]$$

$$q_t = \frac{(Co - Ct) \times V}{m} \quad [3.4]$$

$$\% A = \frac{(C0 - Ce)}{C0} \times 100 \quad [3.5]$$

where q_e and q_t represents the amount (mgg^{-1}) of antibiotic adsorbed at equilibrium and at a time *t* respectively, *V* is the volume (L) of the solution, C_0, C_e and C_t are the initial concentration, equilibrium concentration and final concentration at time *t* (mgL^{-1}) of the solutions respectively and *m* is the mass (g) of adsorbent added (Hameed & Rahman, 2008). All adsorption experiments were carried out in triplicates.

3.6.2 Kinetic Studies

3.6.2.1 Contact Time

The effect of contact time on the removal of antibiotics by the adsorbent was determined by keeping the initial concentration, adsorbent dosage, pH and temperature of the reaction chamber constant. A fixed mass of the adsorbents (0.1 g) was added to 25 ml of the respective adsorbate solutions in 100 ml Erlenmeyer flasks and shaken on a mechanical shaker for different time intervals (i.e. 30 , 60, 90 min, etc) at a constant temperature (23 ^{o}C). After a specified time for each reaction, the mixtures were filtered. The resulting final concentration of the adsorbates in solution after a certain pre-set time was then determined using the HPLC-UV-DAD. All studies were carried out in triplicates.

3.6.2.2 Kinetic Models

Data obtained from the results of effect of contact time on the adsorption efficiency of the adsorbents for AMX, AMP and CHLR was assessed to see which kinetic model(s) better described the rate or mechanism of the sorption processes. Three kinetic models were employed namely the pseudo-first order, pseudo-second order and the Elovich kinetic model. After fitting the data into the various kinetic models, the results obtained were used to determine the kinetic model(s) that better describes reactions rate.

3.6.2.2.1 Pseudo-first order model

Lagergren's first order rate equation was the earliest to be known and used to describe the adsorption rate, based on the adsorption capacity (Ho *et al.,* 1995). Thus the pseudo-first order model is amongst the widely used models for the adsorption of a solute(s) from a liquid solution. The linearized form of a pseudo-first order reaction is presented in equation 3.6:

$$\log (q_e - q_t) = \log (q_e) - k_1 t / 2.303 \qquad [3.6]$$

where q_e is the amount of antibiotics adsorbed at equilibrium (mgg^{-1}), q_t is the amount (mgg^{-1}) of antibiotics adsorbed at a time *t*, and k_1 is the kinetic rate constant (min^{-1}) of pseudo-first order kinetics (Zhang *et al.,* 2012). According to Fierro *et al.* (2008), the rate constant, k_1, and the correlation coefficients (R^2) of the antibiotics under different concentrations would be obtained from the linear plots of log ($q_e - q_t$) versus *t*, which is expected to give a linear plot.

3.6.2.2.2 Pseudo-second order model

A second-order model for the sorption of adsorbate particles onto the adsorbent is based on differentiating the kinetics of a second-order rate expression with respect to the adsorption capacity of the adsorbents (Munagapati & Kim, 2017). This largely relies on the adsorbent dosage (concentration) and on the solute concentration which is represented by a pseudo-

second-order rate expression (Subha & Namasivayam, 2008). The simplest linear expression of the pseudo-second order equation may be written as:

$$t/q_t = 1/(k_2 q_e^2) + t/q_e \quad [3.7]$$

where k_2 is the rate constant ($g.mg^{-1}.min^{-1}$) of the pseudo-second order. If equation 3.7 applies for the adsorption process of the antibiotics, then q_e and k_2 can be determined from the slope and intercept of the plot t/q_t versus *t*, respectively (Zhang *et al.,* 2012).

3.6.2.2.3 Elovich's equation

The Elovich's equation is used to describe activated adsorption. This equation is known to be established through the work of Zeldowitsch who investigated the adsorption of carbon monoxide onto manganese dioxide (Fierro *et al.,* 2008; Idris *et al.,* 2011). The Elovich equation has been successfully applied in describing the adsorption of pollutants from aqueous solutions, and is expressed in its simplest form as:

$$q_t = (1/B) \cdot \ln (A_\varepsilon B) + (1/B) \cdot \ln (t) \quad [3.8]$$

where qt is the amount (mgg^{-1}) of adsorbed antibiotic at a time *t*, A_ε is the initial adsorption rate ($mgg^{-1}min^{-1}$) and B is the desorption constant (gmg^{-1}) during any experiment (Ho & McKay, 2002). Plotting q_t as a function of ln *t* is expected to give a linear plot if this equation subsists.

3.6.3 Effect of adsorbent dosage

The batch equilibration method was applied to assess the effect of adsorbent dose on antibiotics removal from simulated wastewater. Various doses of the adsorbent, (0.1 – 0.4 g) were added to 25 ml of the antibiotic solutions (30 µg/L AMX and 40 µg/L of AMP and CHLR respectively). The solutions were agitated on a mechanical shaker at 120 rpm and room temperature (23 ^oC), neutral pH and variable equilibrium times for the respective adsorbates. The final concentrations of the antibiotic solutions were determined as described previously using the HPLC-UV-DAD. All test samples were performed in triplicates.

3.6.4 Equilibrium Studies

3.6.4.1 Effect of Initial Concentration

Batch equilibrium experiments were carried out in triplicates to determine the effect of initial solution concentration on the adsorption capacity of the adsorbents. The initial concentrations studied for the antibiotic solutions ranged from 30 mg/L to 100 mg/L. A constant mass (0.1 g) of the different adsorbents was added to 25 ml solutions of the

respective adsorbates in a 100 ml Erlenmeyer flask, and temperature, pH and rotation speed kept constant. At equilibrium, the solution mixtures were filtered and analysed using HPLC-UV-DAD. The results obtained were used to determine the equilibrium adsorbed quantity (q_e) by the adsorbent and the data fitted into sorption isotherm models (Langmuir, Freundlich and Temkin isotherms).

3.6.4.2 Adsorption isotherms

3.6.4.2.1 Langmuir Isotherm

This adsorption model is based on the assumption that there exists a maximum limiting uptake which corresponds to a saturated monolayer of adsorbate molecules at the surface of the adsorbent. This simply means that maximum adsorption occurs implying a saturated monolayer of solute molecules on the surface of the adsorbent, with little or no lateral interaction between the sorbed molecules. The Langmuir isotherm model, therefore assumes that all the adsorption sites have the same activation energy (Gong *et al.,* 2005; Hameed *et al.,* 2008). The simplest form of the Langmuir equation is thus

$$C_e/q_e = [1 / (q_M.k_L)] + C_e/q_M \quad [3.9]$$

where C_e is the equilibrium concentration (mgL^{-1}), q_e is the adsorption capacity at equilibrium time (mgg^{-1}), q_M is the maximum adsorption capacity (mgg^{-1}), k_L is the Langmuir constant that is related to adsorption capacity (mgg^{-1}) (Fan *et al.,* 2017).

This model considers several assumptions including that the adsorption is localized, all the active sites on the surface have similar energies, there is no interaction that exists between the adsorbed molecules and that the surface reaction is the limiting reaction step as in the heterogeneous catalytic reaction. The dimensionless equilibrium parameter, R_L, can be used to predict the efficiency of the adsorption process. R_L, is defined by the equation

$$R_L = \frac{1}{1+ \beta.Co} \quad [3.10]$$

where C_0 is the initial concentration of antibiotics in the solution (mgL^{-1}). When $R_L = 0$, the adsorption is considered irreversible, when $0 < R_L < 1$ then adsorption is favourable, when $R_L = 1$ adsorption is linear and it is unfavourable when $R_L > 1$ (Fierro *et al.,* 2008).

3.6.4.2.2 Freundlich Isotherm

The Freundlich isotherm is applied to incorporate the effect of heterogeneous surface energy or rather describe heterogeneous systems. It assumes that adsorption occurs on a heterogeneous surface through a multilayer mechanism of adsorption (Al-Qodah &

Shawabkah, 2009; Dada *et al.,* 2012), and that the adsorbed quantity increases with an increase in concentration as per the equation:

$$\log q_e = \log K_F + (1/N_F) \log C_e \quad [3.11]$$

where K_F is the Freundlich constant (Lg^{-1}) related to the bonding energy. Zhang *et al.,* 2012 reported that K_F can be defined as the adsorption or distribution coefficient and represents the quantity of antibiotics adsorbed onto adsorbent at unit equilibrium concentration, and N_F (dimensionless) is the adsorption intensity or heterogeneity factor. The Freundlich constant N_F gives an indication of how well the adsorption process is favoured. It is a measure of the deviation from linearity of adsorption, which indicates when adsorption process is linear (N_F=1), chemical (N_F<1) or physical (N_F>1) (Kumar *et al.,* 2010; Pezoti *et al.,* 2016).

3.6.4.2.3 Temkin Isotherm

This model assumes that the heat of adsorption of all molecules in the layer would linearly decrease with the surface coverage as a result of the existence of adsorbate-adsorbate interactions as suggested by Temkin and Pyzhev (Hameed *et al.,* 2008; Fan *et al.,* 2017). The Temkin isotherm model mainly describes the chemical adsorption process as electrostatic interaction (Leng *et al.,* 2015; Fan *et al.,* 2017). The Temkin isotherm can thus be adjusted by the equation

$$q_e = B. \ln k_{TEM} + B. \ln C_e \quad [3.12]$$

where B is a constant related to the heat of adsorption and it is defined by the expression $B = RT/b$, b is the variation of the adsorption energy, R is the universal gas constant (8.314 kJ/mol), T is the temperature (K), k_{TEM} is the equilibrium binding constant corresponding to the maximum binding energy and C_e is the equilibrium concentration (mgL^{-1}) (Munagapati & Kim, 2017).

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According to Fierro *et al.* (2008), the single major difference between these three models is the way the heat of adsorption decreases with the surface coverage; Langmuir assumes no decrease at all; Freundlich assumes a logarithmic decrease, while Temkin assumes a linear decrease.

3.6.5 Effect of pH

The effect of pH on adsorption was tested by varying the solutions' pH. Adsorption experiments were carried out at pH 4, 6, 8, 10 and 12. The various pHs of antibiotic solutions were adjusted using the required amount of 0.1 M HCl or 0.1 M NaOH solutions prior to

addition of the adsorbent. Adsorption parameters including adsorbate concentration, temperature, contact time and adsorbent dosage were kept constant during the experiments which were done in triplicates. A portable pH meter (CRISON pH-Meter GLP 21+) was used to determine the pH values of the solutions before addition of adsorbents. The final residual concentrations of the adsorbates in solution were determined using the HPLC-UV-DAD.

3.6.6 Effect of Temperature

A constant mass (0.3 g) for each adsorbent was weighed into 100 ml Erlenmeyer flasks and 25 ml solutions of the respective adsorbates (30 µg/L AMX and 40µg/L AMP and CHLR respectively) added. Temperatures were varied from 20 ^{o}C – 60 ^{o}C in 30 min intervals until equilibrium was reached. After equilibration, the solutions were filtered and analysed by the HPLC-UV-DAD. All experiments were performed in triplicates. Data obtained from this study were utilised to evaluate thermodynamic parameters of the sorption system. The thermodynamic parameters of concern in this study, the enthalpy change (ΔH^0), entropy change (ΔS^0) and the Gibbs free energy change (ΔG^0) were calculated using equations 3.13 – 3.15

$$\Delta G = - RT \ln K_c \quad [3.13]$$

$$\ln K_c = C_a/C_e \quad [3.14]$$

$$\ln K_c = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} \quad [3.15]$$

where R is the gas constant (8.314 $JK^{-1}mol^{-1}$), T is the absolute temperature (K), K_c is the equilibrium constant, C_a is the equilibrium concentration of adsorbates adsorbed onto specific adsorbents (mgL^{-1}), C_e is the equilibrium concentration of adsorbate in solution (mgL^{-1}), ΔH ($kJmol^{-1}$) and ΔS ($JK^{-1}mol^{-1}$) were obtained from the slope and intercept of the van't Hoff plot of ln K_c against 1/T. Also the changes in the ΔH, ΔS and ΔG were used to speculate on the adsorption mechanism (Mitchell *et al.,* 2014).

3.7 Column Study

Column studies enhanced the understanding of adsorption using carbonized biomaterials and they provided greater information for potential application in industry (Biswas & Mishra, 2015). Fixed bed columns were used; a fixed amount of adsorbent was exposed to a continuous flow of adsorbate solutions.

A glass tube (15.0 cm long, internal diameter of 0.5 cm) was packed with 4.50 g of GSB. Cotton wool was fitted at the bottom and the top of the column of the adsorbent to prevent

flotation of the adsorbent. A total volume of 200 ml of the respective feed solutions consisting of 30 mg/L of the respective adsorbates (AMX, AMP and CHLR) were passed through three separate columns and studied for adsorption. The feed solutions were passed through the packed columns maintained at a flow rate of 2 ml/min using a peristaltic pump. The eluents from the column were collected after every 10 min intervals. The eluents were processed through the SPE columns and analysed with the HPLC-UV-DAD.

CHAPTER FOUR

4.0 RESULTS and DISCUSSION

4.1 Optimization of High Performance Liquid Chromatographic (HPLC) Method

A method for the separation and quantitation of three β-lactam antibiotics - amoxicillin (AMX), ampicillin (AMP) and chloramphenicol (CHLR) was developed based on an Ultra-High Pressure Liquid Chromatograph (UHPLC) coupled to an Ultraviolet-Visible-Diode Array Detector (UV-DAD), and validated following the international conference of harmonization (ICH) procedures/principles. These include triplicate runs of 5-set calibration standards for each analyte, plots of calibration curves, determining the equations for the linear regression lines from the calibration curves, determination of the limit of detection (LOD) and limit of quantification (LOQ) for each analyte, evaluation of the reproducibility and repeatability of method selectivity and sensitivity for each analyte.

Figure 4.1 presents the chromatogram of the mixed standards containing the three antibiotics - AMX, AMP and CHLR.

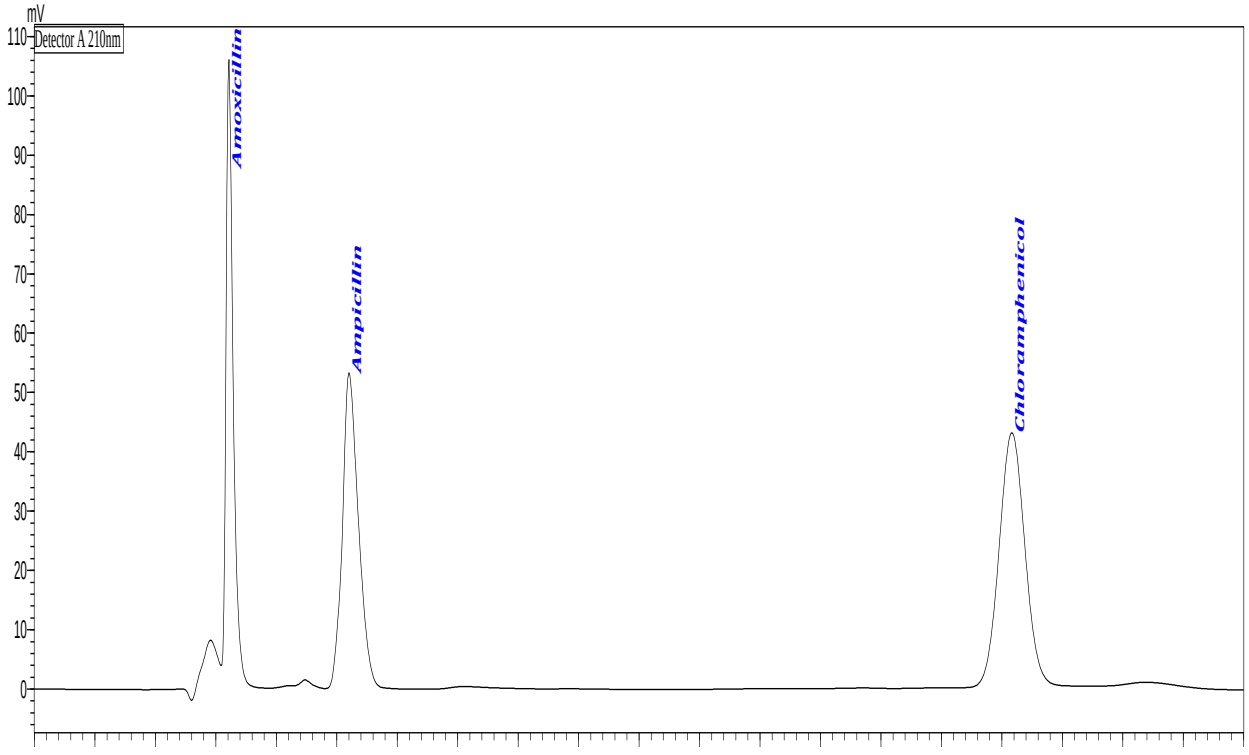

Figure 4.1: Chromatogram of AMX, AMP and CHLR

The instrument response to the detection of each of the β-lactam antibiotics showed linear sensitivity to increasing concentrations with R^2 values > 0.999 for all three analytes (Table 4.1). The method's LOQ and LOD values for each antibiotic were statistically calculated.

Table 4.1: Linear regression parameters for AMX, AMP and CHLR standards

Standard(s)	Linear Regression equation	Retention time (min)	R^2	λ_{max}	LOQ mg/L	LOD mg/L
AMX	y = 21347x + 21709	1.607	0.9999	210nm	0.286	0.857
AMP	y = 11949x – 12397	2.601	0.9998	210nm	0.195	0.584
CHLR	y = 14707x + 8589	8.083	0.9998	254nm	0.224	0.673

4.1.1 Recovery studies for AMX, AMP and CHLR using Solid Phase Extraction (SPE)

The efficiencies of the quantitative recovery of the β-lactam antibiotics (AMX, AMP and CHLR) using the SPE method were evaluated using data from recovery experiments. The analytes were recovered in triplicate from solutions of 20 mg/L, 50 mg/L and 100 mg/L analyte cocktail standards in MilliQ water. The relative percentage recovery of the antibiotics ranged between 95 – 102% for AMX; 88 – 99% for AMP and 93 – 103% for CHLR (Table 4.2)

Table 4.2: Mean recovery percentages for AMX, AMP and CHLR by the SPE method

Standards	Expected concentration (mg/L)	Measured concentration (mg/L)	RSD (%)	Average recovery (%)
AMX	20	18.93	3.31	94.65
	50	49.01	1.89	98.02
	100	102.03	1.06	102.03
AMP	20	17.58	1.27	87.90
	50	48.64	0.63	97.28
	100	99.46	0.48	99.46
CHLR	20	18.54	1.39	92.70
	50	49.88	0.27	99.76
	100	103.36	0.92	103.36

The results showed that, the measure of the affinity efficiency of the HLB cartridges to concentrate the tested antibiotics was high. This suggests that the use of HLB cartridges in solid phase extraction procedure is efficient and satisfactory for the concentration and recoveries of the selected β-lactams from aqueous matrices.

4.1.2 Surface Water Monitoring

4.1.2.1 Physico-chemical characteristics of the selected water bodies

The pH of the Diep River surface water at the different sampling points between sampling station P1 and the downstream sampling station, P5, were generally about circum-neutral and ranged from 7.01 to 7.76 during winter, and from 7.02 to 7.81 in spring (Table 4.3).

The turbidity of the water varied giving a range of 857 – 1839 ppm in winter and 809 – 1896 ppm during spring. High values of total dissolved solids (TDS) in the surface water (> 1000 ppm) were observed at sampling stations P1, P2 and P5 respectively as shown in Table 4.3. The measured levels of TDS in both seasons (winter and spring) did not show any significant difference from each other ($\rho>0.05$).

Table 4.3: Physico-chemical Characteristics of the Diep River surface water in Winter and Spring months of 2016

	pH		TDS (ppm)		Conductivity (µS/cm)		Temperature (°C)	
	Winter	summer	winter	summer	winter	summer	winter	summer
P1	7.41	7.63	1323	1289	2648	2634	13	15
P2	7.01	7.27	1191	1089	2408	2373	14	16
P3	7.06	7.02	903	809	1850	1827	14	19
P4	7.76	7.81	857	869	1720	1727	15	24
P5	7.05	7.21	1839	1896	3833	2518	18	23

The conductivity of the surface water along the Diep River between sampling stations P1 and P5 also varied slightly (1720 – 3833 µS/cm) during winter and (1727 – 2634 µS/cm) during spring. The highest conductivity level (3833 µS/cm), was observed during winter downstream of the Diep River (P5). In contrast, the highest level observed during spring (2634 µS/cm) was at the head stream of the surface water (P1).

The sampling station P5 generally had higher temperature, TDS and conductivity values relative to other stations, while sampling station P4 had the highest pH values. In general, aside from pH values, the physico-chemical data of sampling station P5 showed higher values. This may not be unconnected to the consequential proximal effect of the downstream station water interaction, with marine water intrusions from the ocean.

Levels of the three antibiotics (AMX, AMP and CHLR) studied were variable in the different sampling sites (P1 – P5) of the Diep River. The values were found to be low and in most cases, below detection limit for AMP and CHLR.

AMX was the most frequently detected analyte in all the surface water samples. The levels detected during the winter season in sampling station P1 ranged < DL – 5.84 ± 0.08 µg/L; sampling station P2, 3.50 ± 0.52 – 4.91 ± 0.24 µg/L; sampling station P3, < DL – 9.13 ± 0.13 µg/L; sampling station P4, 1.91 ± 0.42 – 5.60 ± 0.31 µg/L and sampling station P5, <DL – 13.29 ± 0.14 µg/L (Table 4.4). During the spring season the concentration levels of AMX detected from sampling station P1 ranged 0.60 ± 0.32 – 1.95 ± 0.23 µg/L; sampling station P2, 7.71 ± 0.02 – 11.40 ± 0.12 µg/L; sampling station P3, < DL – 7.82 ± 0.16 µg/L; sampling

station P4, 4.09 ± 0.13 – 4.99 ± 0.23 µg/L and at sampling station P5, < DL – 11.55 ± 0.04 µg/L (Table 4.4).

Table 4.4: Levels of Amoxicillin in the Diep River surface water in Winter and Spring months of 2016 (value ± SD) µg/L

Winter					Spring				
P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
<DL	3.53± 0.02	<DL	5.44±0.05	13.29±0.14	1.89±0.12	10.43±0.13	7.82±0.16	4.61±0.23	<DL
2.44± 0.13	4.91±0.24	7.68±0.18	4.12±0.18	6.17±0.07	1.95±0.23	11.40±0.12	6.03±0.10	4.09±0.13	9.55±0.13
5.65± 0.03	3.50±0.52	9.13±0.13	5.60±0.31	9.87±0.12	0.60±0.32	7.71±0.02	<DL	4.50±0.16	11.55±0.04
5.84± 0.08	4.71±0.13	4.73±0.26	1.91±0.42	<DL	1.14±0.17	7.76±0.28	2.45±0.06	4.99±0.23	10.92+0.07

The observed levels may be attributed to the nature and characteristics of the surface water at the different sampling stations. The occurrence of AMX in nearly all the surface water samples may be connected to discharges from domestic and industrial sources as well as wastewater treatment plant effluent into the Diep River. Also, the high levels observed at P5 may be the result of the accumulation of residues on the lower drainage course of the river.

AMP was detected in samples collected from station P5 only during the two seasons, at a concentration range of < DL – 1.24 ± 0.24 µg/L and 1.93 ± 0.28 – 5.27 ± 0.06 µg/L in winter and spring respectively (Table 4.5), while the concentrations were below detection limit in all other surface water samples.

Table 4.5: Levels of Ampicillin in the Diep River surface water in Winter and Spring months of 2016 (value ± SD) ug/L

Winter					Spring				
P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
<DL	<DL	<DL	<DL	0.38±0.13	<DL	<DL	<DL	<DL	1.93±0.28
<DL	<DL	<DL	<DL	1.18±0.08	<DL	<DL	<DL	<DL	3.12±0.23
<DL	<DL	<DL	<DL	1.24±0.24	<DL	<DL	<DL	<DL	4.90±0.21
<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	5.27±0.06

The levels detected at P5 were not unexpected as the sampling station is located by the lower drainage into a marine water intrusion biome zone, thus an accumulation of AMP deposition from upstream.

CHLR was however not detected in any of the surface water samples analysed (Table 4.6). The reason for the non-detection of CHLR in the monitored water is not very clear. Some reports have suggested that CHLR easily degrade via hydrolysis in water (Huang & Sedlak, 2001; Nie *et al.,* 2014) at pH values in the range 2 - 8. Degradation by-products of the

antibiotic include p-nitrobenzaldehyde, arylamine or 2-amino-1-(4-nitrophenyl)propane-1,3-diol (Nie *et al.,* 2014). Degradation is therefore a possibility, since the pH of the surface water samples ranged between 7.01 and 7.81 across all sampling stations.

Table 4.6: Levels of Chloramphenicol in the Diep River surface water in Winter and Spring months of 2016 (value ± SD) ug/L

Winter					Spring				
P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL

The concentration of antibiotics is generally not expected to be high in surface waters. This is because they are known to be prone to hydrolytic degradation over a period of time (Hou & Poole 1969; Huang *et al.,* 2001; Nie *et al.,* 2014). Huang *et al.* (2001) stated that β-lactams mainly undergo hydrolysis fairly quickly under mild conditions. Also there are strong tendencies of the cleavages of the β-lactam rings, resulting in the absence of the parent compounds (Kummerer, 2009). The results of a research done by Mitchell *et al.* (2014) demonstrated that β-lactam antibiotics hydrolyse under ambient pH and temperature conditions. Degradation of β-lactam antibiotics will likely occur over several weeks in most surface waters and over several days in more alkaline systems.

Generally, among all the three β-lactam antibiotics investigated, AMX was found to be the most widespread in occurrence and distribution. Levels of occurrence were in the order AMX > AMP > CHLR. Since β-lactam antibiotics are degraded in the environment (Deschamps *et al.,* 2012; Braschi *et al.,* 2013), it is most likely that the degradation products of these compounds will be persistent in the environment.

Despite the fact that β-lactam antibiotics account for the highest proportion of antibiotics consumption, the release and occurrence of β-lactams in surface waters has not been fully reported (Christian *et al.,* 2003; Kummerer, 2009). A number of research studies on the presence of β-lactams in wastewater have reported that the occurrence of these compounds in treated wastewater samples, generally ranged between non-detection and low concentrations at µg/L levels (Christian *et al.,* 2003; Kummerer, 2009; Deschamps *et al.,* 2012), in spite of the fact that they are among the most commonly prescribed antibiotics in most parts of the world (Hirsch *et al.,* 1999; Le-Minh *et al.,* 2010).

4.2 Characterization of adsorbents produced from grape slurry waste for remediation studies

The grape slurry was carbonized and activated with HCl, H_2SO_4 and KOH to produce GSAa, GSAb and GSB respectively. The un-carbonized and un-activated grape slurry biomass was labelled GS. The four materials were characterized using methods previously described in Chapter 3, Section 3.5.

4.2.1 Moisture Content

The relative percentages of moisture content for the different biomasses used as adsorbents are presented in Table 4.7. The moisture content of the carbonized biomasses activated with acids and alkali (GSAa, GSAb and GSB respectively) were lower than that of the precursor sample (GS) with moisture content of 23.38 %. Adsorbents with low moisture content are often desirable due to the fact that low moisture content facilitates the adsorption process and also improves the adsorption efficiency. The carbonization process probably resulted in approximately 50 % loss of the adsorbent water content with respect to GS, and also it reduced the capacity of the adsorbent to retain moisture.

Table 4.7: Percentage moisture content of GSAa, GSAb, GSB and GS

Adsorbent	% Moisture content
GSAa	10.48
GSAb	9.72
GSB	13.28
GS	23.38

4.2.2 Inorganic Matter

The different biomass adsorbents were heated to a temperature of 500 ^{o}C for 4 h. The ash contents of the adsorbents are presented in Table 4.8. The low ash content for the carbonized adsorbents is indicative of the relative percentage proximate composition of the organic matter contents for GSAa, GSAb, GSB and GS, which were 93.25 %, 98.01 %, 92.68 % and 77.40 %, respectively. The un-carbonized and inactivated adsorbent (GS) had the highest ash content, whilst that of the carbonized adsorbents (GSAa, GSAb, and GSB) were lower than 10 %. This result is consistent with the findings of Al-Qodah & Shawabkah (2009); Ayanda *et al.* (2012) and Fatoki *et al.* (2012), who reported low percentages of ash content in different activated carbonized adsorbents. The low ash content of the carbonized adsorbents may be attributed to the conversion of the inorganic matter content to organic during carbonization, hence, reduction in inorganic matter and consequent increase in the carbon content of the carbonized grape slurry compared to the un-carbonized one.

Table 4.8: Percentage ash content of GSAa, GSAb, GSB and GS

Adsorbent	% Ash content
GSAa	6.75
GSAb	1.99
GSB	7.32
GS	22.60

4.2.3 pH of Adsorbents

The pH of the carbonized and un-carbonized adsorbents appeared to be influenced by the pH of the reagent used for activation. The results presented in Table 4.9 indicate that adsorbents that were activated in acidic media (GSAa and GSAb) had acidic pH values in contrast to alkaline modified and unmodified materials (GSB and GS respectively). The pH range for the four adsorbents (GSAa, GSAb, GSB and GS) was between 4 and 10. This is expected, since adsorbents activated in acidic medium are expected to achieve a final pH in the acidic range whilst those activated in alkaline medium are expected to achieve a basic pH.

Adsorbents with a final pH within the acidic range are considered to have a net positively charged surface due to an excess of H^+ ions on the surface of the adsorbents. This implies that they may effectively remove negatively charged ionic molecules/compounds viz-a-viz electrostatic attraction from solution. Conversely, the basic adsorbents are expected to have net negatively charged surface due to the excess OH^- ions on its surface, hence they are expected to be more electrostatically selective towards the positively charged ionic molecules or compounds (Kosmulski & Saneluta, 2004). However, the un-carbonized adsorbent, GS showed a pH value near neutral (pH of 6.61).

Table 4.9: pH values of the adsorbents

Adsorbent	pH
GSAa	5.27
GSAb	4.43
GSB	9.17
GS	6.61

4.2.4 Energy Dispersive X-Ray (EDX) Analysis

Elemental analysis and quantitation of the different adsorbents (GSAa, GSAb, GSB) as well as the un-carbonized/unmodified adsorbent (GS) were carried out using an EDX technique. The results obtained for the elemental composition of the adsorbents are presented in Table 4.10.

Table 4.10: Percentage elemental composition in adsorbents

	C	O	Na	Mg	Al	Si	P	S	Cl	K	Ca	Fe
GSAa	89.11	9.04	0	0.23	0	0	0	0	1.12	0	0.50	0
GSAb	85.17	12.30	0	0.23	0.28	0.85	0.15	0.30	0.12	0.19	0.43	0
GSB	82.40	15.62	0	0.18	0	0	0	0	0	1.39	0.42	0
GS	56.56	33.93	0.39	0.31	0.41	0.70	0.06	0	0	6.90	0.59	0.15

All of the adsorbents essentially contained carbon and oxygen in different proportions. Carbon content ranged between 89.11 % and 56.56 %. Corresponding values for oxygen ranged between 9.04 % and 33.93 % with GSAa having the lowest oxygen content and GS the highest. All other elements occurred at trace levels. The adsorbent GSAa contained the highest carbon content (89.11 %) and the least oxygen content (9.04 %), while adsorbent GS contained the least carbon content (56.56 %) and the highest oxygen composition (33.93 %). This showed that the process of carbonization and activation of the grape slurry resulted in improved and increased carbon content of the modified adsorbents, relative to that of the un-carbonized and un-modified adsorbent.

4.2.5 Fourier Transform Infra-Red Spectroscopy (FT-IR) Analysis

Fourier transform infra-red spectroscopy (FTIR) was used to elucidate the prominent functional groups on the four adsorbents (GSAa, GSAb, GSB and GS) tested in sorption experiments. This is because, the carbonization and chemical activation of an adsorbent material using different modifying agents have been reported to produce different effects in the functionality of the material, with possible significant differences in the FTIR absorption spectra line patterns (Stuart, 2004). An understanding of the characteristic surface functionalities of the adsorbents may provide insight on the mechanisms of sorption, such as physisorption or chemisorption that have been suggested as responsible for adsorbate removal.

The infra-red spectroscopy results showing the prominent functional groups on the surfaces of GSAa, GSAb, GSB and GS are presented in Figures 4.2 to 4.5. Peak fitting of the wavelength of absorption on the FTIR spectra of the adsorbents was done by cross match linking the spectra obtained for each adsorbent with characteristic absorption wavelength bands of functional groups reported in literature (Mozgawa *et al.,* 2004; Mozgawa *et al.,* 2005; Mozgawa *et al.,* 2011; Xie *et al.,* 2012).

Five prominent wavelengths of absorption spectra lines of absorption were observed in the FTIR spectrum of GS. The distinct adsorption lines were noted at 2920.77 cm^{-1}, 1818.44 cm^{-1}, 1032.56 cm^{-1} and 426.19 cm^{-1} and 405.09 cm^{-1}. Peak assignment/fitting suggests that the

wavelength of absorption at 2920.77 cm^{-1} represents asymmetric C–H bonds bending vibrations of methyl group, and the symmetric CH_2 bonds bending vibration of methylene groups (Peak *et al.,* 2003; Roggo *et al.,* 2007; Wahab *et al.,* 2012). Wavelength band observed at 1818.44 cm^{-1} represents a vibration stretching for C=O functional group. The sharp adsorption band at 1032.56 cm^{-1} might be characteristic asymmetric stretching vibration of C-C(O)-C or C-O functional group (Figure 4.2):

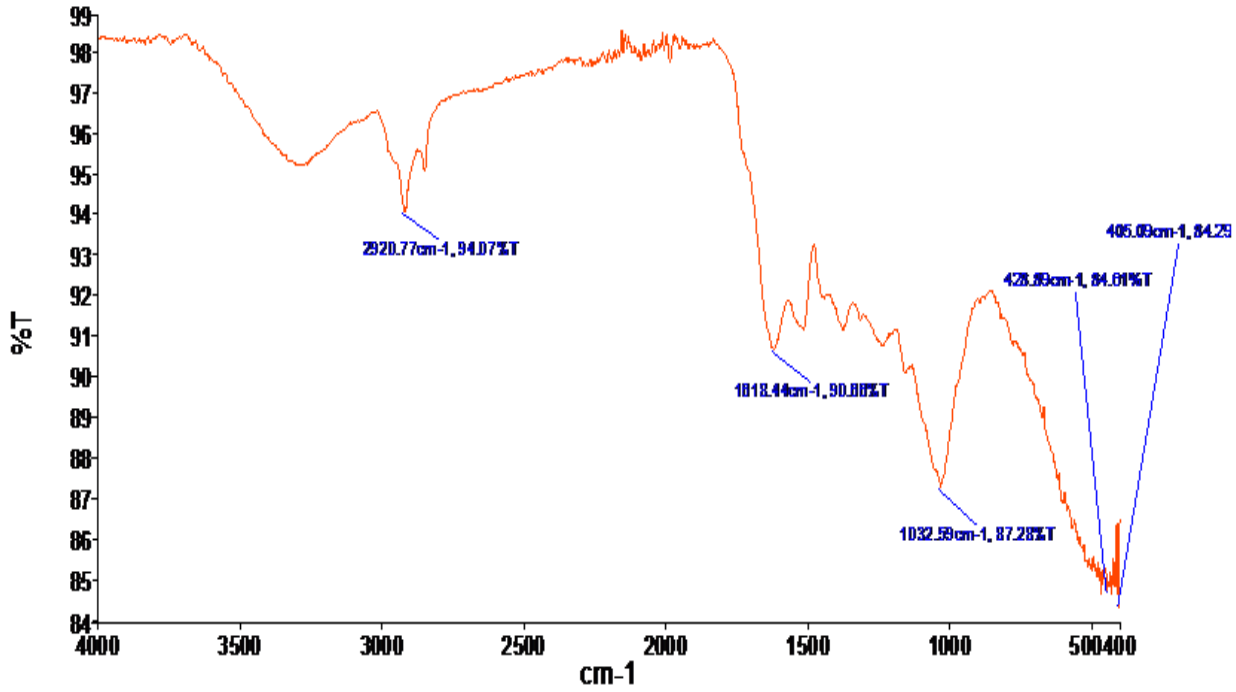

Figure 4.2: FTIR spectra of the un-carbonized /unmodified grape slurry adsorbent (GS)

The prominent adsorption bands observed on the IR spectra of adsorbent GS were nulled in the IR spectra of GSAa, GSAb and GSB. This may be attributed to degradation reactions, such as dehydration, depolymerisation and decarboxylation arising from partially or significant destruction/conversion of lignin-cellulose upon thermal treatment and modification (Peak *et al.,* 2003; Demirbas, 2004; Roggo *et al.,* 2007). This probably accounted for the reduced functional groups on the surfaces of GSAa, GSAb and GSB, but consistent increase in carbon content after activation.

The IR spectra of the hydrochloric acid modified adsorbent (GSAa) showed a low intensity spectra line at 418.25 cm^{-1} (Figure 4.3) in contrast to the prominent spectra peaks, observed on the IR spectra of the un-carbonized and unmodified grape slurry adsorbent (GS). The suppression of the absorption at this wavelength suggested the deformation and/or destruction of functional groups present on the adsorbent during carbonization and chemical activation.

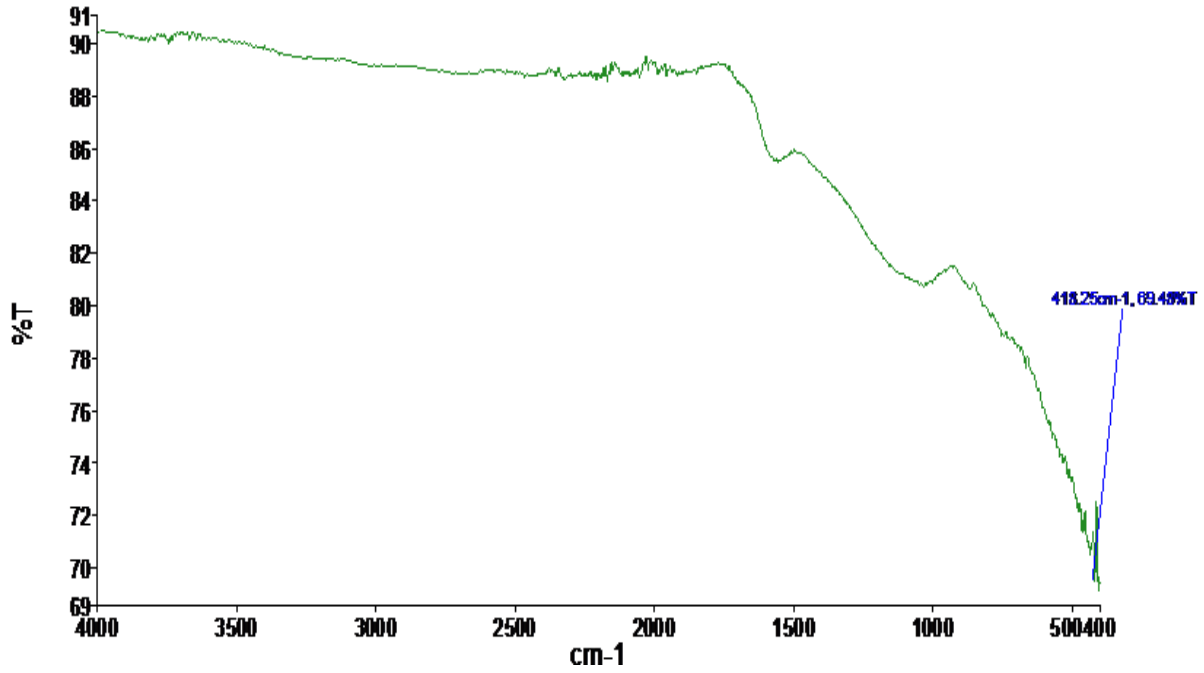

Figure 4.3: FTIR spectra of hydrochloric acid (HCl) modified grape slurry adsorbent GSAa

Absorption wavelength stretches and distinctive absorption bands occur at about 1590 cm^{-1} – 1700 cm^{-1}, 1100 cm^{-1} – 1320 cm^{-1}; and 1600 cm^{-1} and 1100 cm^{-1}. These bands may be those of the asymmetric C=O stretching vibrations of carbonyl, and symmetric C-C or C=C stretching vibrations of alkenes.

The absorption wavelength stretches and distinctive absorption band lines observed on the FTIR absorption spectra of GSAb (Figure 4.4) is very similar to those noted on the absorption spectra of GSAa (Figure 4.3). However, there were slight variations between the weak absorption spectra lines of GSAa and GSAb in the lower spectra band regions. While 426.19 cm^{-1} and 405.09 cm^{-1} were noted in the IR spectra of GSAa, weak 417.94 cm^{-1} and 403.43 cm^{-1} absorption lines were observed in the lower region of GSAb IR spectra. The similarity in the absorption patterns of GSAa and GSAb may be said to be as a result of the comparatively similar effect of the acids used in the modification of the adsorbents for activation purposes.

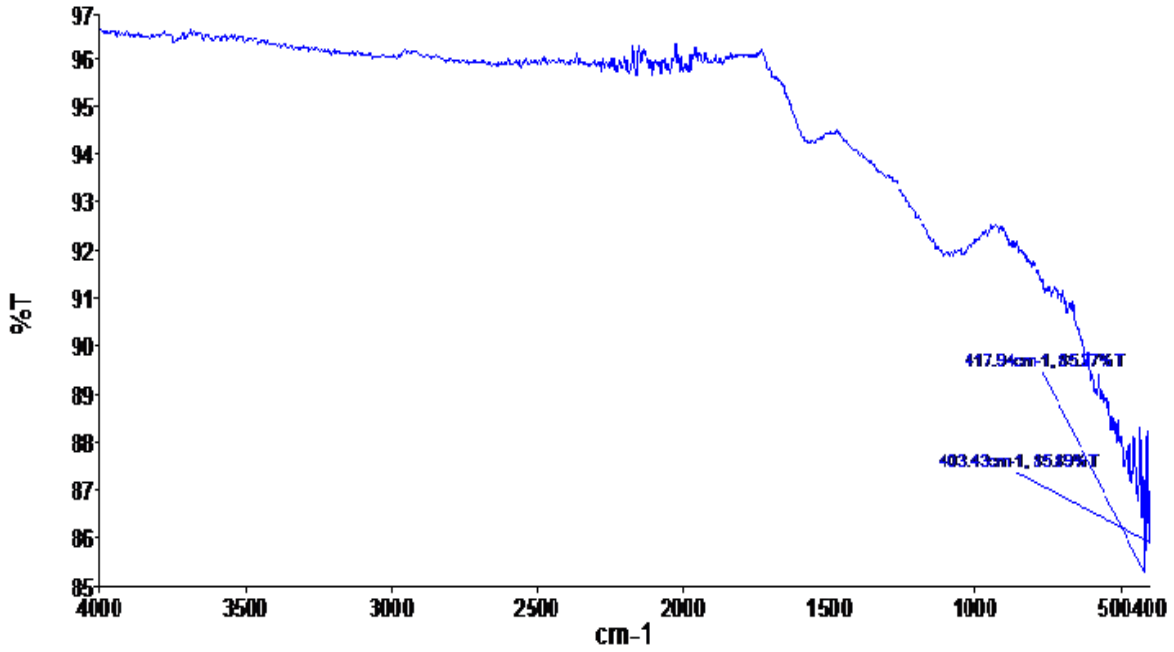

Figure 4.4: FTIR spectra of sulphuric acid (H_2SO_4) modified grape slurry adsorbent GSAb

Some spectra lines of adsorption were obtained around 1700 cm^{-1}, and 1032.56 cm^{-1}, which may have been due to asymmetric stretching vibrations of C=O bonds and symmetric C=C stretching vibrations of alkenes on the surface of GSAb. Also, two weak spectra lines of absorption at 417.94 cm^{-1} and 403.43 cm^{-1} were observed on the IR spectra pattern of GSAb, close to the 426.19 cm^{-1} and 405.09 cm^{-1} spectra lines of absorption observed on the spectra line pattern of GS.

The absorption spectra lines, which appeared at 2920.77 cm^{-1} on the FTIR spectra of GS were absent on the FTIR spectra of GSAb. This suggests the probable loss of C-H and CH_2 stretching vibrations in GSAb due to the combustion of hydrogen in carbonization.

The modification of the carbonized grape slurry adsorbent in potassium hydroxide (KOH) (GSB) resulted in the production of a different absorption pattern on the FTIR spectra compared with acid modified patterns. Some absorption bands observed along the spectral line of GS at the higher absorption region were not completely nulled (suppressed) in the GSB spectrum (Figure 4.5).

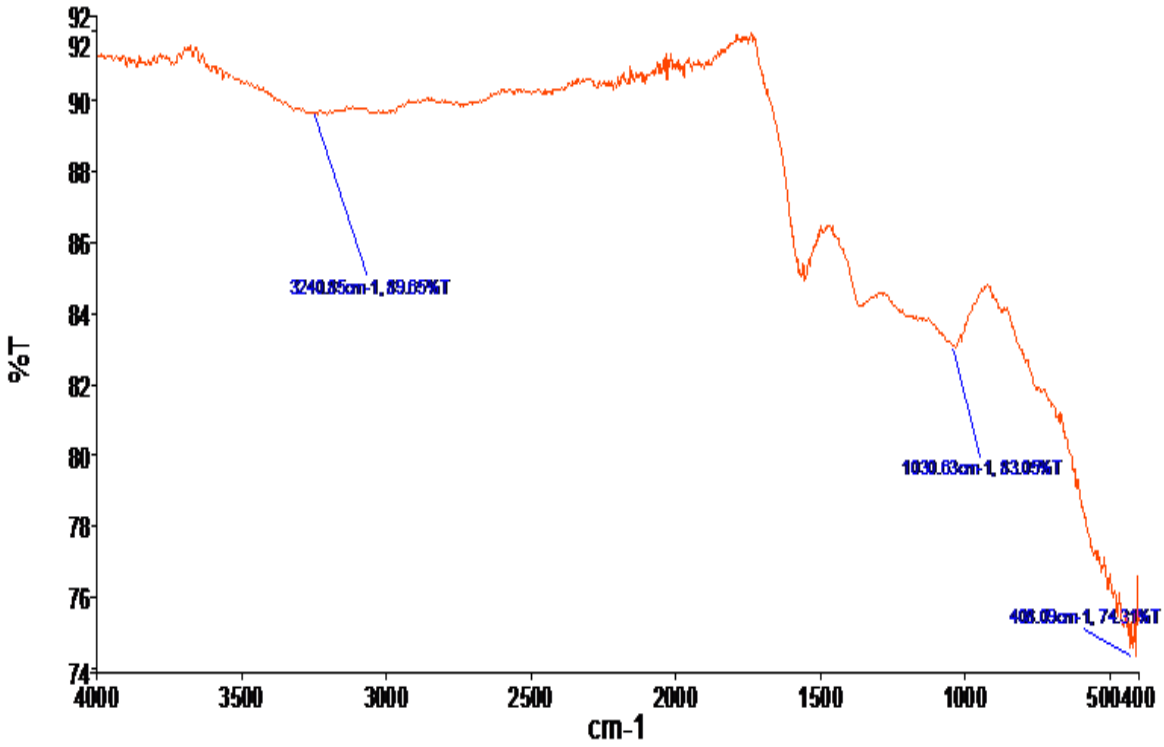

Figure 4.5: FTIR spectra of potassium hydroxide (KOH) modified grape slurry adsorbent GSB

A shift in absorption from 2920.77 cm^{-1} was observed at absorption band 3240.85 cm^{-1}. Absorption stretch at wavelength between 3200 and 3500 cm^{-1} has been reported to be characteristic of O-H stretch, perhaps from surface waters or the alkaline solution used for adsorbent modification (Zhang & Chuang, 2001).

Absorption wavelength stretch 1600 cm^{-1} – 1700 cm^{-1}, 1420 cm^{-1} – 1490 cm^{-1}, 1000 cm^{-1} – 1100 cm^{-1}, and distinctive absorption at 406.09 cm^{-1} were also observed to occur along the absorption spectra line of GSB. The stretching vibration band at 1600 cm^{-1} – 1700 cm^{-1} may be that of the asymmetric C=O, and symmetric C=C stretching vibrations of carbonyl or olefin compounds. A broad band was observed at 1030.63 cm^{-1}: and this can be assigned to stretching vibration of the C-O functional group (Mozgawa *et al.*, 2005, Roggo et al., 2007; Mozgawa *et al.*, 2011, Xie *et al.*, 2012).

4.3 BATCH SORPTION EXPERIMENTS

Milli-Q water spiked with 40 mg/L of AMX, AMP and CHLR, was used for batch sorption experiments, using the four adsorbents (i.e. GSAa, GSAb, GSB and GS). The sorption parameters - contact time, adsorbent dose, pH and temperature were optimized to determine their effect on the adsorption efficiencies and capacities of the adsorbents for the antibiotics.

4.3.1 Kinetic Studies

4.3.1.1 Effect of Contact Time

The effect of contact time on adsorption of the antibiotics (AMX, AMP and CHLR) from 40 mg/L standard solutions of each of the antibiotics onto the four different adsorbents (GSAa, GSAb, GSB and GS) was investigated. The masses of the adsorbents were kept constant at 0.1 g for every 25 ml of standard solutions of the different antibiotics. All solutions were kept at room temperature (25 ^{o}C) and shaken on an orbital mechanical shaker (Labotec OrbiShake, Germany) for a total period of 5 h with a revolution of 120 rpm.

Figure 4.6 shows the effect of contact time on AMX, AMP and CHLR removal from aqueous solutions using GSAa. The equilibrium times obtained for the sorption of AMX, AMP and CHLR onto GSAa were 120 min, 150 min and 90 min, respectively. This indicates that the GSAa adsorbent showed relatively better selectivity towards CHLR, followed by AMX and then AMP. These equilibrium times were applied for all further batch experiments carried out. The sorption quantities of AMX, AMP and CHLR adsorbed at equilibrium were 2.964 mg/g, 2.608 mg/g and 3.007 mg/g respectively.

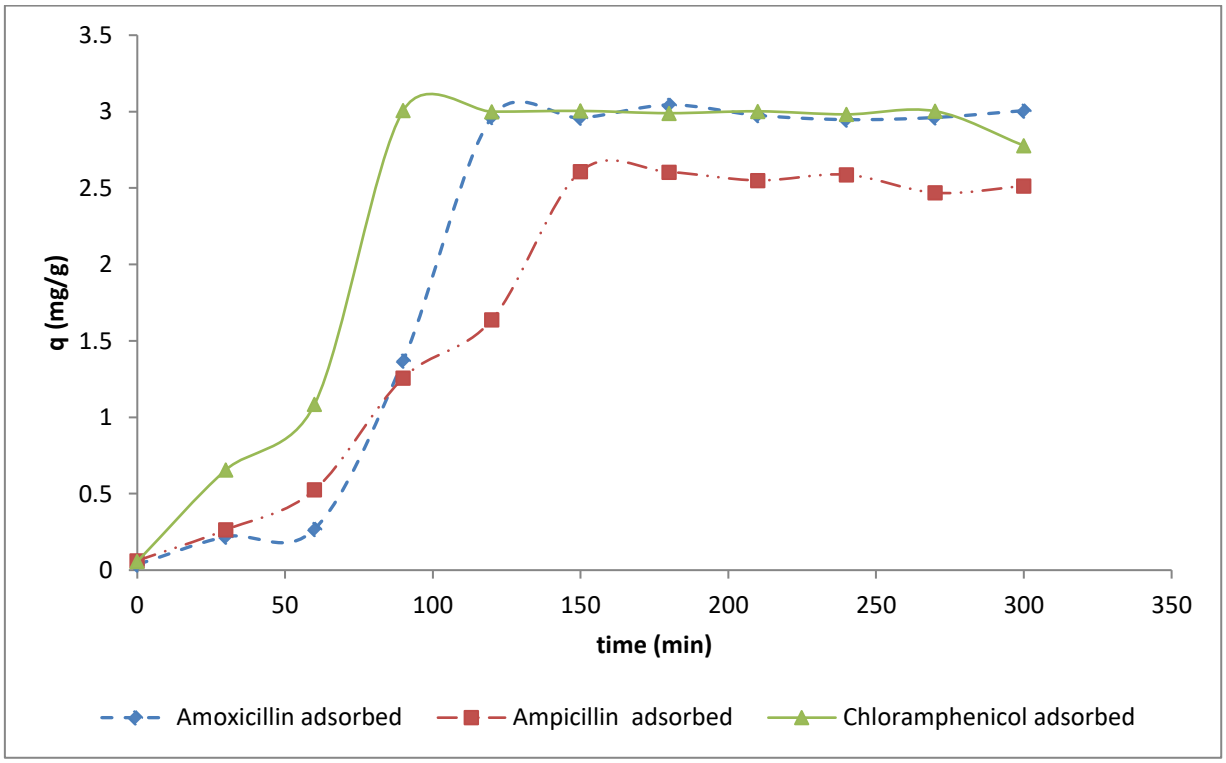

Figure 4.6: Effect of contact time on the sorption of AMX, AMP and CHLR from solution using GSAa

Similar sorption trend was observed for the adsorption AMX, AMP and CHLR onto GSAb (Figure 4.7). Although the process proceeded faster for CHLR, the equilibrium times for all adsorbates did not shift compared to those obtained using GSAa. However, there were

slight increases in the amount of AMX and AMP adsorbed from solution onto GSAb at equilibrium (2.992 mg/g and 2.700 mg/g, respectively). A slight decrease in the quantity adsorbed was observed for CHLR (2.798 mg/g). The adsorbent showed a better selectivity towards AMX relative to AMP and CHLR.

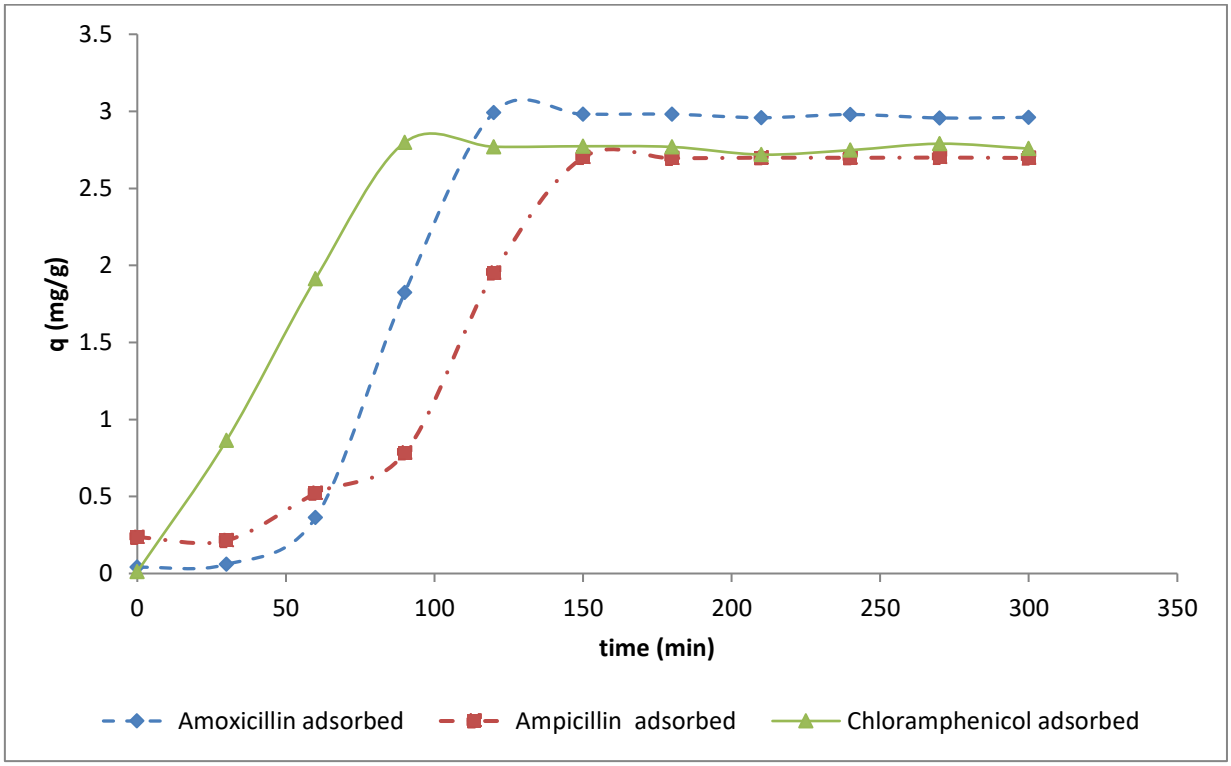

Figure 4.7: Effect of contact time on the sorption of AMX, AMP and CHLR from solution using GSAb

The selectivity and equilibrium contact time for the adsorption of AMX, AMP and CHLR onto GSB varied. The sorption processes' equilibria for AMX, AMP and CHLR were attained at 120, 90 and 150 min respectively. Figure 4.8 shows that the removal of CHLR by GSB was better with 3.104 mg/g adsorbed at a contact time of 150 min, compared to AMX and AMP with corresponding values of 2.971 mg/g and 2.838 mg/g, respectively.

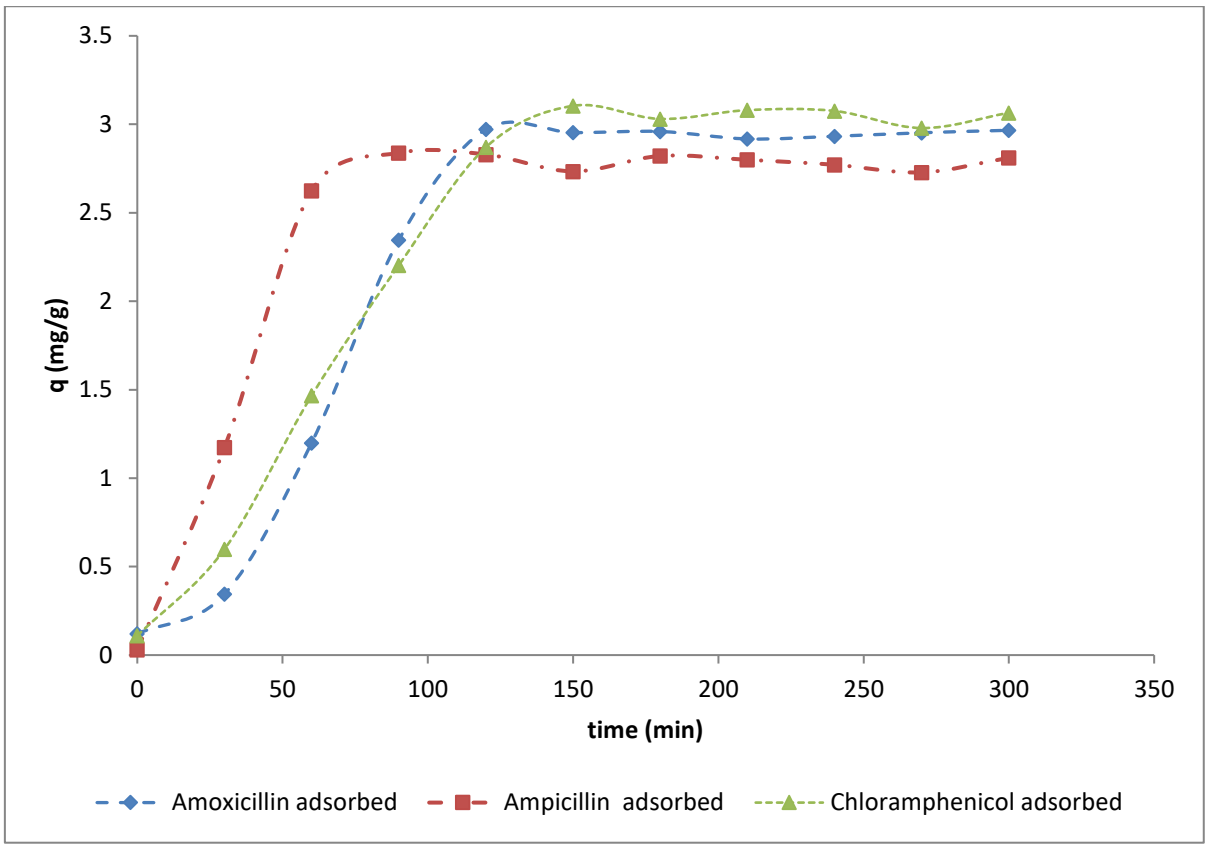

Figure 4.8: Effect of contact time on the sorption of AMX, AMP and CHLR from solution using GSB

GS showed a very poor sorption capacity for AMP and CHLR (Figure 4.9). However, it gave better sorption selectivity towards AMX, with equilibrium sorption quantity of 2.940 mg/g at 150 min. The maximum amounts of AMP and CHLR adsorbed by GS were 1.724 mg/g at 150 min and 1.650 mg/g at 90 min, respectively. These values were relatively low compared to those observed for GSAa, GSAb and GSB.

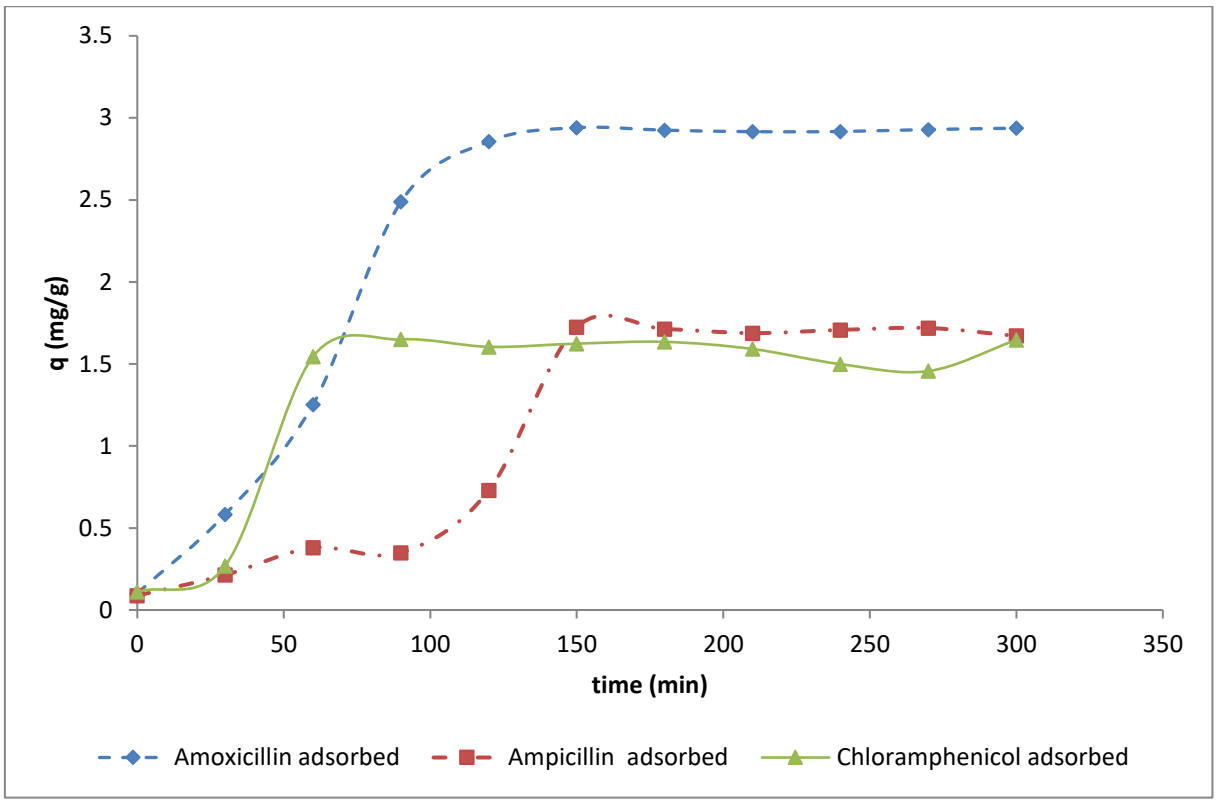

Figure 4.9: Effect of contact time on the sorption of AMX, AMP and CHLR from solution using GS

The sorption of AMX onto GSAa, GSAb and GSB attained equilibrium at 120 min, while with GS it was 150 min; CHLR onto GSAa and GSAb reached equilibrium at 90 min, with that for GS and GSB at 60 min and 150 min respectively. The adsorption of AMP onto all adsorbents attained equilibrium time at 150 min except for GSB with an equilibrium time of 90 min.

The contact time required for the adsorption processes to reach equilibrium is variable for the different adsorbents. The process proceeded rapidly for CHLR on the different adsorbents, reaching sorption equilibrium after an average time of 90 min, while AMX and AMP attained an average equilibrium time of 120 min and 150 min respectively for all adsorbents (Figures 4.6 - 4.9). Steep slopes indicating sharp increases in adsorption of the adsorbates were observed in the diagrams before equilibrium was attained, after which flat trend lines followed. The sorption trend lines are consistent with the findings of Ayanda *et al.* (2013) who reported tributyltin adsorption from contaminated water using fly ash, activated carbon and fly ash/activated carbon composite, and that of Mohammad *et al.* (2014), who worked on fluoride adsorption using modified *Lemna minor*. The adsorption of adsorbates on the adsorbents proceeded fast and increased gradually until equilibrium was reached. This may probably be due to the availability of large numbers of functional sites for adsorption on the surface of the sorbent materials. Over time, the active surface sites become occupied thus slowing the intra-particle diffusibility (pore diffusion) sorption of the adsorbate onto sorption sites of the adsorbents (Shaibu *et al.,* 2014). This might result in an increase in the repulsive

forces between the solid molecules and the bulk phase, thereby pushing the adsorption process towards equilibrium.

There was no significant difference in the sorption capacity of the adsorbents for all the three antibiotics beyond the sorption contact equilibrium time. Hence any further increase in contact time after equilibrium would not facilitate significant difference on the sorption capacity of the adsorbents. It is important to note that, modification of the adsorbent materials is inconsequential to the adsorption of AMX onto all the adsorbents, whereas the sorption of AMP and CHLR were enhanced by the modification of the adsorbents.

4.3.1.2 Comparison of Sorption Efficiencies of Adsorbents

The rate of adsorption for molecules of the adsorbates onto the adsorbent surfaces is expressed using the kinetic process. A comparative evaluation on the effectiveness of the use of different adsorbents in the removal of antibiotics from solution was conducted in order to understand the kinetics of the adsorption processes. Comparative sorptions of AMX, AMP and CHLR onto the different adsorbents are presented in Figures 4.10, 4.11 and 4.12.

The sorption of AMX onto all of the adsorbents was rapid, with sharp increases in the adsorption of AMX from solution. The adsorbents had good removal efficiency for AMX, with equilibrium sorption time of 120 min for the four adsorbents (Figure 4.10).

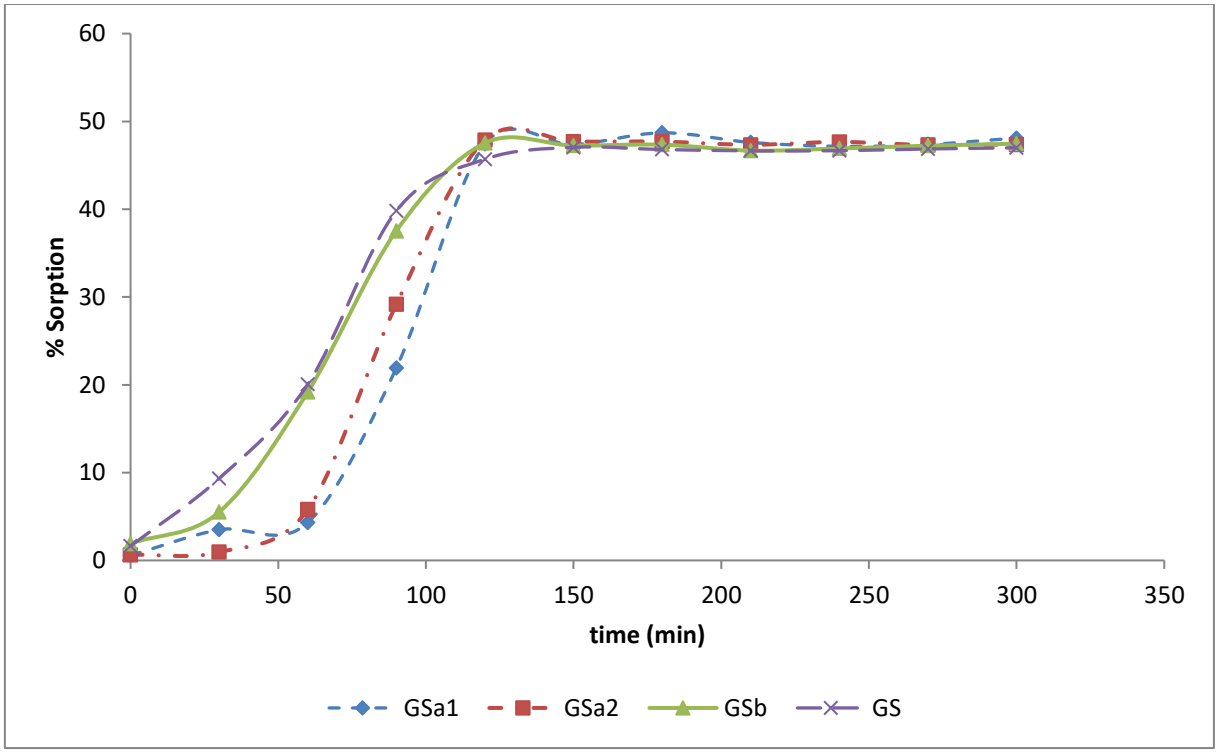

Figure 4.10: Sorption kinetics of AMX from solution using GSAa, GSAb, GSB and GS

However, the rates at which the sorption process of AMX attained equilibrium onto each of the adsorbents were not significantly different from each other. At equilibrium, the sorption efficiencies of the adsorbents for the removal of AMX ranged between 45.70 % for GS and 47.87 % for GSAb. This implies that all the adsorbents were competitive in the removal of AMX from solution.

The adsorption of AMP from solution onto the different adsorbents is presented in Figure 4.11. The different adsorbents showed variable sorption capacities, and different equilibrium times for the sorption of AMP. GSB was selective towards the removal of AMP relative to the other three adsorbents (GSAa, GSAb and GS). Equilibrium was attained using GSB at 90 min with a sorption quantity of 32.43 % (Figure 4.11)

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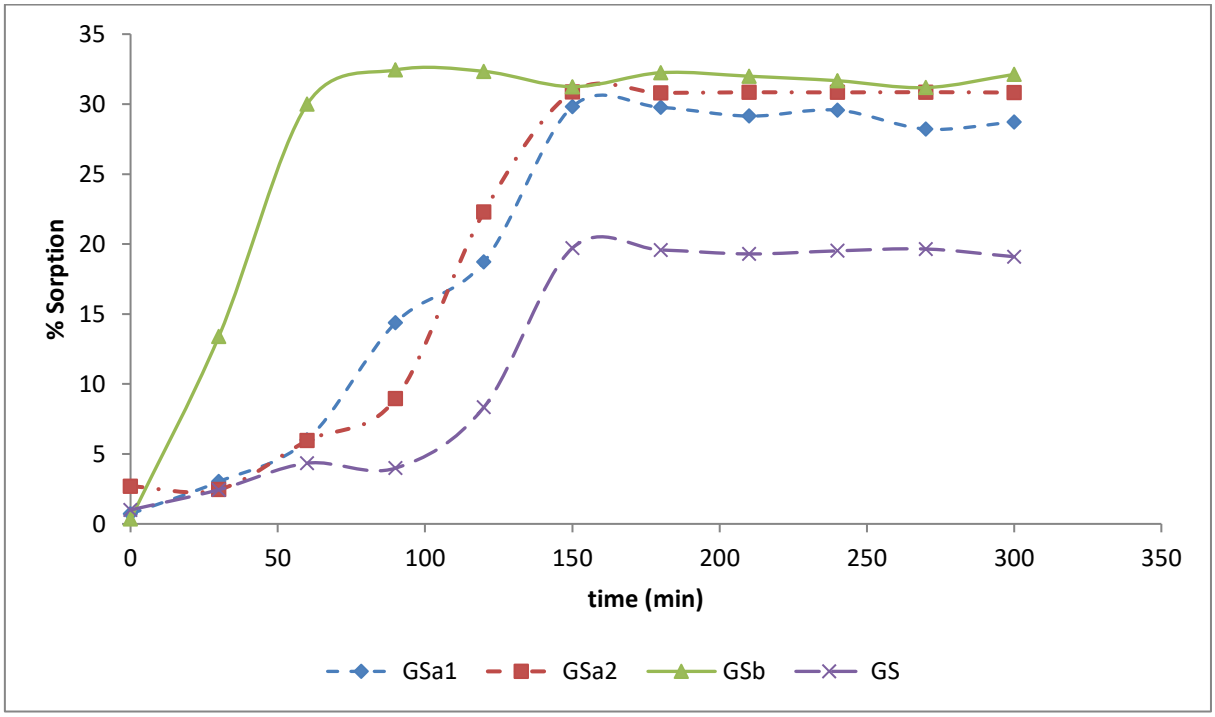


Figure 4.11: Sorption kinetics of AMP from solution using GSAa, GSAb, GSB and GS

GS however gave the lowest adsorption capacity of 19.70 % for AMP and reached equilibrium at 150 min. AMP sorption equilibria was attained at 150 min using GSAa and GSAb. The selectivity of the adsorbents for AMP were generally in the order GSB > GSAb > GSAa > GS

The adsorption pattern for CHLR from solution differs greatly with the four adsorbents, each attaining equilibrium at different times (Figure 4.12). The sorption of CHLR onto GS was the fastest, reaching equilibrium at 60 min. GS however had the lowest sorption capacity for CHLR implying its poor efficiency in removing the adsorbate from solution.

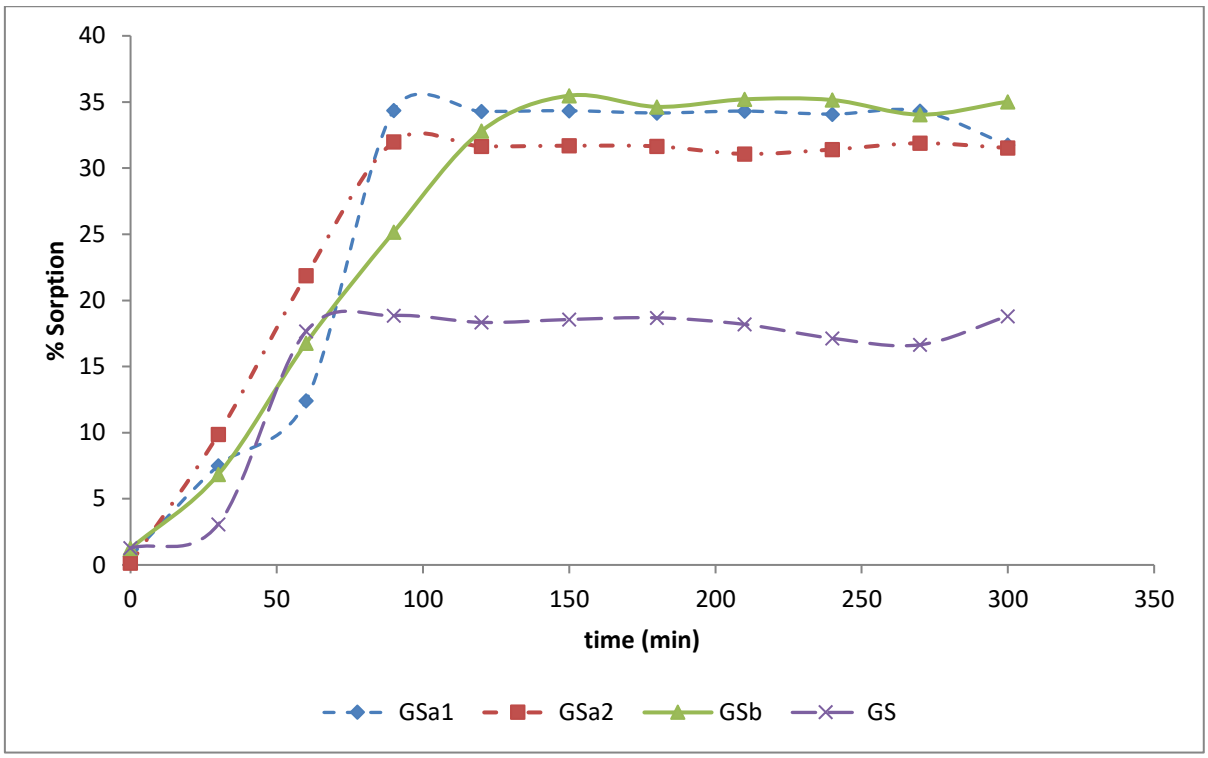


Figure 4.12: Sorption kinetics of CHLR from solution using GSAa, GSAb, GSB and GS

GSB had the highest sorption capacity for CHLR, but attained equilibrium last (150 min). In contrast GSAa and GSAb reached equilibria at about 90 min. The highest percentage sorption capacities obtained for CHLR onto GS, GSAb, GSAa and GSB were 18.85 %, 31.98 %, 34.28 % and 35.48 % respectively.

4.3.1.3 Rate Kinetics

The sorption of solutes onto the different adsorbents can proceed by different mechanisms governed by the physico-chemical conditions under which the adsorption process takes place, as well as the heterogeneity of reactive sites. It is therefore important to understand the sorption mechanism that is aligned with each particular adsorbate, in order to be able to describe and design effectively and efficiently, future feasibilities of the adsorption at large scale. In order to investigate the mechanism controlling the adsorption process, the pseudo-first-order, pseudo-second-order and the Elovich kinetic rate models were tested to model the kinetics of AMX, AMP and CHLR adsorption onto the various adsorbents.

The calculated constant parameters for the pseudo-first-order, pseudo-second-order and the Elovich models obtained by non-linear regression for the adsorbates using GSAa are summarized in Table 4.11.

Table 4.11: Kinetic parameters for the sorption of AMX, AMP and CHLR from solution by GSAa

GSAa		k_1	q_e	R^2
Pseudo-first Order	AMX	1.497×10^{-2}	4.107	0.3121
	AMP	1.612×10^{-2}	2.846	0.8050
	CHLR	1.842×10^{-2}	4.576	0.7842
		k_2	q_e	R^2
Pseudo-second Order	AMX	6.882×10^{-6}	3.017	0.9879
	AMP	4.514×10^{-4}	2.657	0.9969
	CHLR	4.403×10^{-3}	3.235	0.8174
		Aε	B	R^2
Elovich	AMX	0.0263	1.571	0.6342
	AMP	0.0316	1.922	0.6760
	CHLR	0.0383	1.668	0.7495

The kinetic data for the adsorption of AMX onto GSAa revealed that, the sorption process may be better explained by using pseudo-second-order and the Elovich models, with good correlations to the kinetic data (R^2 of 0.9879 and 0.6342, respectively) compared to the pseudo-first-order model. Furthermore, the values of q_e obtained from the pseudo-second-order model are not significantly different to the experimental values obtained. According to Sikarwar & Jain (2016), the mechanism for the removal of an adsorbate by an adsorbent is a complex if the linear portion of the curves do not pass through the origin (y = 0.36628x + 0.7589, pseudo-second-order model; and y = 0.6364x - 0.7733, Elovich model). Thus, these two models assume that chemisorption is dominant and that it controls the adsorption (Ho, 2006). The results are in agreement with the findings of Budyanto *et al.* (2008); Moussavi *et al.* (2013); and Chayid & Ahmed (2015) on the sorption of AMX onto activated carbons prepared from coconut shells, pomegranate wood and *arundo donaxl linn* respectively.

Results for the sorption kinetics of AMP onto GSAa (Table 4.11) showed that the kinetic data obtained for the adsorption process are better represented by all the kinetic models tested. However, the pseudo-first order and the pseudo-second order had high R^2 values compared to the Elovich model. Moreover, slight deviations between the calculated and experimental adsorption capacity, q_e, reflects good fit of experimental data into the kinetic models. These results suggest that the adsorption mechanism involves both the physical and chemical processes (Queiroz *et al.,* 2001).

The kinetic data for the adsorption of CHLR using GSAa followed similar pattern with those of AMP. The correlation values (R^2) for the pseudo-first-order, pseudo-second-order and the

Elovich models were not significantly different ($p < 0.05$) from one and other, with the R^2 values ranged between 0.7495 and 0.8174 for the three models. However, there was a large deviation between the calculated and experimental adsorption capacity with the pseudo-first-order equation as seen from the table reflecting poor pseudo-first-order fit to the experimental data. The pseudo-second-order and Elovich model thus explains the reaction mechanism for the adsorption of CHLR from solution, suggesting the adsorption proceed largely by chemisorption. The results agree with the previous findings of Ma *et al.* (2015) on the sorption of CHLR using shell molecularly imprinted polymers.

The results of the kinetic parameters, and the correlation coefficients (R^2) for the adsorption processes of AMX, AMP and CHLR onto GSAb, are presented in Table 4.12.

Table 4.12: Kinetic parameters for the sorption of AMX, AMP and CHLR from solution by GSAb

GSAb		**k_1**	**q_e**	**R^2**
Pseudo-first Order	AMX	2.050×10^{-2}	2.446	0.5395
	AMP	3.362×10^{-2}	3.280	0.6963
	CHLR	1.704×10^{-5}	2.906	0.8629
		k_2	**q_e**	**R^2**
Pseudo-second Order	AMX	9.718×10^{-4}	3.068	0.9913
	AMP	7.721×10^{-5}	2.656	0.9820
	CHLR	1.223×10^{-2}	2.879	0.9608
		Aε	**β**	**R^2**
Elovich	AMX	0.0374	1.565	0.6488
	AMP	0.0082	1.893	0.5977
	CHLR	0.0374	1.833	0.8685

The correlation coefficients (R^2) obtained for the sorption of AMX from solution onto GSAb using the non-linear regression of the pseudo-first-order, pseudo-second-order and the Elovich kinetic models were all greater than 0.50. However, the R^2 value of the pseudo-second-order model was significantly higher ($R^2 = 0.9913$) than those of the pseudo-first-order ($R^2 = 0.5395$) and the Elovich ($R^2 = 0.6488$) models. This suggests chemisorption as the predominating mechanism for the adsorption process. The theoretical q_e value obtained from this model was closer to the experimental value relative to that of the pseudo-first-order model. Thus, the pseudo-second-order model described the adsorption of AMX onto GSAb. The adsorption of AMX onto GSAb can therefore be assumed to proceed via the chemisorption. Firdaous *et al.* (2017) noted that adsorption processes, proceeding via chemisorption are as a result of the adsorbent surfaces having active sites exhibiting

different activation energies due to surface heterogeneity resultant of the Elovich model contribution to the kinetics mechanism

The kinetic data obtained for the adsorption of AMP onto GSAb (Table 4.12) is probably better explained by the non-linear pseudo-second-order model, which gave the highest correlation coefficient value ($R^2 > 0.98$), followed by the pseudo-first-order ($R^2 > 0.68$) and then the Elovich model ($R^2 > 0.59$). In addition, there was a good agreement between the calculated q_e value derived from this model and the experimental one in comparison to that obtained with the pseudo-first-order. Based on this result, the sorption of AMP onto GSAb could be considered a second-order reaction (Munagapati & Kim, 2017).

Fitting the kinetic data for adsorption of CHLR onto GSAb into the tested kinetic models all gave high coefficients of correlation ($R^2 > 0.86$). Furthermore, the pseudo-first order and the pseudo-second order models gave q_e values that were in agreement with the experimental values. This indicates that both models described the kinetics of the adsorption process well. Since the pseudo-second-order explains the external liquid film diffusion, surface adsorption and intra-particle diffusion processes (Vadivelan & Kumar, 2005), this model provided a more comprehensive and accurate reflection of the adsorption mechanism of CHLR onto GSAb than did the pseudo-first-order. The Elovich model describes the heterogeneous diffusion process, which is comprehensively regulated as the reaction rate and diffusion factor. The adsorption of CHLR was an integrative process that was controlled by reaction rate and diffusion, a conclusion that is consistent with the results of the pseudo-second-order kinetic model (Fan *et al.,* 2017).

Table 4.13 shows the calculated correlative parameters and correlation coefficients for the three kinetic models applied in the adsorption study of AMX, AMP and CHLR onto GSB.

Table 4.13: Kinetic parameters for the sorption of AMX, AMP and CHLR from solution by GSB

GSB		k_1	q_e	R^2
Pseudo-first Order	AMX	2.188×10^{-2}	3.107	0.8174
	AMP	1.313×10^{-2}	0.897	0.8157
	CHLR	1.635×10^{-2}	3.953	0.7137
		k_2	q_e	R^2
Pseudo-second Order	AMX	1.573×10^{-3}	4.671	0.9447
	AMP	2.828×10^{-2}	2.930	0.9851
	CHLR	3.555×10^{-3}	3.211	0.9818
		Aε	β	R^2
Elovich	AMX	0.0283	1.670	0.7548
	AMP	0.0583	1.904	0.8780
	CHLR	0.0473	1.648	0.8111

The pseudo-second-order model gave a high coefficient of correlation ($R^2 > 0.94$) for the sorption of AMX onto GSB, compared to those obtained for the pseudo-first-order ($R^2 > 0.81$) and the Elovich ($R^2 > 0.75$) models. The theoretical q_e value obtained with the pseudo-first-order was too high than the experimental value, indicating that the adsorption of AMX onto GSB is not a first-order reaction. Thus, the fit of the kinetic data and high coefficient of correlation ($R^2 > 0.94$) is suggestive that the sorption of AMX onto GSB could be considered a pseudo-second order reaction. The process is therefore assumed to follow a chemisorptive diffusion process since the Elovich model also slightly contributed to the mechanism. This observation is consistent with the findings of Pezoti *et al.* (2016) on the sorption of AMX onto NaOH-activated carbon.

High correlation coefficient values (R^2) ranging from 0.8157 to 0.9851 were obtained for the adsorption of AMP onto GSB using all three models. The pseudo-second order and the Elovich models better described the rate kinetic and mechanism for the adsorption of AMP. This is because the experimental q_e value and the theoretical q_e obtained using the pseudo-first-order model was not in agreement. This implies that the sorption of AMP proceeds via a heterogeneous chemical diffusion process and that the pseudo-second-order is the rate limiting step (Sun *et al.,* 2014). Similar results were reported by Martins *et al.* (2015), using a base-activated carbon for adsorption of tetracycline.

Similar results were obtained for the sorption of CHLR onto GSB. High values for the correlation coefficients ($R^2 > 0.71$) were obtained using all three kinetic models. The pseudo-second-order and the Elovich models with $R^2 > 0.98$ and $R^2 > 0.81$, respectively, describe the

reaction rate and mechanism for the adsorption of CHLR. This is because the q_e value obtained using the pseudo-first-order equation did not agree with the experimental q_e value, thus limiting it as a probable model for CHLR adsorption kinetics. Based on the results obtained, adsorption of CHLR onto GSB was predominantly a chemisorption process.

The kinetic parameters and coefficients of correlation calculated from the non-linear regression equations of the pseudo-first-order, pseudo-second-order and the Elovich kinetic models for the sorption of the antibiotics onto GS are presented in Table 4.14.

Table 4.14: Kinetic parameters for the sorption of AMX, AMP and CHLR from solution by GS

GS		**k_1**	**q_e**	**R^2**
Pseudo-first Order	AMX	2.303×10^{-2}	3.172	0.9047
	AMP	1.958×10^{-2}	1.812	0.8736
	CHLR	1.336×10^{-2}	0.802	0.4664
		k_2	**q_e**	**R^2**
Pseudo-second Order	AMX	3.722×10^{-3}	3.801	0.0869
	AMP	3.529×10^{-2}	5.637	0.0769
	CHLR	1.196×10^{-2}	1.774	0.7990
		Aε	**B**	**R^2**
Elovich	AMX	0.0340	1.7062	0.7954
	AMP	0.0074	3.002	0.5629
	CHLR	0.0092	3.385	0.7242

From the results, the adsorption of AMX onto GS can be assumed to follow the pseudo-first-order and the Elovich models since they gave better fits for the experimental data. The coefficients of correlation for the two models were $R^2 > 0.7954$ and $R^2 > 0.9047$, respectively. In addition, the pseudo-first-order gave a theoretical q_e value that was close to the experimental value. This indicated that the adsorption process of AMX is a first-order reaction. This implies that the adsorption process proceeds via a physical heterogeneous diffusion process (physisorption). In physisorption processes, adsorbents could be recovered since no chemical reaction occurred between the adsorbent surface and the adsorbate molecules (Olatunji *et al.* 2016). Budyanto *et al.* (2008) reported similar observation for the sorption of AMX from simulated wastewater onto activated carbon and natural bentonite.

A similar trend was obtained for the adsorption of AMP onto GS. The pseudo-first-order and the Elovich models gave high correlation coefficients (R^2 of 0.8736 and 0.5629 respectively). Due to the significant difference between the correlation coefficient values of these two models, the pseudo-first-order was considered to be the rate limiting step. This implies that

the adsorption process was controlled by physisorption. In this case, the adsorption of AMP onto GS took place as a result of physical interactions such as Van der Waals forces between the surface group of adsorbent and the adsorbate molecule as described by Rahardjo *et al.* (2011).

The experimental data for the adsorption of CHLR fitted better into the pseudo-second-order and the Elovich model giving R^2 values in the range 0.7242 to 0.7990. The theoretical q_e value that was in agreement with the experimental q_e value was obtained for the pseudo-second order model, and thus used to describe the adsorption rate and mechanism. From these findings it could be ruled out that the sorption of CHLR onto GS followed a chemical diffusion process on a heterogeneous surface as reported by Fan *et al.* (2017). Generally, the pseudo-second-order model is also known to be a special kind of Langmuir kinetics, which assumes that adsorbate concentration is constant in relation to time and the total number of binding sites, and dependent on the amount of adsorbate adsorbed at equilibrium (Gupta & Bhattacharyya, 2011; Pezoti *et al.,* 2016).

4.3.2 Effect of adsorbent dosage

Every adsorbent reaches an equilibrium optimum capacity for the sorption of a compound at a specific adsorbent mass. This parameter is important because it influences adsorption of adsorbates from aqueous solutions. For the respective antibiotics, 40 mg/L standard solutions were prepared. Variable masses (0.1 g to 0.4 g) of the four adsorbents were weighed and added to the respective standard solutions (25 ml), with other parameters (temperature, rotation speed and pH) kept constant. The solutions were stirred at room temperature, using a horizontal mechanical shaker at a rotation speed of 120 rpm for the variable optimized equilibrium times.

The effect of adsorbent dose on the removal of AMX, AMP and CHLR from solution using GSAa, is shown in Figure 4.13.

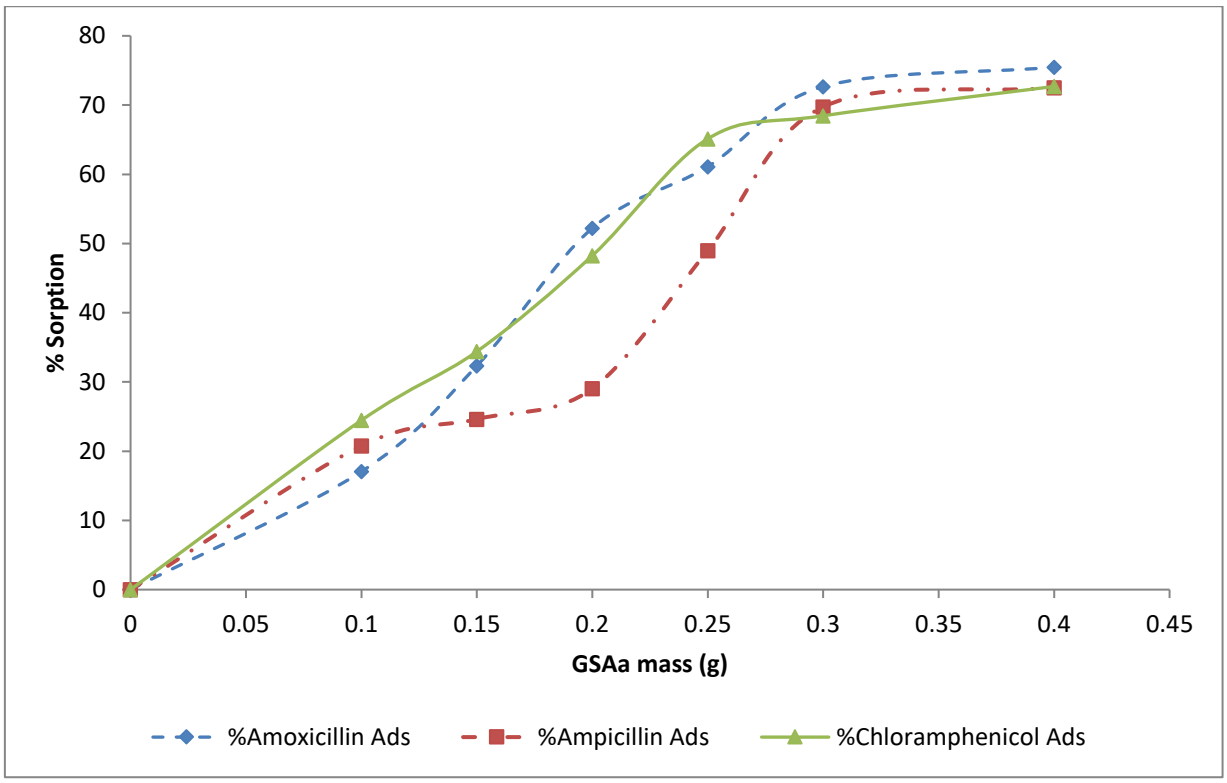


Figure 4.13: Effect of GSAa mass on the sorption of AMX, AMP and CHLR from solutions

There were sharp increases in the sorption capacities of the adsorbents for the antibiotics with increasing adsorbent dose from 0.1 g to about 0.3 g for every 25 ml solute. This may be credited to the availability of more surface active sites as the adsorbent dose is increased. According to Fakhri & Adami (2014), an increase in dose and conglomeration of the adsorbent results in increased availability of surface sites for adsorption. Further increases in the adsorbent dose from 0.3 g to 0.4 g resulted in no significant differences in the enhancement of sorption for the antibiotics. The maximum percentage adsorption recorded for AMX, AMP and CHLR were 75.46, 72.51 and 72.69 respectively.

Adsorption efficiency of GSAb for the sorption of AMX, AMP and CHLR showed steady increases with increased adsorbent dosage (Figure 4.14). GSAb showed a better affinity for AMP removal relative to AMX and CHLR. The maximum sorption percentages observed for AMX, AMP and CHLR were 59.46, 62.09 and 58.99 respectively for a GSAb dosage of 0.4 g.

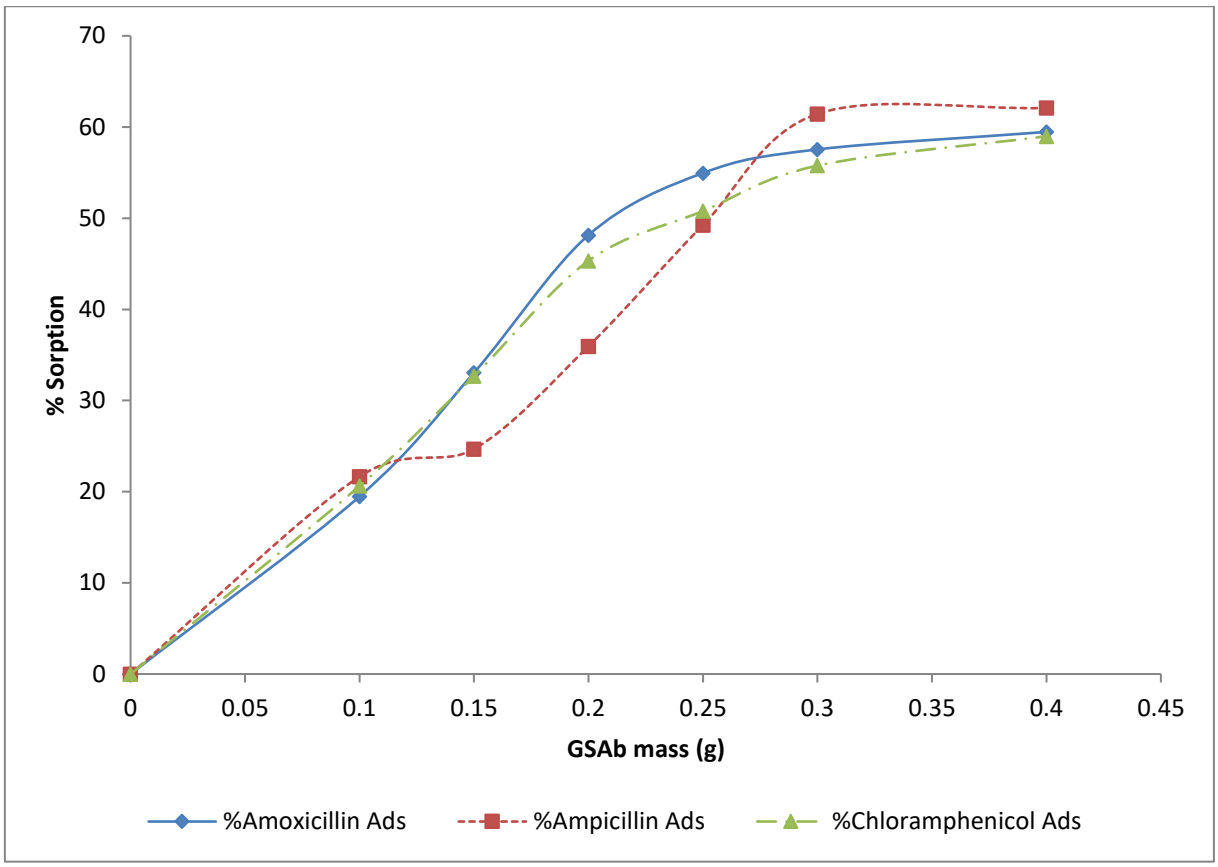


Figure 4.14: Effect of GSAb mass on the sorption of AMX, AMP and CHLR from solution

Increased percentage adsorption of all analytes was observed with increasing adsorbent dosage. However, the maximum percentages of antibiotics adsorbed from solution using GSAb were lower than previously obtained using GSAa. Demirbas *et al.* (2004) reported that the use certain reagents such as H_2SO_4 for the activation of adsorbents may result in reduced number of activation sites on the adsorbent surface. This in turn limits the maximum amount of adsorbates that could be removed from solution, thus limiting efficiency of adsorption (Aminu *et al.,* 2010; Ahile *et al.,* 2015).

Figure 4.15 presents the plot of the effect of adsorbent dosage on the adsorption efficiency of AMX, AMP and CHLR onto GSB. The sorption of the antibiotics also consistently increased with an increase in GSB dose up to an optimum mass of 0.3 g. At an adsorbent dose above 0.3 g, no significant increase in the sorption of antibiotics was obtained.

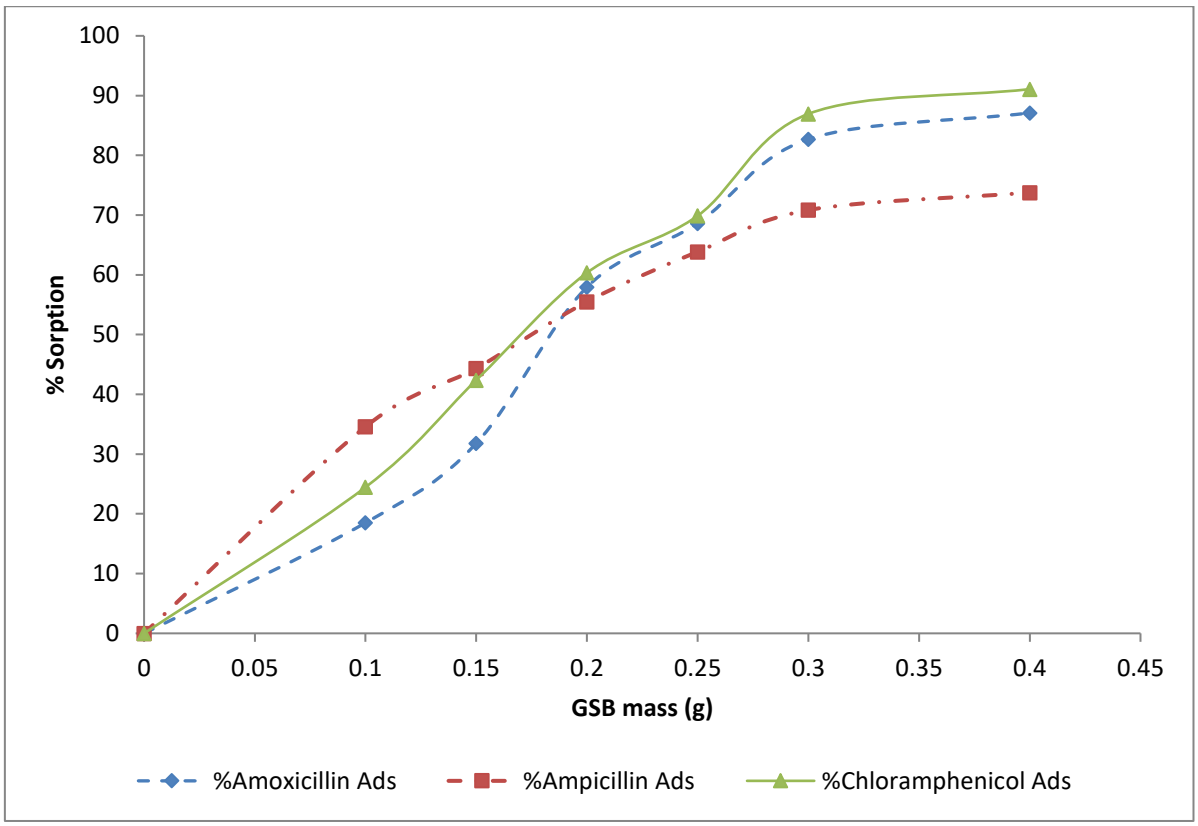


Figure 4.15: Effect of GSB mass on the sorption of AMX, AMP and CHLR from solution

Increasing the dosage of GSB showed enhanced removal efficiencies of the antibiotics from aqueous solutions compared to the acid modified adsorbents (GSAa and GSAb). The maximum adsorption capacities of the adsorbent for the antibiotics were 87.10 %; 73.77 % and 91.08 % for AMX, AMP and CHLR respectively. The enhanced adsorption suggests that GSB had more active binding sites, thus presenting an increase in available surface area for adsorption. Asgari *et al.* (2012) as well as Guler & Sarioglu (2014) noted that enhanced sorption may be the result of availability of active binding sites due to increased surface area brought about by adsorbent activation or modification.

Although increasing the dose of GS resulted in an increase in the adsorption of AMX, AMP and CHLR, the sorption efficiencies of the adsorbates from aqueous solution were poor (Figure 4.16). The maximum percentage sorption values recorded for AMX, AMP and CHLR were 35.33, 43.09 and 39.25 respectively. The poor adsorption may be due to low organic matter content and high moisture content of GS relative to other adsorbents. Ghasemi *et al.* (2014) reported that poor sorption could be a product of low active functional sites on the surface of the adsorbent and pore spaces that are not well defined.

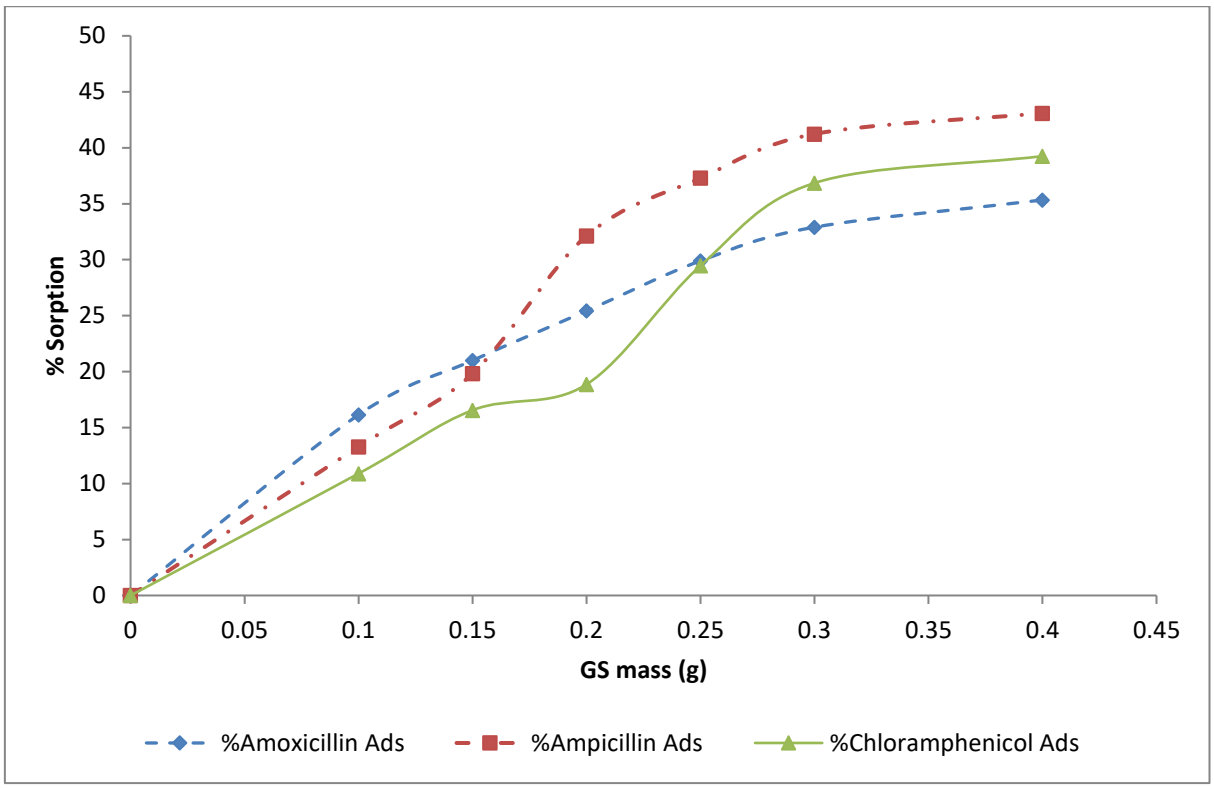


Figure 4.16: Effect of GS mass on the sorption of AMX, AMP and CHLR from solution

It has been reported that an increase in the amount of the adsorbent dosage is expected to increase percentage of adsorbates adsorbed (Shaibu *et al.,* 2014). This is because increasing the adsorbent dosage increases the amount of surface area available for the adsorbates to be adsorbed out of solution (Mahmoodi *et al.,* 2011; Shaibu *et al.,* 2014). At higher adsorbent to antibiotic concentration ratios, there was an increase in the magnitude of the percentage removal of the antibiotics. Zhang *et al.* (2012) suggested that a fixed dose of adsorbent can only adsorb a certain amount of adsorbate. Therefore, increase in adsorbent dose would result in increase in the quantity of adsorbate that can be sorbed out of solution. Generally, the uptake of the antibiotics using various dosages (0.1 – 0.4 g) of the four adsorbents increased reaching a maximum equilibrium uptake capacity (q_e) at 0.3 g, after which there was no significant increase in sorption. According to a report by Nandi *et al.* (2008), the decrease in significant variations in the antibiotics uptake value (q_e) may be due to the splitting effect of the flux (concentration gradient) between the antibiotics and adsorbent. GSB had a better sorption capacity for the three antibiotics relative to GSAa, GSAb, and GS.

4.3.3 Equilibrium Studies

4.3.3.1 Effect of initial concentration

An important driving force required to facilitate adsorption process is provided for by the initial concentration, in order to overcome the mass transfer resistance of the antibiotics between the solute and the aqueous phase (Hameed & Rahman, 2008; Shaibu *et al.,* 2014).

When the adsorbate particles succeed in overcoming the mass transfer resistance, adsorption is favoured and the percentage removal of the adsorbates molecules increases. This process is also determined by the pore size and the ability of the adsorbent surface to retain the adsorbate molecules.

Plots showing the effect of initial adsorbate concentration on the sorption efficiencies of adsorbents for AMX, AMP and CHLR are presented in Figures 4.17 to Figure 4.20. It was observed that the quantity of antibiotics removed by the adsorbents decreased with an increase in the initial concentration of the antibiotics. This is because, at lower adsorbate concentrations, there were more vacant adsorption sites available on the adsorbent for the adsorbate molecules to sorb on, until the adsorbent surface was saturated. There was therefore an inverse relationship between sorption capacity of the adsorbents and the initial concentration of the antibiotics.

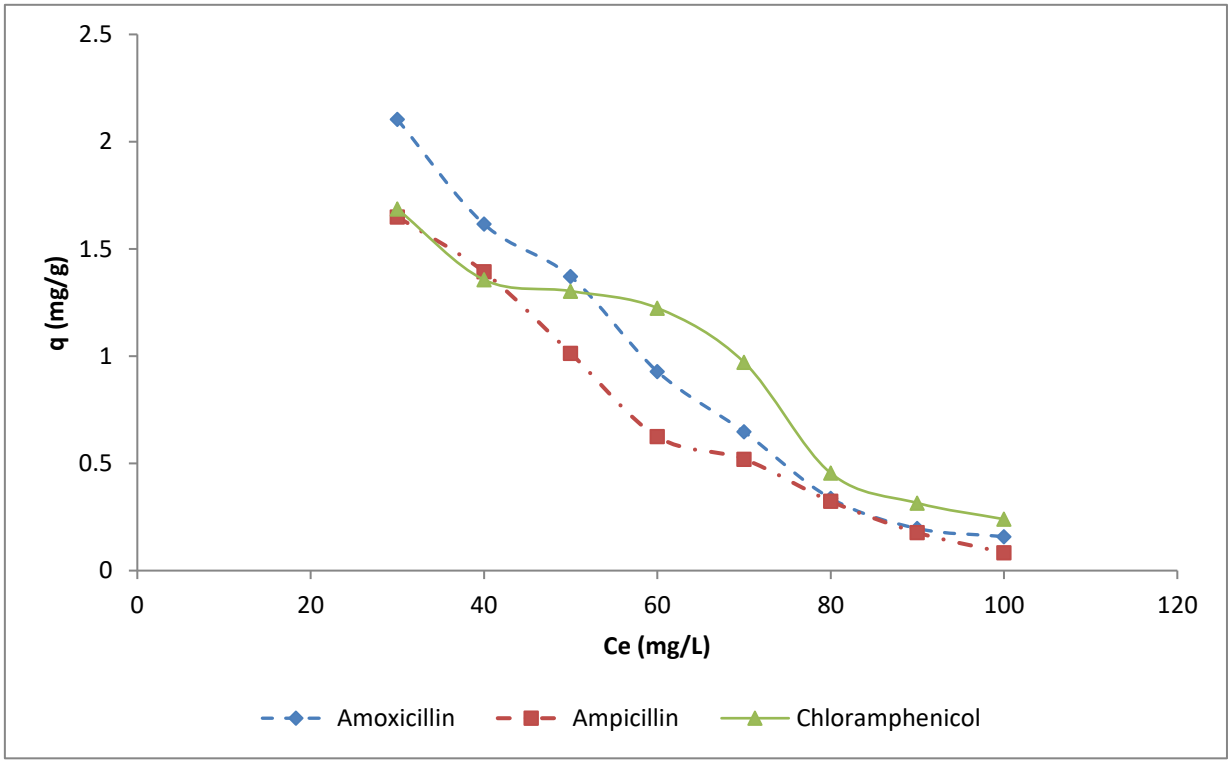


Figure 4.17: Effect of initial adsorbate concentration on the sorption of AMX, AMP and CHLR from solution using GSAa

As the concentration of antibiotics increased, the adsorption efficiency of antibiotics onto GSAa dropped significantly (Figure 4.17). Removal of antibiotics at the initial concentration of 30 mg/L was 2.104 mg/g, 1.650 mg/g, and 1.686 mg/g for AMX, AMP and CHLR respectively, and this decreased to 0.159 mg/g, 0.084 mg/g and 1.356 mg/g, respectively, when the initial concentration of antibiotics was increased step wise to 100 mg/L. This is because as the number of adsorbates increase with increasing concentration, the number of

active sites for adsorption becomes limited resulting in reduced adsorption quantity. Moussavi & Khosravi (2010) and Moussavi et al. (2013) noted that as long as the adsorbent concentration remains constant during sorption (adsorbent mass/volume of solution), a decrease in the removal efficiency with increased initial adsorbate concentration is related to limited availability of adsorption sites for an increased amount of adsorbate molecules.

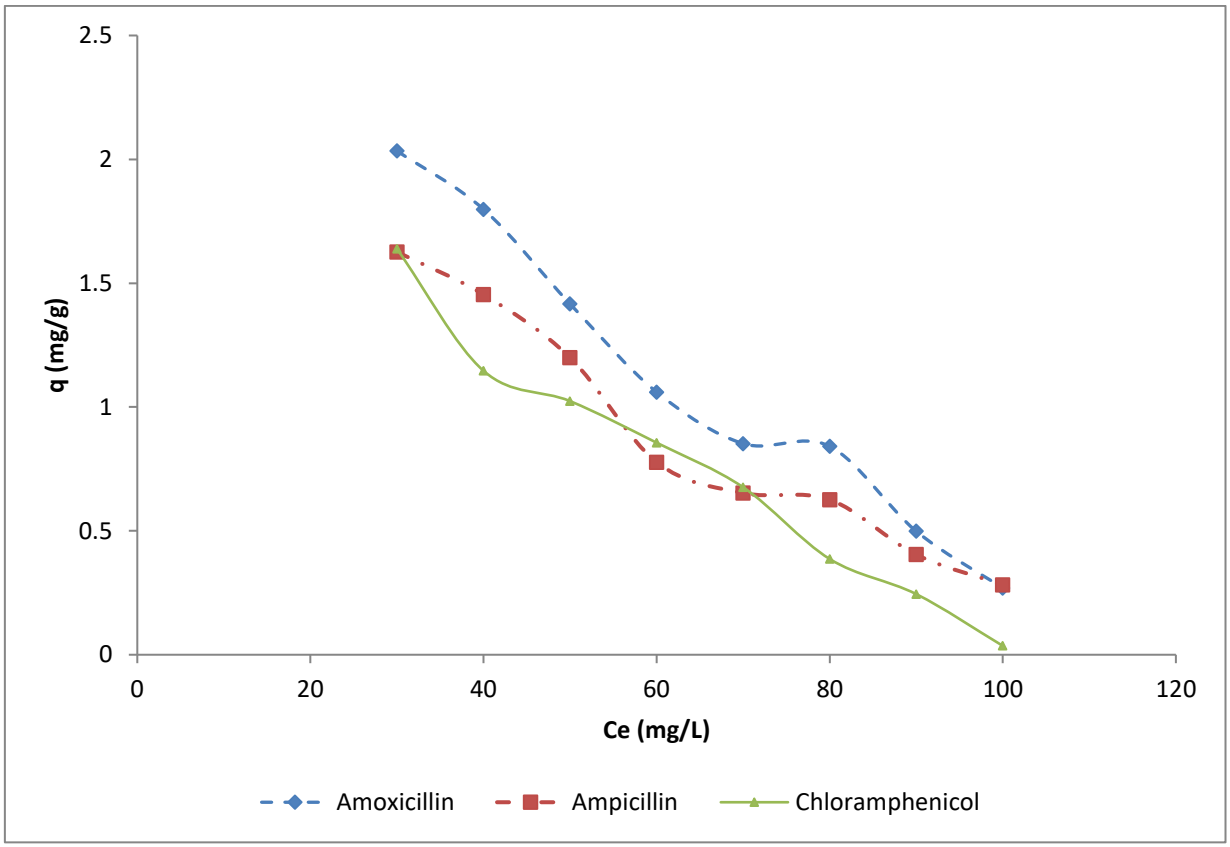


Figure 4.18: Effect of initial adsorbate concentration on the sorption of AMX, AMP and CHLR from solution by GSAb

GSAb showed a greater affinity for AMX in solution compared to AMP and CHLR with the highest adsorbed quantity of 2.304 mg/g (Figure 4.18). The maximum quantities of AMP and CHLR removed from the solution during the adsorption process using GSAb were 1.639 mg/g for both compounds. However, the percentage uptake of antibiotics decreased with increase in initial concentrations due to the presence of finite number of active sites on GSAb. According to Adriano et al. (2005) and Mohd-Din et al. (2015), a decrease in the amount of adsorbate sorbed from solution as concentration increases may be attributed to the adsorbent surfaces being saturated quickly, thus limiting uptake of the adsorbates.

Figure 4.19 shows the effect of adsorbate initial concentration on the sorption efficiency of GSB for AMX, AMP and CHLR.

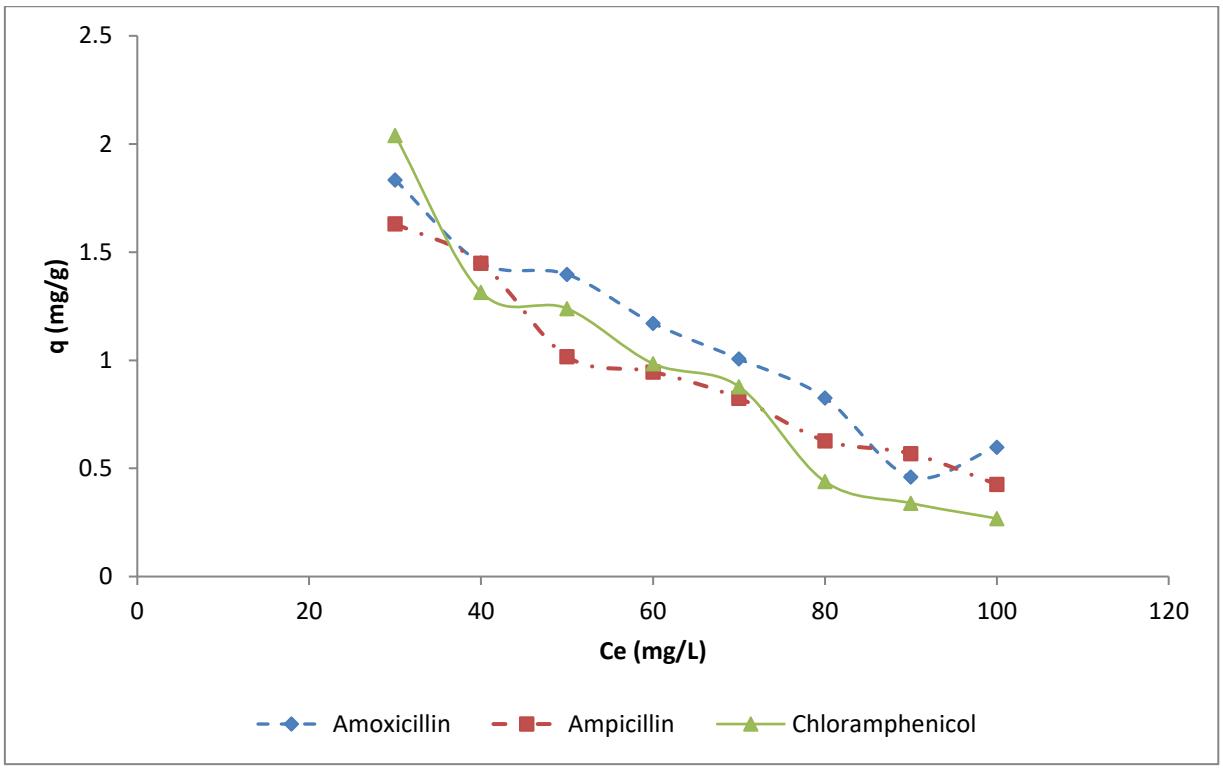


Figure 4.19: Effect of initial adsorbate concentration on the sorption of AMX, AMP and CHLR from solution by GSB

The result showed that GSB had a better selectivity towards CHLR compared to the other two adsorbates. While the antibiotics removal for AMX, AMP and CHLR was 1.834 mg/g, 1.631 mg/g and 2.040 mg/g for a 30 mg/L initial concentration, it decreased to 0.599 mg/g, 0.427 mg/g and 0.269 mg/g, respectively at 100 mg/L. This was assumed to be due to the surface of GSB being saturated with the antibiotic molecules as a result of limited active sites for adsorption.

Results for the removal efficiencies of AMX, AMP and CHLR by GS are shown in Figure 4.20.

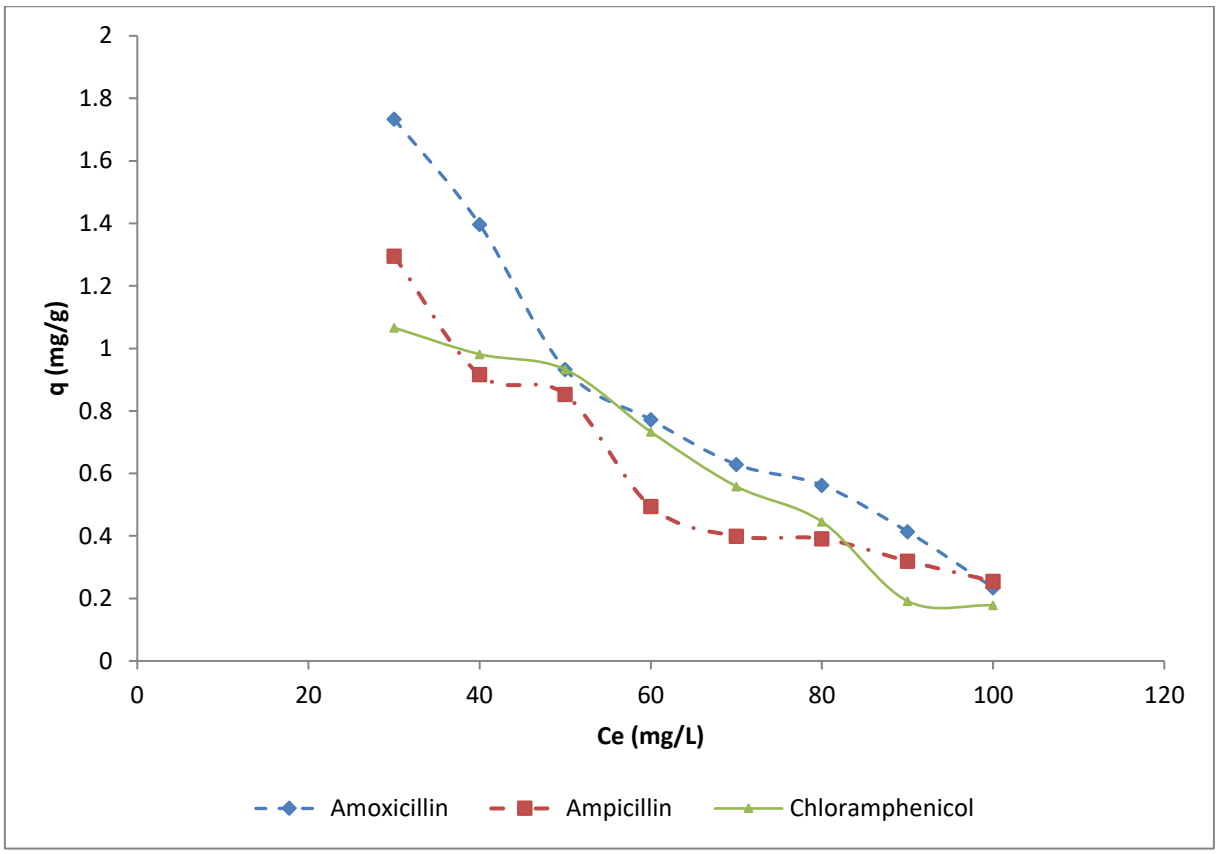


Figure 4.20: Effect of initial adsorbate concentration on the sorption of AMX, AMP and CHLR from solution by GS

GS was found to be more selective towards AMX relative to the other antibiotics. The removal efficiency of the adsorbent for antibiotics decreased rapidly with an increase in initial concentration from 30 mg/L to 100 mg/L. The highest equilibrium adsorbed quantities of the adsorbates (AMX, AMP and CHLR) were 1.734 mg/g; 1.295 mg/g and 1.066 mg/g respectively at 30 mg/L. Furthermore, the assumed consequence for reaching sorption equilibrium was that the amount of adsorbed molecules depends on conditions such as the initial adsorbate concentration and the driving force to overcome resistance of mass transfer for adsorbates, with the number of available adsorption sites being a limiting factor. The drop in adsorption efficiency could be a result of adsorption reaching saturation at a high antibiotic concentration due to a limited number of surface binding sites.

Of the three antibiotics studied, AMX was found to be easily removed from solution relative to AMP and CHLR using GSAa, GSAb and GS. On the other hand, GSB showed a better affinity for CHLR removal from solution. Based on the result obtained from the effect of initial antibiotic concentration on the efficiency of adsorption using GSAa, GSAb, GSB and GS, it was observed that lower initial concentration favours the antibiotics adsorption processes.

4.3.3.2 Adsorption isotherms

Adsorption isotherms are very important for the optimization of the adsorption system. They indicate how the adsorption molecules distribute between the liquid phase and the solid phase when the adsorption process reaches equilibrium (Tan *et al.,* 2007). Analysis of the isotherm data fit into the different isotherm models is an important step to finding the suitable model that can be used for design purposes (El-Guendi, 1991; Fytianos *et al.,* 2000). There are several isotherm models available for analysing experimental data and for describing the equilibrium of adsorption. These include Langmuir, Freundlich and Temkin isotherm equations which were also tested in this work. The applicability of the isotherm models to the adsorption studies done was compared by evaluating the correlation coefficients, R^2 values.

Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites of uniform strategies of adsorption with no transmigration of adsorbate in the plane of surface (Weber & Chakkravorti, 1974). The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless equilibrium parameter (R_L) (Weber & Chakkravorti, 1974). The value of R_L indicates the type of the isotherm to be either favourable ($0 < R_L < 1$), unfavourable ($R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$).

On the other hand, the Freundlich isotherm assumes heterogeneous surface energies, in which the energy term in Langmuir equation varies as a function of the surface coverage (Tan *et al.,* 2007). The Freundlich parameter, commonly known as the heterogeneity factor, N_F is used to determine the mechanisms responsible for the sorption processes. Kumar *et al.* (2010) and Pezoti *et al.* (2016) explained that the Freundlich parameter index N_F is a measure of deviation from linearity of adsorption, which indicates that an adsorption process is linear when $N_F=1$, chemical when $N_F<1$ and physical when $N_F>1$. Also N_F values above 10 indicate that the adsorption process was irreversible (Do, 1998).

The Temkin isotherm considers the effect of indirect adsorbate/adsorbate interactions on adsorption isotherms. The model assumes that the heat of adsorption of all the molecules in the layer would decrease linearly with coverage due to adsorbate/adsorbate interactions (Dada *et al.,* 2012). A Temkin constant, B, which is related to the heat of sorption (J/mol), indicates the kind of mechanism that influences the sorption.

All data obtained from the effect of initial concentration on the efficiency of adsorption of GSAa for AMX, AMP and CHLR from solution were fitted into the Freundlich, Langmuir and Temkin isotherms and the results are presented in Table 4.15.

Table 4.15: Isotherm parameters for the sorption of AMX, AMP and CHLR from solution using GSAa

GSAa		N_F	k_F		R^2
	AMX	0.995	0.197		0.9946
Freundlich	AMP	0.874	0.208		0.9869
	CHLR	0.648	0.218		0.9763
		q_M	R_L	k_L	R^2
	AMX	2.283	0.33 – 0.61	0.071	0.9905
Langmuir	AMP	1.868	0.25 – 0.87	0.060	0.9896
	CHLOR	1.713	0.19 – 0.44	0.042	0.9585
		B	k_{TEM}		R^2
	AMX	42.695	1.168		0.9032
Temkin	AMP	41.515	1.643		0.8585
	CHLR	52.710	0.983		0.9588

Experimental data for the sorption of AMX using GSAa was better fitted into the Langmuir and Freundlich isotherm models with correlation coefficient values (R^2) > 0.99. This result suggests that the sorption of AMX using GSAa occurred on a monolayer heterogeneous surface having a maximum limited uptake of the adsorbate molecules corresponding to a saturated adsorbent surface. The Temkin model gave a low R^2 value (0.9032), therefore the Freundlich and Langmuir models were used to explain the mechanisms of sorption and the isotherm data.

The heterogeinity factor (N_F) calculated for the sorption of AMX from the intercept of the Freundlich linear plot was lower than 1. This result suggests that the sorption of AMX was influenced by chemical reactions resulting from the interactions of AMX molecules with some functional groups on the adsorbent surface. The Langmuir dimensionless parameter R_L which measures the efficiency of the adsorption process is indicative of the preference of the adsorption process in the corresponding adsorbent/adsorbate system. Since the R_L values for the sorption of AMX onto GSAa ranged between 0 and 1, the sorption of the adsorbate with the adsorbent was favourable. The maximum quantity for AMX removed from the solution as determined using the Langmuir isotherm was 2.283 mg/g. This value is close to the experimental value obtained.

The correlation coefficient values for the adsorption of AMP using GSAa appear more satisfactory with the Langmuir and Freundlich isotherm models (R^2 > 0.98) than with the Temkin isotherm model (R^2 = 0.8585) (Table 4.15). Correlation coefficients indicated that both the Langmuir and Freundlich models better described the adsorption process well. Hence, the adsorption of AMP onto GSAa occurred largely by monolayer sorption onto a heterogeneous surface with the adsorbent sorption capacity reaching a limit at the saturation

of the monolayer surface. The N_F value for AMP adsorption (0.874) showed that the process was feasible proceeding via chemisorption mechanism. In addition, the R_L values which ranged between 0.25 and 0.87 implied the favourability of the reaction. The maximum AMP uptake value of 1.868 mg/g is close to the experimental value, thus suggesting the fitness of the experimental data into the Langmuir isotherm model.

The fitting of experimental data for CHLR sorption produced good correlation to the Freundlich model with R^2 = 0.9763 (Table 4.15), implying that the sorption of CHLR molecules occurs on a heterogeneous surface. The experimental data also showed good fit with the Langmuir and Temkin models with correlation coefficient values (R^2 > 0.95), hence the sorption of the adsorbate may to a good extent be explained by Langmuir and Temkin model indices. The result suggests that the sorption may have also occurred on a monolayer surface having a limit of sorption due to saturation of the heterogeneous adsorbent surface. The Temkin model also assumes a possibility for a linear decrease in the heat of adsorption process as the adsorbent surface gets saturated. Rahardjo *et al.* (2011) explained that, if the experimental data fits well into the Temkin isotherm model it implies that the heat of adsorption for the sorption decreases as the adsorbent surface coverage is enhanced. Thus, the sorption process for CHLR was assumed to proceed via a combination of chemisorption and physisorption onto heterogeneous surfaces, and attaining saturation at some point. However, the N_F value for the sorption of CHLR onto the adsorbent was <1, thus suggesting chemisorption to be the dominant mechanism responsible for the sorption of the adsorbate molecules. Also, the low R_L values obtained supports a favourable heterogeneous chemisorption process for the CHLR sorption onto GSAa. The Langmuir isotherm q_M value obtained for the sorption of CHLR is in agreement with the determined experimental value.

The experimental data for the sorption of the antibiotics using GSAb were tested using the three isotherm models. The resulting values for the correlative parameters are presented in Table 4.16.

Table 4.16: Isotherm parameters for the sorption of AMX, AMP and CHLR from solution using GSAb

GSAb		N_F	k_F		R^2
Freundlich	AMX	0.968	0.213		0.9843
	AMP	0.956	0.191		0.9873
	CHLR	0.819	0.176		0.9862
		q_M	R_L	k_L	R^2
Langmuir	AMX	2.135	0.05 – 0.16	0.174	0.9816
	AMP	1.706	0.84 – 0.92	0.095	0.9860
	CHLOR	1.213	0.63 – 0.90	0.303	0.9841
		B	k_{TEM}		R^2
Temkin	AMX	74.870	0.764		0.9131
	AMP	64.778	0.822		0.9430
	CHLR	43.376	2.953		0.7675

For the equilibrium sorption data of AMX using GSAb, the correlation coefficient values of the Langmuir and Freundlich isotherms (R^2 > 0.98) were greater than that of the Temkin isotherm (R^2 >0.91). This means that adsorption of AMX molecules onto GSAb occur on a heterogeneous monolayer surface with the reaction attaining saturation after equilibration. The Temkin isotherm also suggests a decrease in the heat of adsorption with surface coverage of GSAb with AMX. The values obtained for the Freundlich constant N_F for AMX (N_F = 0.968) shows that the adsorption process is controlled by chemisorption since N_F value is below 1. The calculated values for the dimensionless Langmuir constant, R_L, falls between 0 and 1, indicating that AMX adsorption onto GSAb was favourable. The maximum monolayer adsorption capacity of AMX onto GSAb based on Langmuir model was 2.135 mg/g. Findings from the studies of Putra *et al.* (2009) and Moussavi *et al.* (2013) suggests that both Langmuir and Freundlich models could equally well-fit the results of tests of adsorption of AMX onto a commercial activated carbon with maximum adsorption capacity at 222 mg/g. Moussavi et al. (2013) further achieved higher adsorption capacity (262 mg/g) for the sorption of AMX onto NH_4-Cl induced carbon which is significantly greater than with the adsorbents previously tested.

The Langmuir and Freundlich models provided the best fit for the adsorption of AMP onto GSAb, hence the sorption of AMP onto GSAb can be described to proceed on a heterogeneous surface with a monolayer coverage that has a limited adsorption capacity. Subramanyam & Das (2009) and Xia *et al.* (2013) reported that where there is an agreement between the Langmuir isotherm equation and the experimental data, it could be assumed that the sorption process may occur via chemisorption. The kinetics experiment of AMP sorption onto GSAb showed that this result corresponds to the hypothesis of the pseudo-

second order model. Calculated values of N_F (0.956) and R_L values indicated that the sorption process was influenced by chemisorption mechanisms and it was favourable.

Adsorption of CHLR onto GSAb is compatible with both Langmuir and Freundlich isotherms (R^2 > 0.98). The correlation coefficient value obtained using the Temkin isotherm model could not be strongly related in the description of the sorption mechanism since the R^2 value (0.7675) was too low compared to those of Langmuir and Freundlich isotherms. Application of Langmuir and Freundlich isotherms in describing the adsorption of CHLR onto GSAb suggest that adsorption involved monolayer coverage and occurred on heterogeneous surfaces. The Freundlich heterogeinity value (N_F=0.819) was below 1 indicating that the CHLR adsorption onto GSAb was a chemically favoured reaction. The Langmuir values of R_L between 0 and 1 also showed that the sorption of CHLR onto GSAb was a favourable process. From the Langmuir model, the maximum adsorption capacity (q_M) was determined to be 1.213 mg/g, and was in agreement with the experimental value. This is relatively smaller than the reported value in the previous findings of Liao et al. (2013), who reported a q_M value of 8.10 mg/g for CHLR adsorption on bamboo charcoal; and Zhang et al. (2013) who reported 147.84 mg/g for CHLR adsorption on BSA/Fe_3O_4 magnetic composite microspheres.

The results obtained from the sorption process of AMX, AMP and CHLR from solution onto GSB was fitted into the Freundlich, Langmuir and Temkin models and presented in Table 4.17.

Table 4.17: Isotherm parameters for the sorption of AMX, AMP and CHLR from solution using GSB

GSB		**N_F**	**k_F**		**R^2**
	AMX	0.985	0.195		0.9865
Freundlich	AMP	0.902	0.174		0.9885
	CHLR	0.969	0.197		0.9769
		q_M	**R_L**	**k_L**	**R^2**
	AMX	1.505	0.14 – 0.67	0.004	0.9987
Langmuir	AMP	1.316	0.75 – 0.91	0.014	0.9824
	CHLOR	2.551	0.32 – 0.61	0.022	0.9779
		B	**k_{TEM}**		**R^2**
	AMX	48.966	0.621		0.9607
Temkin	AMP	45.903	0.660		0.9624
	CHLR	64.797	0.850		0.9211

Data for the sorption of AMX using GSB fitted well into all isotherm models that were studied (R^2 > 0.96). However, the Langmuir isotherm gave the highest R^2 value (0.9987) suggesting that physical sorption was the most influential mechanism for the sorption process of AMX onto GSB. Based on this data, the adsorption of AMX can be described to involve a monolayer surface coverage that is limited by saturation of the adsorbent surface. However, the Freundlich and Temkin isotherm models also influenced the sorption process of AMX since they gave high correlation coefficients (R^2>0.96). According to Yang *et al.* (2015) and Pezoti *et al.* (2016); adsorption data fitting well into the Freundlich and Temkin isotherm models suggest that such sorption process occurs on a heterogeneous surface with a reduction in the heat of adsorption as the sorption process proceeds, provided that the adsorbent surface sites consists of a spectrum of different binding energies. Furthermore, the Freundlich constant parameter N_F (0.985) implied that the sorption of AMX was influenced strongly by chemisorption (N_F<1). The process was favourable as indicated by the R_L values ranging between 0.14 and 0.67 for the concentration range studied. GSB showed a maximum monolayer chemisorptive adsorption capacity for AMX of 1.505 mg/g, which was in agreement with the experimentally determined value.

The Freundlich and Langmuir isotherms better described the adsorption process of AMP onto GSB (R^2>0.98). This means that adsorption occurred on a heterogeneous surface with the limit of uptake of the adsorbate molecules from solution, determined by the saturation of the monolayer of the adsorbate molecules. Data also fitted into the Temkin isotherm model (R^2 = 0.9624) implying that the sorption process was significantly influenced by a linear reduction in the heat of adsorption with increased surface coverage. Dutta *et al.* (1997) suggested that if data of a sorption process fits well into the Temkin isotherm model, an electrostatic interaction mechanism may be involved in the sorption of the adsorbate. Hence, there could be electrostatic interaction between AMP molecules and the GSB surface. The parameters calculated from the Langmuir and Freundlich models are presented in Table 4.17. The value of the Freundlich parameter N_F was <1 implying a strong influence of affinitive chemical reactions in the sorption process. This supports the similar effect deduced from the influence of the Freundlich and Temkin isotherm models. The Langmuir's dimensionless constant, R_L (0.75 – 0.91) revealed that the adsorption of AMP onto GSB was favourable. According to the Langmuir model, the maximum adsorption capacity of AMP (q_M) was 1.316 mg/g, which was slightly lower than the equilibrium experimentally adsorbed quantity of AMP.

The highest correlation coefficients obtained for the adsorption of CHLR using GSB were derived from the Freundlich and Langmuir isotherm plots (R^2 > 0.98). Both models better described the adsorption processes of the adsorbate onto GSB. This suggests that the

adsorption process occurred on a heterogeneous surface with the CHLR molecules forming a monolayer on GSB reaching a maximum sorption limit after saturation. Pietrzak & Bandosz (2007) explained that a high value of N_F represents more highly affinitive adsorption process; hence the sorption of CHLR onto GSB is a slightly affinitive chemical process since the calculated N_F value for CHLR was lower than 1. Low values of Langmuir constant, R_L (0.32 – 0.61), indicate that the chemisorption of CHLR was favourable.

Table 4.18 shows the results obtained from the calculations of the Freundlich, Langmuir and Temkin isotherm constants from the adsorption of AMX, AM and CHLR using GS.

Table 4.18: Isotherm parameters for the sorption of AMX, AMP and CHLR from solution using GS

GS		**N_F**	**k_F**		**R^2**
	AMX	2.715	0.115		0.8579
Freundlich	AMP	1.654	0.091		0.8720
	CHLR	0.591	0.191		0.9580
		q_M	**R_L**	**k_L**	**R^2**
	AMX	1.479	0.57 – 0.12	0.092	0.9425
Langmuir	AMP	1.326	0.30 – 0.59	0.023	0.9663
	CHLOR	1.093	0.16 – 0.39	0.052	0.9550
		B	**k_{TEM}**		**R^2**
	AMX	23.747	0.868		0.9115
Temkin	AMP	32.627	1.005		0.9590
	CHLR	46.477	1.437		0.9486

The experimental data for the sorption of AMX using GS conforms better to the Langmuir and Temkin isotherms (R^2>0.91). Adsorption of AMX using GS could thus be said to have proceeded via a monolayer surface coverage with limited uptake of adsorbate as the heat of adsorption decreases due to continued sorption. Although the correlation coefficient value of the Freundlich isotherm model was lower (R^2=0.8579) than those of Langmuir and Temkin models, the sorption of AMX may also be considered to occur on a heterogeneous surface. In the case of AMX adsorption onto GS, the N_F constant value was above 1 (N_F=2.715), indicating a strongly affinitive favourable physical adsorption sorption process occurring on a heterogeneous surface. R_L values of AMX using GS ranged from 0.12 to 0.57 showing that the physisorption of AMX was favoured. Also, a fairly low maximum uptake value for the sorption of AMX from solution using GS was obtained. The value lies closer to the experimental value suggesting that the Langmuir isotherm better fitted the experimental data.

Langmuir and Temkin isotherm models fitted well to the experimental data for AMP sorption. The Langmuir model suggests that sorption proceeded with the adsorbates covering a monolayer of the adsorbent surface until a maximum limit uptake was attained. On the other hand, the Temkin isotherm suggests that as the adsorbent surface coverage is enhanced, the heat of adsorption for the sorption decreases. An N_F value greater than 1 was calculated, suggesting the sorption was governed by a high affinity physisorption mechanism. The efficiency of adsorption was tested by calculating the R_L values, and it showed that the process was favourable. The values obtained with all the parameters for the different isotherms imply that AMP adsorption onto GS is a physical process occurring on a monolayer surface with a limited uptake after equilibrium is achieved.

CHLR sorption data fitted into all the isotherm models with insignificant differences in their R^2 values ($\rho<0.02$). The Freundlich and Langmuir models fitted the adsorption isotherms as supported by their high R^2 values, implying a monolayer sorption on a heterogeneous surface with limited sorption capacity after equilibrium is attained. Also, the heat of the adsorption process decreased linearly as the surface of the adsorbent was covered with CHLR. A measure of the heterogeneity for the adsorption process was determined by calculating the N_F value. The result showed that the sorption of CHLR was controlled by chemisorption since N_F value (0.591) was lower than 1. The range of R_L values was low (<0.50) showing that it was a strongly favoured reaction. These results suggest that CHLR adsorption is better described as a heterogeneous monolayer surface adsorption process influenced by chemical adsorption.

In general, the majority of the adsorption processes for the adsorbates onto the different adsorbents (GSAa, GSAb, GSB and GS) proceed via chemisorption than physisorption onto heterogeneous adsorbent surfaces where the adsorbates formed monolayers. The isotherm parameters also assisted and were observed to be in agreement with the determination of the feasibility of the adsorption processes.

4.3.4 Effect of pH

The pH of a solution is a critical factor in adsorption as it influences the adsorption capacity of the adsorbate onto the adsorbent (Fakhri & Adami, 2014). Solution pH remains one of the most crucial factors affecting the adsorption capacity of activated carbons, because it influences both the chemistry of the adsorbent surface groups and the adsorbate functional groups (Guedidi *et al.,* 2013). When the pH value is higher than pKa value of the adsorbate molecule, the molecule will be present in its anionic form and vice versa (Guedidi *et al.,* 2013). Generally, the adsorption of ionizable organic contaminants like antibiotics is affected by pH due to the varied species (Zhang *et al.,* 2010; Li *et al.,* 2014). The adsorbates under

study (AMX, AMP and CHLR) have positively charged (cationic), negatively charged (anionic), and/or zwitterionic species at different pHs due to their different p*K*a values (Gu & Karthikeyan, 2005; Li *et al.,* 2014).

In instances where opposite charges exist between the adsorbent surface and the adsorbate molecule's functional groups, electrostatic attractions are assumed to be the most influential mechanism for sorption. Electrostatic interactions which control the sorption of ionic compounds have been successfully used to interpret the sorption of some antibiotics onto activated carbon surfaces (Yang & Xing, 2010; Zhang *et al.,* 2010; Li *et al.,* 2014). Where both the adsorbent surface and the adsorbate molecules carry similar charges, electrostatic repulsion is expected to occur. However, an enhanced sorption may sometimes be observed under such circumstances, and this can be explained by assuming the possibility of mechanisms, involving π–π electron-donor-acceptor (EDA) interaction, hydrogen bonding or π-hydrogen bonding. For chemical compounds containing olefinic groups, benzene ring(s) and other aromatics; one of the driving forces for their sorption involves π–π electron-donor-acceptor (EDA) interactions. Mechanisms involving hydrogen bonding widely exists in the sorption processes of polar organic pollutants onto activated carbon (Yang & Xing, 2010; Li *et al.,* 2014). Functional groups of organic chemicals can act as hydrogen bonding donors and form hydrogen bonds with the adsorbent surface groups, where the benzene rings can act as hydrogen bonding acceptors (Yang *et al.,* 2008). The β-lactam antibiotics (AMX, AMP and CHLR) contain C=O, -OH, $-NH_2$ and -NH groups which could interact with oxygen-containing functional groups on the surface of the adsorbents through hydrogen bonding as hydrogen bonding donors (Wang *et al.,* 2010).

In this study, the efficiency of adsorption for the antibiotics from solutions with variable pH values onto GSAa, GSAb, GSB and GS was investigated. This was essential since the solution pH affects both the surface binding sites of the adsorbents and the aqueous chemistry (Ali *et al.,* 2012; Shaibu *et al.,* 2014). Researchers such as Al-Degs *et al.* (2008) and Ayanda *et al.* (2013) who studied the adsorption of reactive dyes and tributyltin respectively onto activated carbon reported supporting that the pH of the solution governed the adsorption of adsorbates onto the adsorbents. This is because pH affects both the adsorbent and adsorbate chemistry in a solution. Thus, the degree of ionization of the adsorbates is primarily affected by the hydrogen ion concentration (pH), which in turn affects the surface properties of the adsorbent (Ali *et al.,* 2012; Shaibu *et al.,* 2014).

Figure 4.21 shows influence of solution pH on AMX, AMP and CHLR adsorption onto GSAa. The sorption of the antibiotics was highly dependent on the initial pH of the solution.

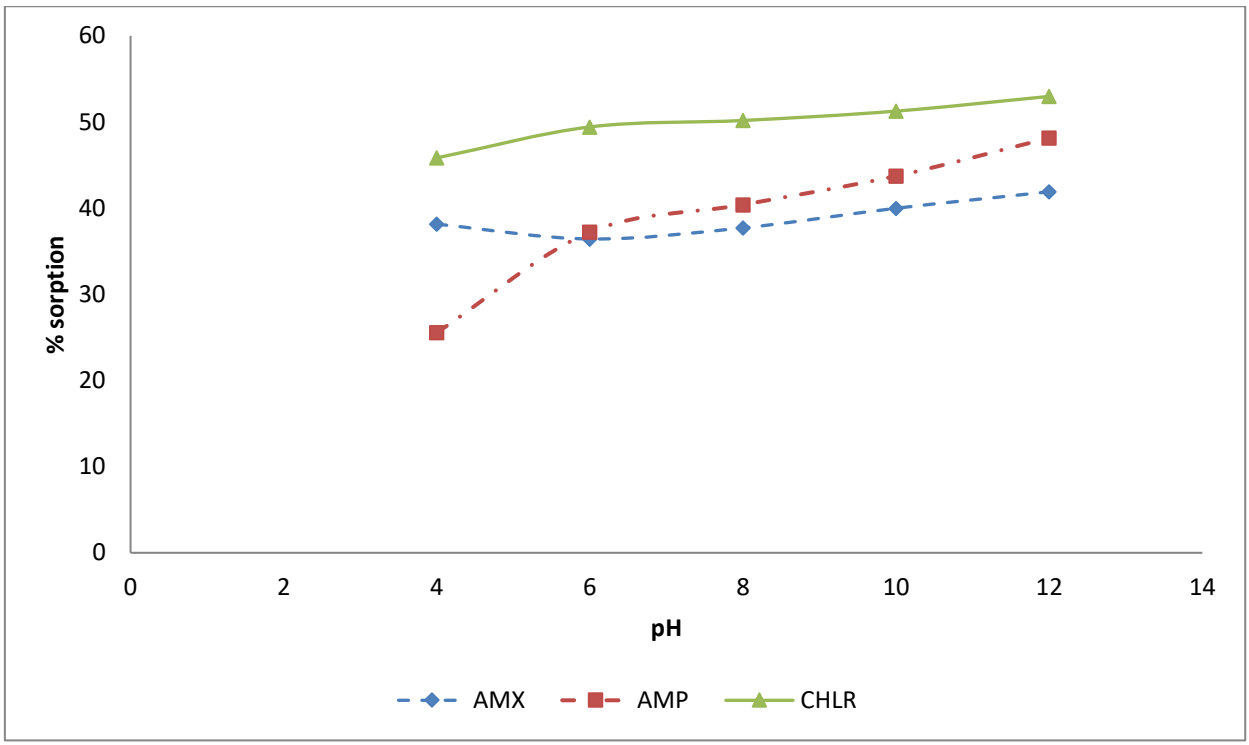


Figure 4.21: Effect of solution pH on the adsorption of AMX, AMP and CHLR from solution using GSAa

A semi-parabolic curve was obtained in testing the effect of pH on the sorption of AMX from solution at different pHs. The quantity of AMX sorbed onto GSAa increased from 38.15 % at pH 4 to 41.90 % at pH 12 except for pH 6 which gave the least adsorption of 36.43 %. This showed that as the solution pH increase, the sorption capacity of the adsorbent increases. At lower pH levels (pH 4), the adsorbent surface carries a net positive charge due to an excess of H^+ ions in solution. According to Zhu *et al.* (2000), at pH 4 the carboxyl group (-COOH) present in the AMX molecule gets dissociated into a carboxylate ($-COO^-$) (solution pH > pKa of AMX: 2.67). Thus, electrostatic attraction is likely to occur between the GSAa surface (positively charged) and the anionic AMX molecules (through $-COO^-$ functional group). This electrostatic attraction could be the predominant mechanism influencing the sorption of AMX with GSAa. At pH 6 the surface of the adsorbent is assumed to have a fair concentration of cations and anions while the AMX molecules exist as zwitter-ions at pH values between 5.36 and 7.27 (Putra *et al.,* 2009). Hence a drop noted in the sorption quantity of AMX at pH 6 may be a result of electrostatic repulsions between the AMX zwitter-ions and the negative charges on GSAa surface. As the solution pH was increased above 6, increased quantities of AMX adsorbed could probably be due to the possibility of electron pairing between the AMX molecules and the functional groups present on the GSAa surface. Adriano *et al.* (2005) who worked on the sorption of AMX using chitosan beads reported that in highly alkaline solutions, electron-donor-acceptor (EDA) π-π interactions occurred between the deprotonated amine groups on AMX ($-NH_3 \rightarrow NH_2$) and the carbonyl groups, anionic groups or the negative charges on the adsorbent surfaces resulting from excess OH^- ions in solution.

The adsorption trend of AMP onto GSAa indicates that the amount of adsorbate retained on the adsorbent increased with increasing solution pH (from 25.56 % at pH 4 to 48.14 % at pH 12). Thus basic medium is favourable for the adsorption process of AMP with GSAa.

The sorption of CHLR with GSAa was higher compared to the other adsorbates at all solution pH values. The highest quantity of CHLR obtained with GSAa was 52.97 % at pH 12. The enhanced affinity of GSAa for CHLR may be explained by considering the influence on sorption from the functional groups found on the structure of the adsorbate.

In addition to the benzene ring in the molecular structure of CHLR, the molecule consists of carbon atoms which are organised in such a way that the nitro group enhances the aromatic structure. Therefore, some π-π electron interaction between CHLR and the functional groups on GSAa surface could thus exert a major influence on the adsorption process. Also, the high organic content on the surface of GSAa could aid in the improved sorption capacity of GSAa for CHLR for the solution pH range concerned. Pan & Xing (2008) and Fan *et al.* (2010) reported that the nitro group in CHLR aided in the sorption of the molecule via EDA π-π interactions provided there were electron-donor groups on the surface of the adsorbent. Based on this, the nitro group in CHLR and the carbonyl or alky groups in GSAa may have functioned as electron-acceptor and electron-donor respectively, leading to an enhanced sorption of CHLR onto the adsorbent.

The effect of pH on the quantity of adsorbates adsorbed from solution using GSAb is shown in Figure 4.22. The quantity of AMX, AMP and CHLR adsorbed from solution increased with increasing pH. This implies that the adsorption efficiency of GSAb for the antibiotics improved with an increasing solution pH. The highest amounts of AMX, AMP and CHLR adsorbed by GSAb at pH 12 were 39.63 %; 49.73 % and 49.05 % respectively.

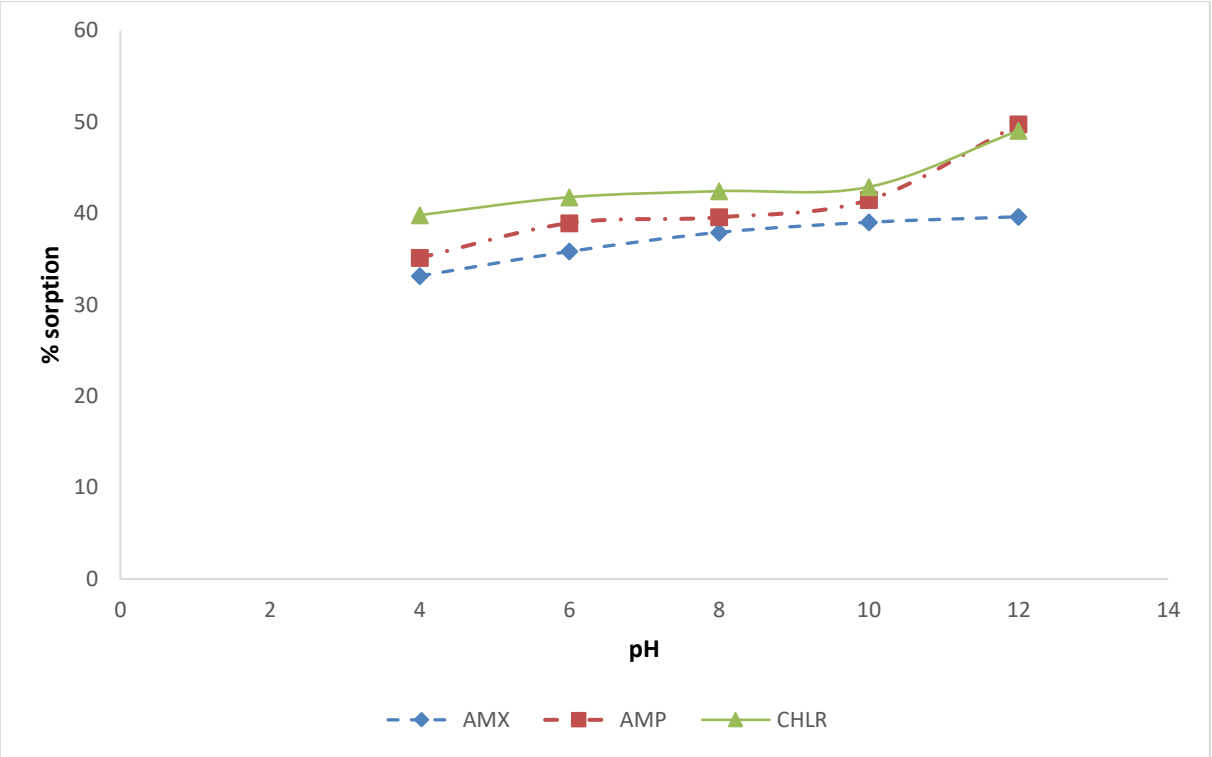


Figure 4.22: Effect of solution pH on the adsorption of AMX, AMP and CHLR from solution using GSAb

Adriano *et al.* (2005) explained that AMX often has a net positive charge below isoelectric point, and has a net negative charge above isoelectric point. Goddard *et al.* (1996), suggested that this may be due to the ionisation of its functional groups, i.e. the carboxyl (pKa=2.68); amine (pKa=7.49) and phenolic hydroxyl (pKa=9.63). Therefore the increasing adsorption of AMX with increase in pH may be due to the structure of AMX shifting its charges on the functional groups of the molecule from a cation to a zwitter-ion to an anion at different pHs. The mechanisms that could assist the sorption of AMX in this state would be electron sharing, π-bonding or hydrogen bonding. Furthermore, AMX adsorption showed an insignificant increase when solution pH was increased from 8 to 12. This may be due to AMX having a net negative charge as solution pH increased, leading to a reduced interaction between AMX and GSAb due to repulsion.

The influence of solution pH on the adsorption of AMP using GSAb is shown to be dependent on the solution pH (Figure 4.22). At solution pH 4, the low quantity of AMP that was retained from solution is assumed to be due to a repulsive force prevailing between the cationic species of the AMP molecules in solution and the net-positive charge on the surface of GSAb. The adsorbed amount of AMP increased between solution pH 6 (38.94 %) and pH 10 (42.89%). Fan *et al.* (2016) explained that over this pH range, the antibiotic exist as a zwitter-ionic specie. As a result the adsorption of AMP at pH between 6 and 10 is assumed to be promoted onto the GSAb surface via hydrogen bonding. Fan *et al.* (2016) reported that

where an adsorbate assumes a zwitter-ionic form at neutral to high pHs, adsorption is facilitated either through hydrogen bonding or EDA π-π dispersion interaction between the aromatic ring of AMP and the delocalised π electrons present on the adsorbent surface. The sharp increase in the sorption of AMX at pH 12 may thus be attributed to the π-π interactions or hydrogen bonding between the negatively charged AMP molecules and the excess OH^- ions on the surface of GSAb. In addition to the hydroxyl ions was the high concentration of organic content on the surface of GSAb which could also have aided in the enhancement in the sorption process by improving the functionality of the adsorbent.

Sorption capacities of GSAb for CHLR were fairly similar for the solution pH range 4 – 10, after which a sharp increase in the amount of CHLR retained from solution was observed at pH 12. The adsorption was assumed to have been strengthened by hydrogen bonding or electrostatic attractions between CHLR and GSAb due to the enhanced oxygen and organic content of the adsorbent since it was higher than that of GSAa (Pan & Xing, 2008).

Figure 4.23 shows influence of solution pH on sorption at equilibrium using GSB. The amount of adsorbates adsorbed (q_e) onto GSB is a function of the effect of the initial pH of solutions. However, the quantity of AMX, AMP and CHLR adsorbed over the pH range 4 – 12 was nearly constant with no significant differences with further increase in pH.

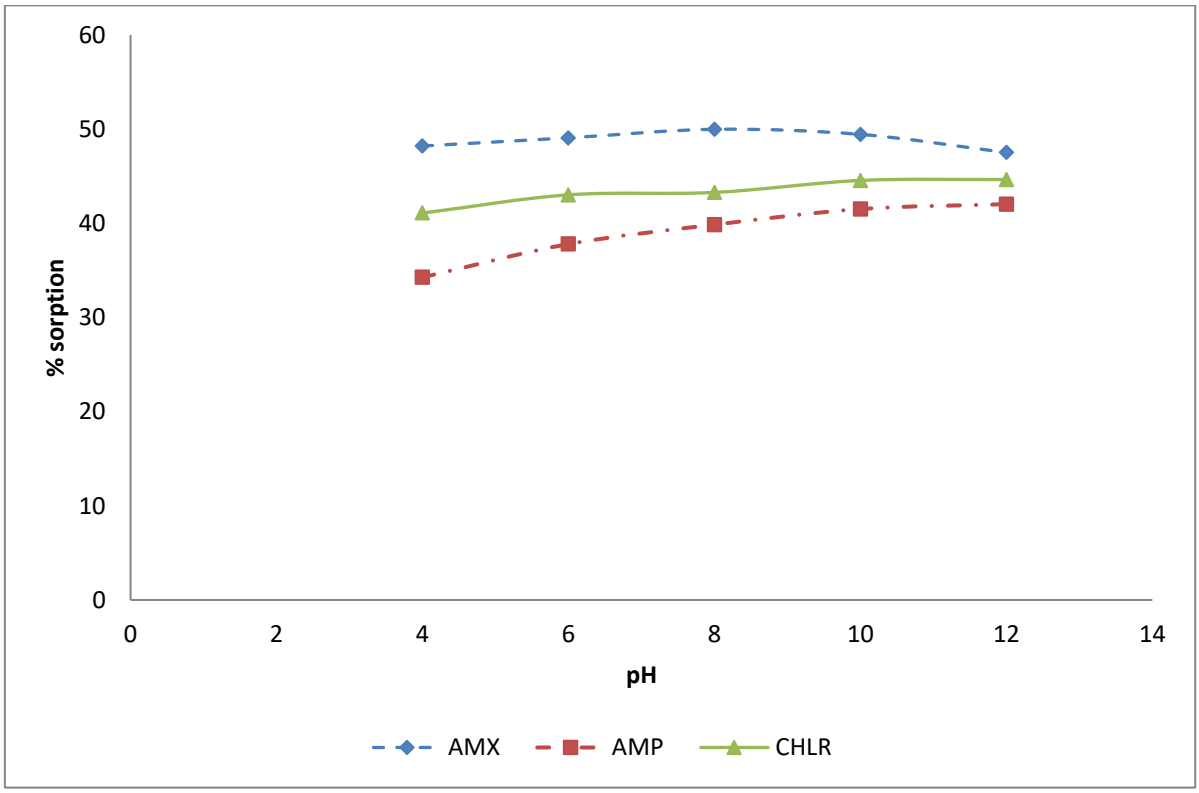


Figure 4.23: Effect of solution pH on the adsorption of AMX, AMP and CHLR from solution using GSB

GSB showed a steady increase in the sorption of AMX when the solution pH was varied between 4 and 8, which then decreased as solution pH was raised from 8 to 12. This suggests that AMX sorption was slightly better enhanced at near neutral pH values. The enhanced sorption of AMX at pH between 4 and 8 could possibly be as a result of electrostatic attraction between the AMX anionic molecules and the positively charged surface of GSB. According to Dousa & Hosmanova (2005), a reduction in the sorption quantity of an adsorbate in highly alkaline solutions may be a result of increased competition for adsorption sites on the surface of the adsorbent between the anions in solution. As the pH of the solution was increased from 8 to 12, the concentration of the OH^- ions also increased. Thus the reduced sorption efficiency of GSB for AMX in solutions of high pH could be due to the subsequent competition between the COO^- anions on AMX molecules and the OH^- ions for active adsorption sites on the surface of GSB. Increased in electrostatic repulsion between the anions of AMX and the negative charge on the surface of GSB may also result in decreased sorption efficiencies at high pH.

The steady increase obtained in the quantity of AMP removed from solution with increasing solution pH may be related to mechanisms including hydrogen bonding and π-π EDA interactions between the ionic species of AMP and the GSB surface which constitutes of the O-H and C=O; C=C or C-O functional groups as observed on the FTIR spectrum of GSB.

Furthermore, the hydroxyl (O-H) group on the surface of GSB (due to treatment with KOH) might influence the observed increase in sorption of CHLR using GSB. The mechanism of sorption may involve interactions between oxygen molecule of the hydroxyl group and the π - π electrons of the CHLR benzene ring leading to an EDA interaction between GSB and CHLR. Besides the π-π interaction, hydrogen bonding could also have exerted an influenced on the adsorption of CHLR. This is because various functional groups in the molecule of CHLR and the surface functional groups on GSB can form hydrogen bonding (i.e. between N-H, O-H-NH_2 groups in CHLR and –OH, C=O, C=C groups on GSB).

The effect of solution pH on the adsorption capacity of GS for AMX, AMP and CHLR is presented in Figure 4.24.

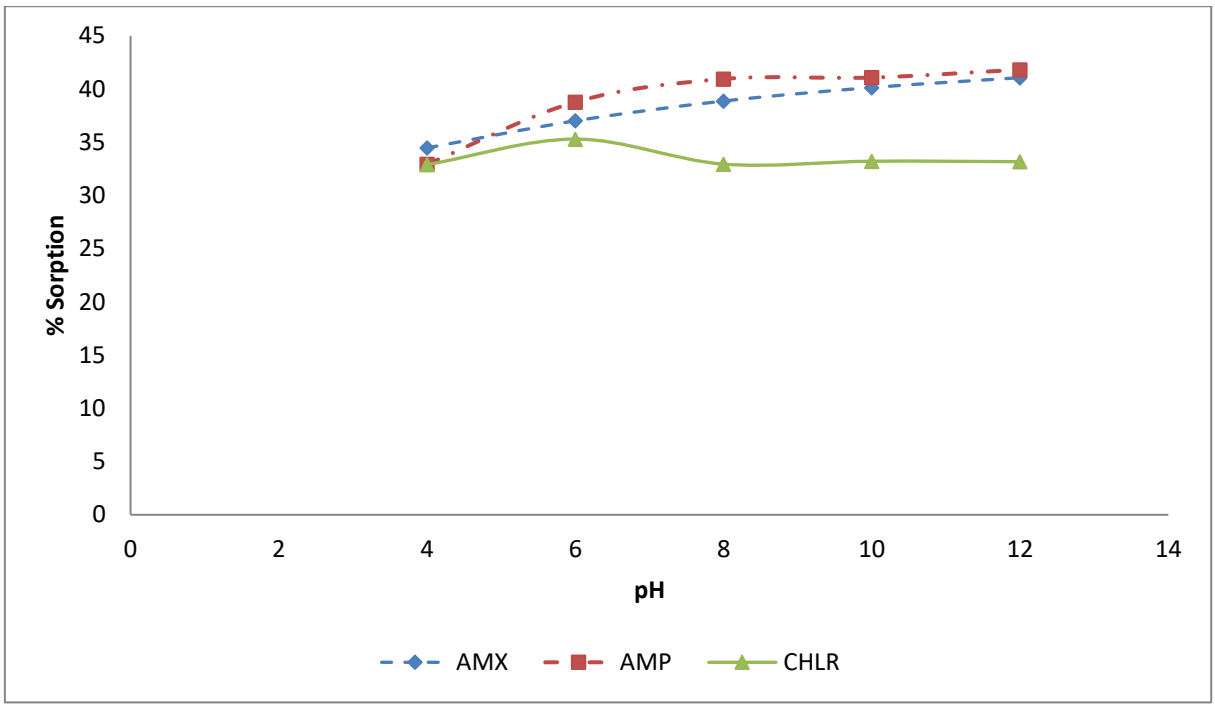


Figure 4.24: Effect of solution pH on the adsorption of AMX, AMP and CHLR from solution using GS

The sorption of AMX and AMP increased steadily from 34.47 % and 32.90 % at pH 4 to 41.07 % and 41.82 % at pH 12 respectively. The adsorbed quantity of CHLR increased from 32.90% to 35.30 % between pH 4 and 6, after which it dropped significantly as pH increased above pH 6. However, no appreciable difference was observed in the quantity of CHLR adsorbed onto GS when the solution pH was increased between 8 and 12 (32.95 % - 33.17 %).

The surface of GS contains various functional groups including CH_2, C=O and C-O groups, with high oxygen content which is capable of improving adsorption processes via mechanisms like π-bonding or electron-pair donation. The interaction between AMX in solution and the GS surface is assumed to involve electrostatic attraction between the ionic forms of AMX and the charged surface of GS. Mechanisms including the π-π EDA interaction and hydrogen bonding may also contribute in the sorption process. This probably explains the observed trend in the sorption of AMX as solution pH was increased.

AMP adsorption showed a sharp change in sorption quantity between pH 4 and 8. At pH lower than 6, the AMP molecules and the surface of GS were positively charged. This adsorption was assumed to have occurred through electron pairing between the adsorbent surface and AMP ions. At solution pH 8, the antibiotic existed in its zwitterionic form, thus, hydrogen bonding and EDA π-π interactions could probably have promoted the adsorption of AMP. However, there was no significant difference in the quantity of AMP retained from

solution at pH values above 8. In a report published by Adriano *et al.* (2005), it was noted that AMP molecule shifts from a zwitter-ion to having an anionic form at pH values above 8. Therefore at pH > 8, AMP molecule was assumed to shift into an anion, causing a reduction in GS and AMP molecules interaction due to electrostatic repulsion between the adsorbent and adsorbate molecule.

The adsorption of CHLR was observed to have been strongly affected by changes in solution pH. The highest adsorbed CHLR quantity was obtained at solution pH 6. This was close to the original pH of the adsorbent (6.61). CHLR is made up of a nitrobenzene ring, an amide bond and an alcohol functional group. Once dissolved in water, the dissociation of the hydroxyl leaves CHLR in the state of positive charge that is attracted to the negative charge of the GS surface. This 'pairing' activity consequently caused a higher accumulation of CHLR on the GS surface when solution pH was at 6. As the alkalinity of the solutions increased between pH 8 and 12, there was increased repulsion between the negatively charged nitro group on CHLR and the negatively charged surface of GS. Another possible explanation for the reduction in sorption capacity at high pH values could be the increased competition between the anionic form of CHLR (using the nitro group $–NO_2^-$) and the OH^- ions in solution for adsorption sites on GS, since OH^- ion concentration is increased in highly alkaline solution.

From all the pH studies conducted, it was noted that the initial pH of a solution has a great influence on the adsorption process of the adsorbates by the various adsorbents. A higher sorption capacity was recorded for higher solution pH, i.e. the alkaline medium was more favourable for adsorption compared to the acidic medium for generally all adsorbents.

4.3.5 Effect of temperature

The effect of temperature on adsorption capacity of adsorbent under optimized conditions is indicative and index whether the process is endothermic or exothermic (Munagapati & Kim, 2017). Temperature effect on the adsorption of the adsorbates (AMX, AMP and CHLR) onto the different adsorbents (GSAa, GSAb, GSB and GS) was investigated in batch experiments. Apart from the potential capacity of temperature to influence the adsorption process either positively or negatively, all antibiotics studied are temperature sensitive as most others that belong to the β-lactam pharmaceutical group.

The percentage removal of AMX, AMP and CHLR from solution by GSAa decreased with an increase in temperature (Table 4.19). Maximum sorption percentages for all adsorbates were obtained at 20 ^oC while the lowest sorption percentages were attained at 60 ^oC. Percentage removal decreased with increasing temperature; hence the adsorption of the antibiotics using

GSAa was better enhanced at low temperatures than at higher temperatures. Based on the results, the sorption of the antibiotics onto the adsorbent is exothermic.

Table 4.19: Effect of temperature on percentage removal of AMX, AMP and CHLR using GSAa

GSAa	% AMX	% AMP	% CHLR
20	37.62	33.74	38.16
30	29.66	27.11	26.04
40	12.95	15.08	21.22
50	7.82	10.12	13.31
60	4.82	7.97	9.78

Munagapati & Kim (2017) suggested that if the sorption efficiencies of an adsorbent for removal of adsorbates from solution decrease with increasing temperature, then that process is exothermic. Jorgensen & Halling-Sorensen (2000) and Xu *et al.* (2006) noted that antibiotic compounds tend to decompose at higher temperatures and this could possibly be responsible for the observed decrease in the adsorbed antibiotics quantities as temperature increases.

The thermodynamic spontaneity and feasibility of the adsorption process was assessed by calculating the three basic thermodynamic parameters viz., Gibbs free energy (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0) (Munagapati & Kim, 2017). The linear regression lines obtained from the Van't Hoff plots were used to determine ΔH^0 and ΔS^0 which in turn were used to determine ΔG^0. Figures 4.25 to 4.27 present that Van't Hoff plots for the adsorption of AMX, AMP and CHLR onto GSAa.

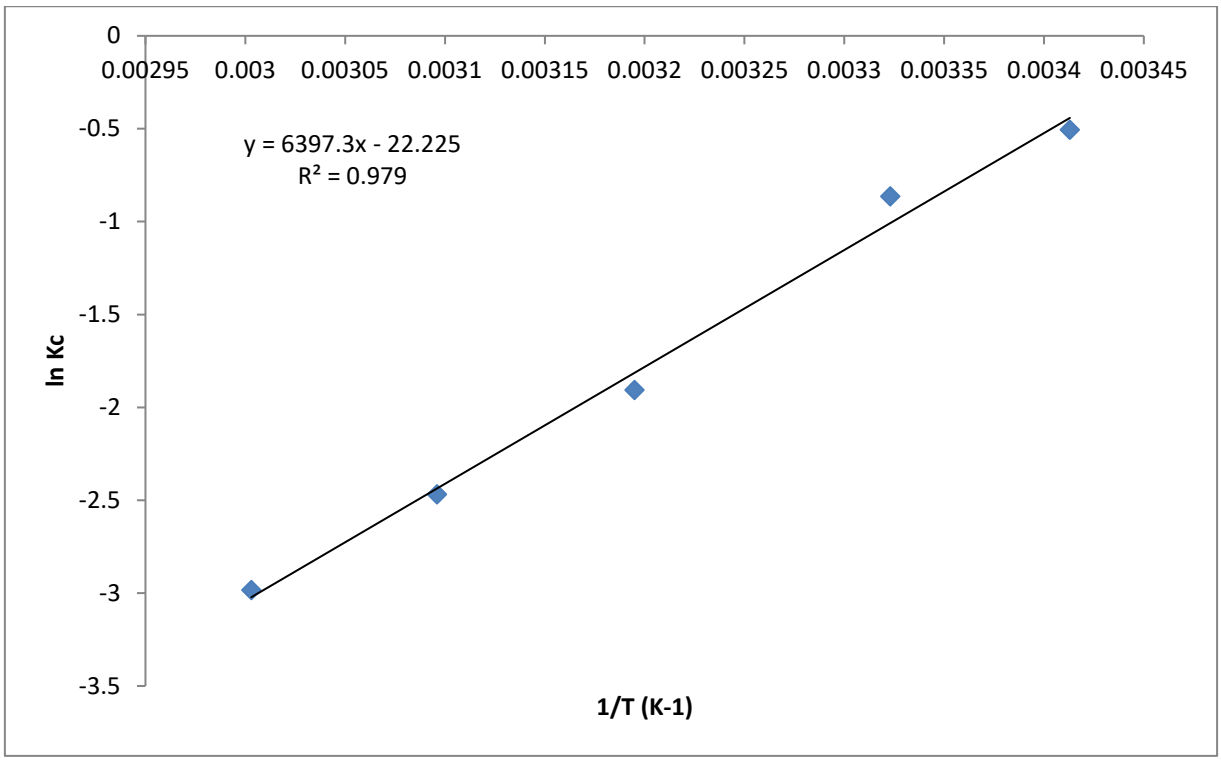


Figure 4.25: Van't Hoff plot for adsorption of AMX using GSAa

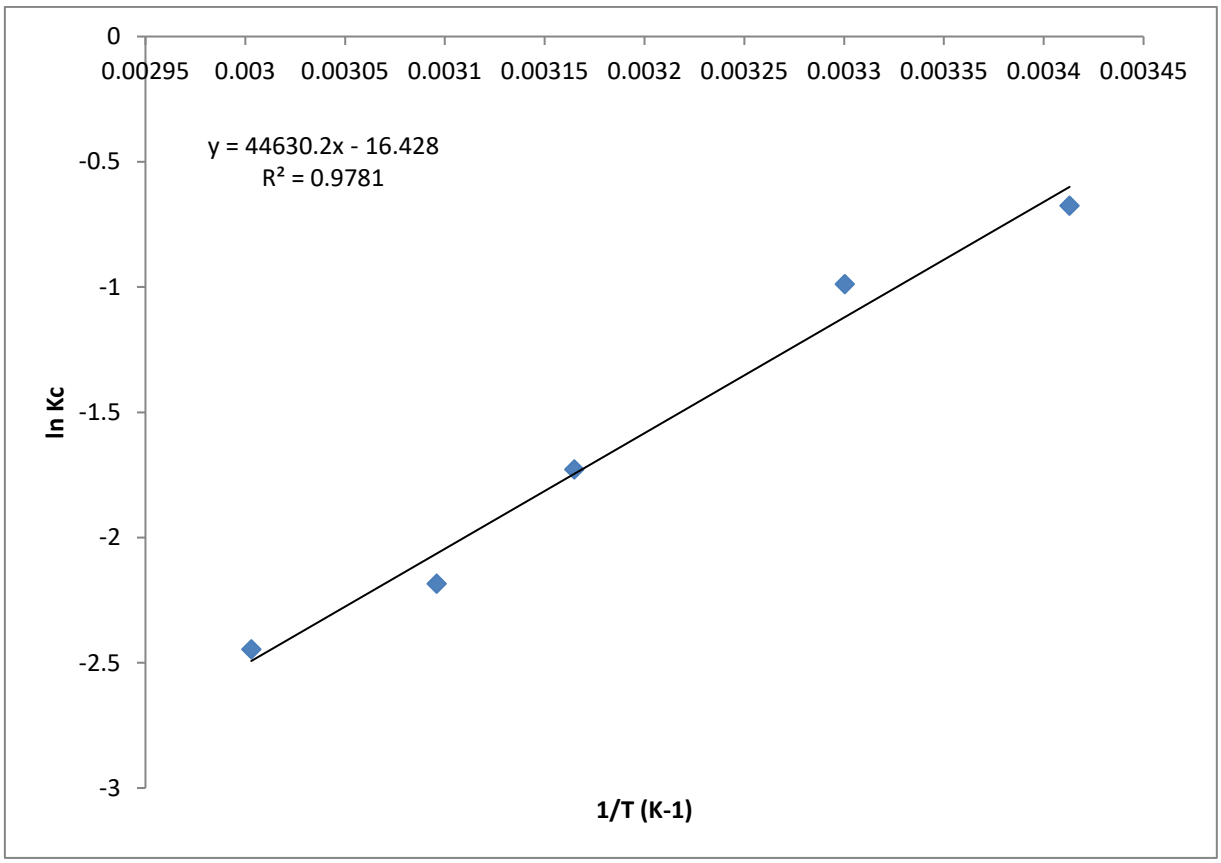


Figure 4.26: Van't Hoff plot for adsorption of AMP using GSAa

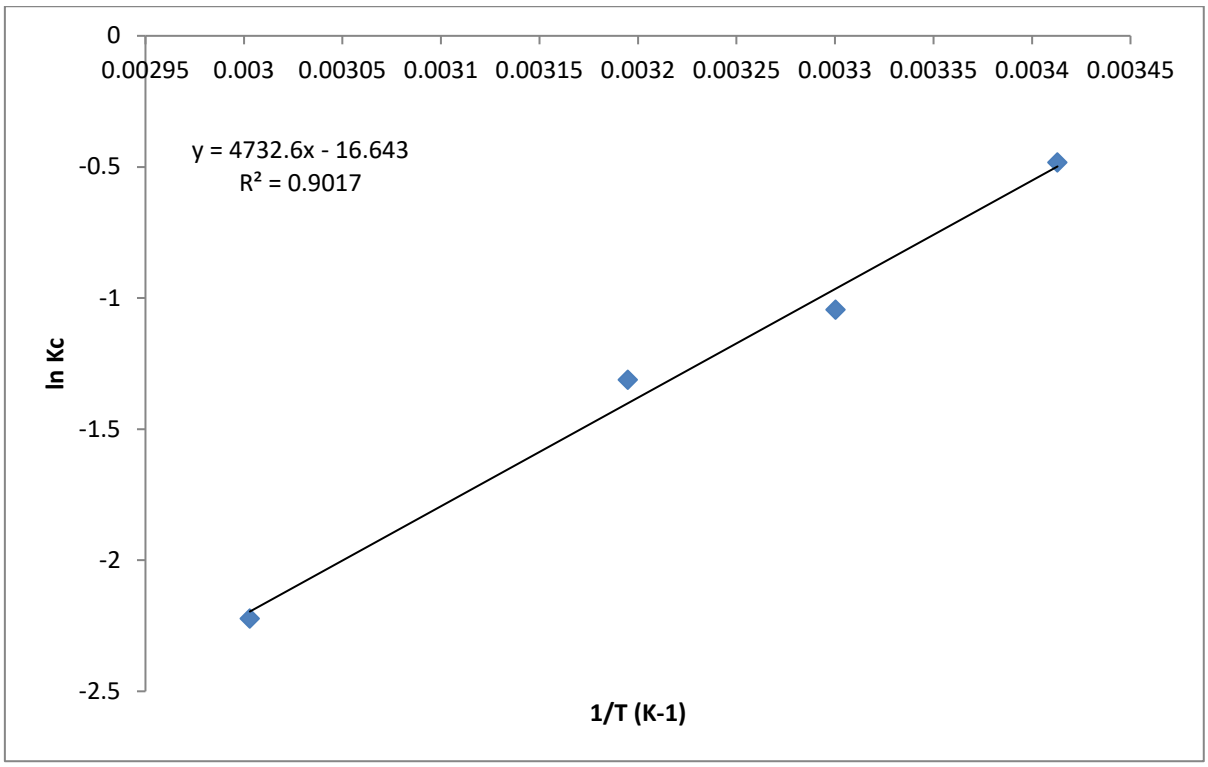


Figure 4.27: Van't Hoff plot for adsorption of CHLR using GSAa

The enthalpy change (ΔH^0), entropy change (ΔS^0) and Gibbs free energy (ΔG^0) values calculated for the sorption of the three antibiotics from solution onto GSAa are presented in Table 4.20.

Table 4.20: ΔH^0, ΔS^0 and ΔG^0 Values of Sorption Studies of AMX, AMP and CHLR using GSAa

Analyte	Conc. (mg/L)	ΔH^0 (kJ/mol)	ΔS^0 (J/Kmol)	ΔG^0 (kJ/mol)			
				293 K	303 K	313 K	323 K
AMX	30	-53.187	-184.779	0.9532	2.8010	4.6488	6.4966
AMP	30	-48.495	-136.582	1.5235	2.8893	4.2552	5.6210
CHLR	30	-67.250	-138.370	1.1964	2.5801	3.9638	5.3475

The negative values of all the adsorption enthalpy, ΔH^0, (which is the net result of enthalpy change, for molecular diffusion and repulsion between adsorbed molecules and desorption of solvent) indicate the adsorption process is exothermic with an affinitive binding of adsorbate molecules onto the adsorbent surface (Roosta *et al.,* 2014). In addition, Demirbas *et al.* (2004) reported that the ΔH^0 values for adsorption processes are approximately equal to the activation energy (E_a). Generally, a physisorption process usually involves activation energies in the range of 0 – 40 kJ/mol while higher activation energies (40 – 800kJ/mol) indicate chemisorption (Guo *et al.,* 2014; Fan *et al.,* 2017).

The ΔH^0 values obtained for the sorption of AMX, AMP and CHLR using GSAa were all negative. According to Roosta *et al.* (2014), the negative values of ΔH^0 suggest that the sorption of the adsorbates onto GSAa is exothermic. The results also agree with the observed negative effect on the efficiency of the adsorbent to retain antibiotics from solution as the solution temperature increased. Also, the ΔH^0 values obtained for the adsorption of the adsorbates were all above 40 kJ/mol, which indicate that the adsorption processes via a chemisorption reaction. Similar findings were reported by Chern & Wu (2001) and Li *et al.* (2002) on the sorption of dyes onto granular activated carbon and grapheme oxide respectively.

Negative values of ΔS^0 means there is a reduction in entropy whilst positive values of ΔS^0 implies that there is an increase in entropy. The entropy change (ΔS^0) values for the sorption studies of AMX, AMP and CHLR using GSAa, were all negative. Thus the entropy of the antibiotic molecules decreased during uptake onto adsorbent. El-Shafey *et al.* (2012) explained that reduction in entropy could lead to a decrease in the randomness at the solid-solution interface during the sorption of adsorbates onto the adsorbent surface.
Li *et al.* (2012) reported that a positive value of the Gibbs free energy (ΔG^0) implicates a non-spontaneous reaction/process whilst a negative value implicates a spontaneous reaction/process. The Gibbs free energy (ΔG^0) values obtained for the adsorption process of the antibiotics were all positive suggesting that adsorption of AMX, AMP and CHLR onto GSAa was non-spontaneous. However, due to the small magnitude of the ΔG^0 values, it is assumed that the non-spontaneous sorption processes were feasible.

The effect of solution temperature on the adsorption of the antibiotics from solution using GSAb was studied and results are presented in Table 4.21.

Table 4.21: Effect of temperature on percentage removal of AMX, AMP and CHLR using GSAb

GSAb	% AMX	% AMP	% CHLR
20	52.98	43.26	30.97
30	42.96	41.65	26.04
40	31.12	24.67	14.43
50	11.72	18.10	5.72
60	4.94	14.95	1.61

Results showed that GSAb gave a better affinity for AMX removal relative to AMP and CHLR. An increase in solution temperature results in reduced sorption percentages for the adsorbates. Sorption trends with temperature variation were similar to those observed for GSAa. Lower temperatures therefore enhance the sorption capacities of the adsorbent for the antibiotics compared to higher temperatures. Based on the observed sorption trends, the

sorption of antibiotics onto GSAb is an exothermic process. According to Karadag *et al.* (2006) and Moussavi *et al.* (2013), there is a possibility of desorption of adsorbates into solution from an adsorbent surface as temperature is increased, provided the adsorption process is exothermic. The decrease in sorption capacity of GSAb with increasing temperature may be due to desorption of the adsorbed antibiotics from the adsorbent surface back into solution.

Figures 4.28 - 4.30 presents that Van't Hoff plots for the adsorbates from solution onto GSAb. The values of the linear regressions obtained from the plots were used to determine the ΔH^0, ΔS^0 and ΔG^0.

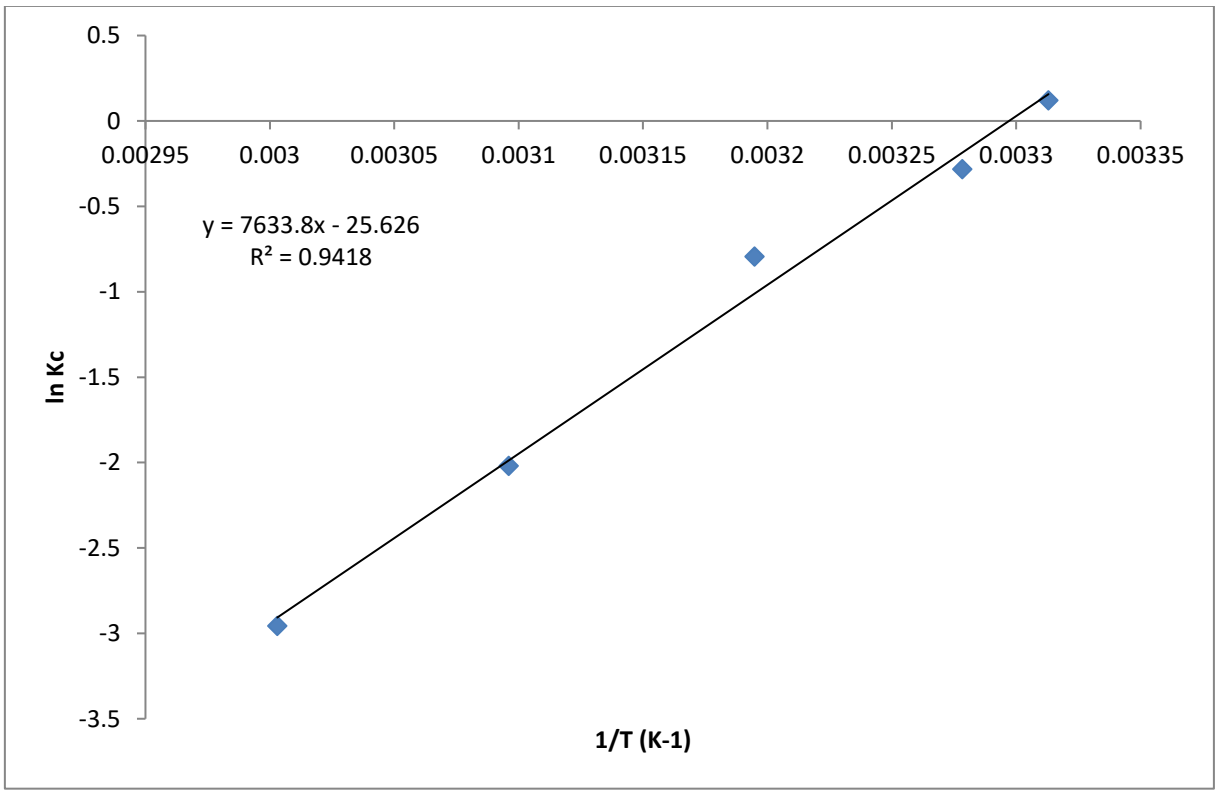


Figure 4.28: Van't Hoff plot for adsorption of AMX using GSAb

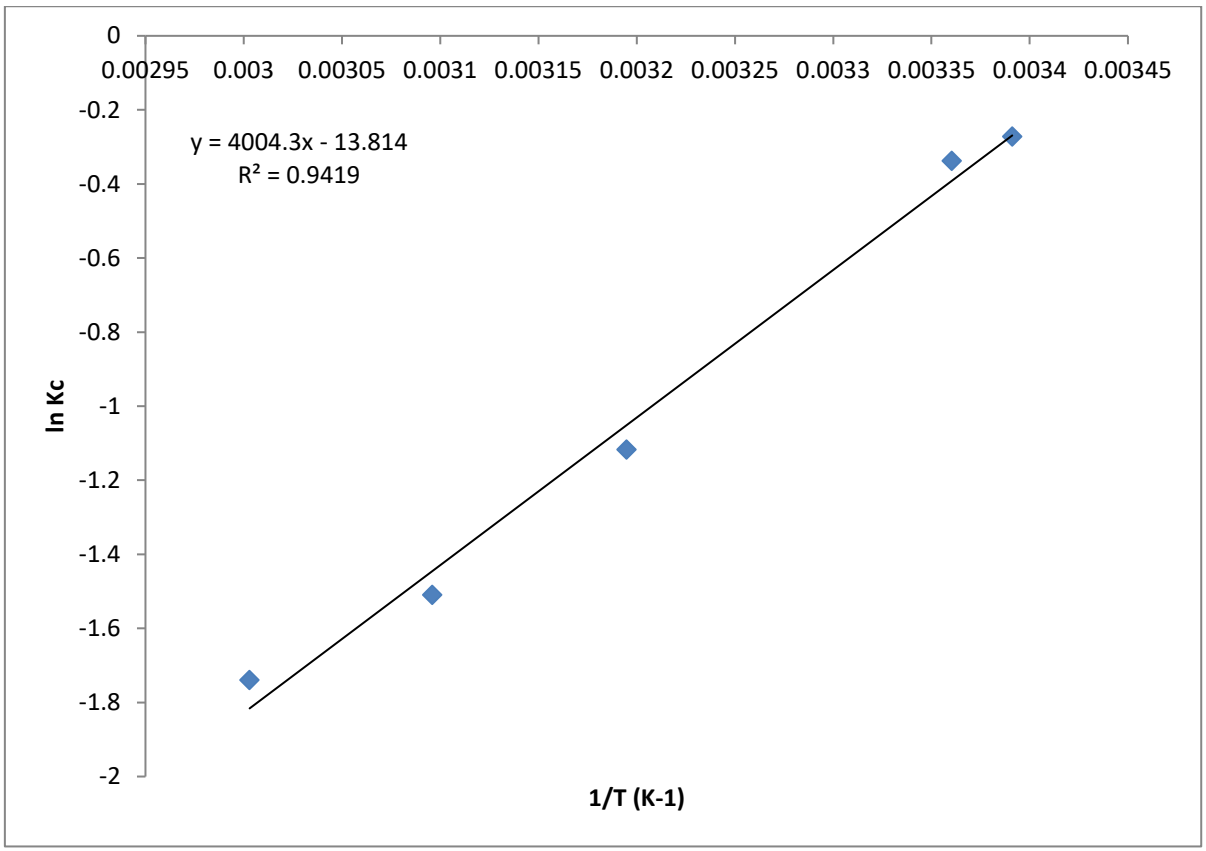


Figure 4.29: Van't Hoff plot for adsorption of AMP using GSAb

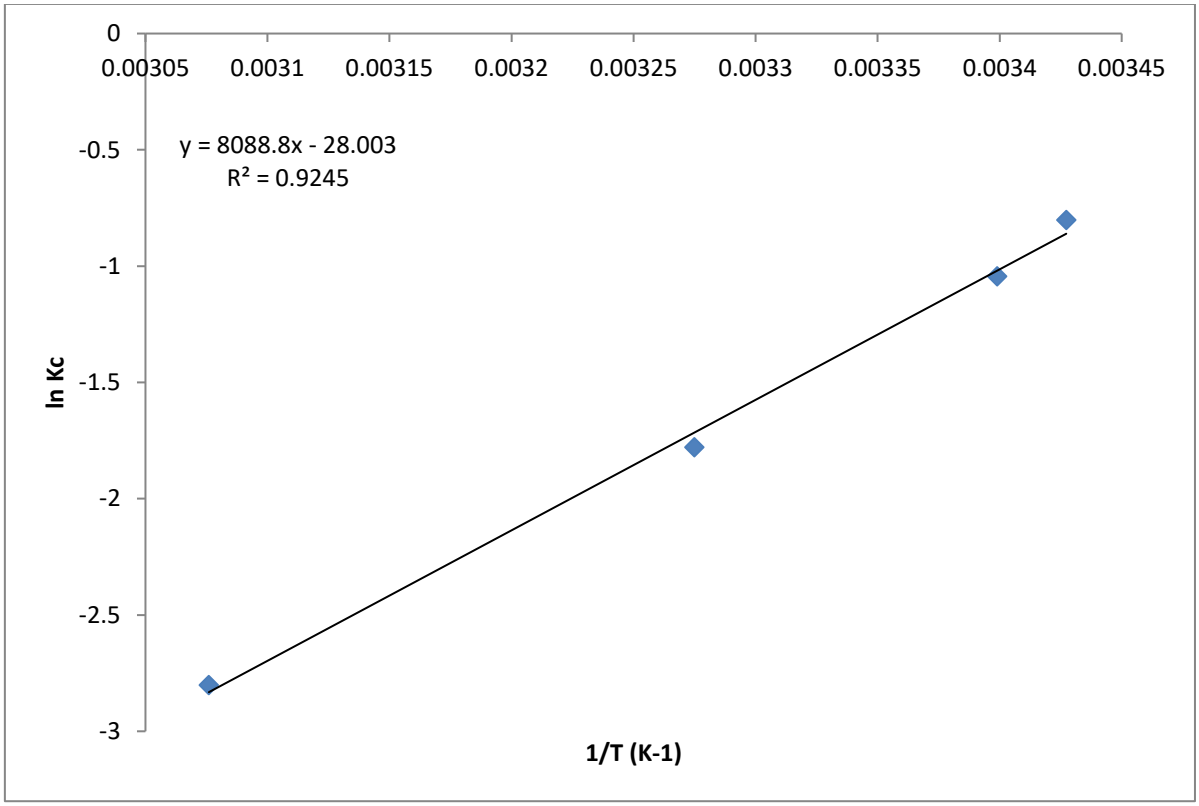


Figure 4.30: Van't Hoff plot for adsorption of CHLR using GSAb

The enthalpies and entropies of sorption of AMX, AMP and CHLR onto GSAb were calculated and the results presented in Table 4.22.

Table 4.22: ΔH^0, ΔS^0 and ΔG^0 Values of Sorption Studies of AMX, AMP and CHLR using GSAb

Analyte	Conc. (mg/L)	ΔH^0 (kJ/mol)	ΔS^0 (J/Kmol)	ΔG^0 (kJ/mol)			
				293 K	303 K	313 K	323 K
AMX	30	-63,467	-213,055	-1.0419	1.0887	3.2192	5.3498
AMP	30	-51,291	-114,85	0.3601	1.5086	2.6571	3.8056
CHLR	30	-49.346	-232,817	0.9654	3.2936	5.6217	7.9499

Negative values of ΔH^0 were obtained for the sorption of all three antibiotics onto GSAb. This indicates that the adsorption process of the antibiotics onto GSAb was exothermic. The result supports the observed trend of reduction in the adsorption capacity of the adsorbent for the antibiotics with increase in temperature. Unuabonah *et al.* (2007) and Boparai *et al.* (2011) suggested that ΔH^0 values ranging from 40 to 800 kJ/mol are indicative of a chemisorption process while values ranging from 5 to 40 kJ/mol indicate physisorption. According to Mohd-Din *et al.* (2015), chemisorption processes are specific involving stronger forces and consequently demands for higher activation energy. Physical adsorption process (physisorption mechanism) may be attributed to reversible and rapid adsorption processes which are involved with small energy requirements (Mohd-Din *et al.,* 2015). Enthalpy change values (ΔH^0) calculated for AMX, AMP and CHLR were all higher than 40 kJ/mol (Table 4.22). Based on this result, the adsorption of the antibiotics onto GSAb proceeded via chemisorption.

The negative values of ΔS^0 obtained for the sorption of the antibiotics onto GSAb as presented in Table 4.22, implies small degrees of freedom for the adsorbates at the adsorbent-liquid interface. This means that there was a decrease in the amount of adsorbates sorbed onto the surface of the adsorbent as temperature increased, resulting in reduce spontaneity of the antibiotic molecules.

Positive values for ΔG^0 with small magnitudes were obtained for the adsorption of antibiotics from solution using GSAb. This suggested that the sorption process of the antibiotics was feasible and non-spontaneous. However, an exception was noted with the ΔG^0 value for the sorption of AMX 293K with negative value (ΔG^0, -1.0419 kJ/mol). This indicated that the sorption of AMX at 293 K was feasible and spontaneous. According to Tan *et al.* (2009), an increase in the positive value of ΔG^0 with an increase in temperature indicates that the adsorption process is more favourable at lower temperatures. The trend of Gibbs free energy values suggests that the affinity of the GSAb surface for the antibiotics decreased with an

increase in temperature. Jiwalak *et al.* (2010) also reported a similar trend of reduction in affinity of the adsorbent for the adsorbate as temperature is increased.

Table 4.23 presents data for the effect of solution temperature on the sorption efficiency of the antibiotics from solution using GSB.

Table 4.23: Effect of temperature on percentage removal of AMX, AMP and CHLR using GSB

GSB	% AMX	% AMP	% CHLR
20	59.64	44.71	41.65
30	42.21	32.41	34.10
40	37.83	20.46	26.57
50	16.51	16.32	16.06
60	7.77	14.21	13.23

A decrease in percentage removal of the adsorbates from the respective solutions by GSB was noted. The reduction in the quantity of antibiotics adsorbed as temperature is increased indicates the exothermic nature of the adsorption process. Bansal (2012) summarised that exothermic adsorption processes are characterized by a decrease in the quantity of adsorbates sorbed onto an adsorbent as temperature increases.

As observed with GSAb, GSB also removed AMX better from solution at lower temperatures between 20˚C and 40˚C. There was a continuous decline in the percentage of antibiotics adsorbed by GSB at equilibrium with increasing temperature of solutions. Yang *et al.* (2009) noted that this may be as a result of the weakening of the Van der Waals forces of attraction between the adsorbate molecules and the adsorbent surface. The maximum adsorption percentages for AMX (59.64), AMP (44.71) and CHLR (41.65) were recorded at the lowest temperature considered (20 ^{0}C). The data also revealed that changes in temperature influences the affinity between antibiotics and adsorbent surface. An increase in solution temperature generally imposes a negative influence on the affinity of the antibiotics, restricting them from reaching the adsorbent surface, hence a reduction in sorbed quantities of adsorbates.

The Van't Hoff plots for the adsorption of AMX, AMP and CHLR onto GSB adsorbent are as presented in Figures 4.31 - 4.33.

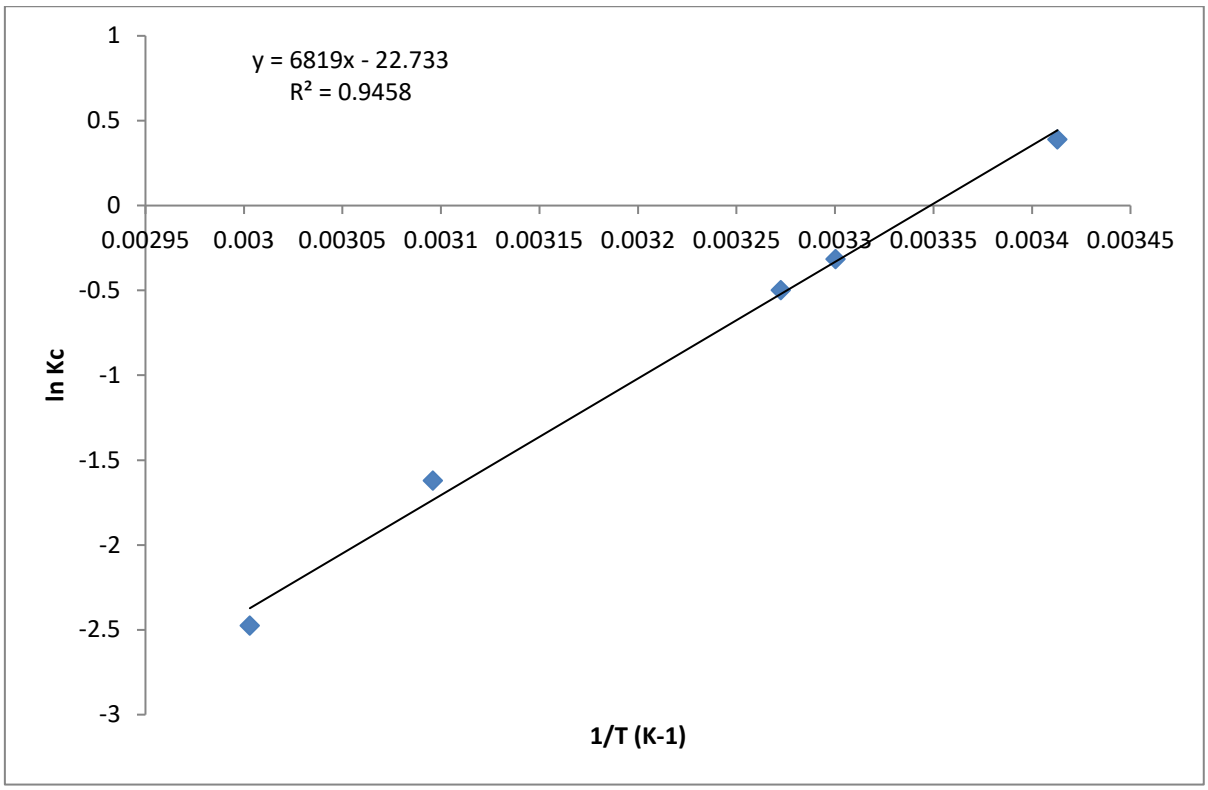


Figure 4.31: Van't Hoff plot for adsorption of AMX using GSB

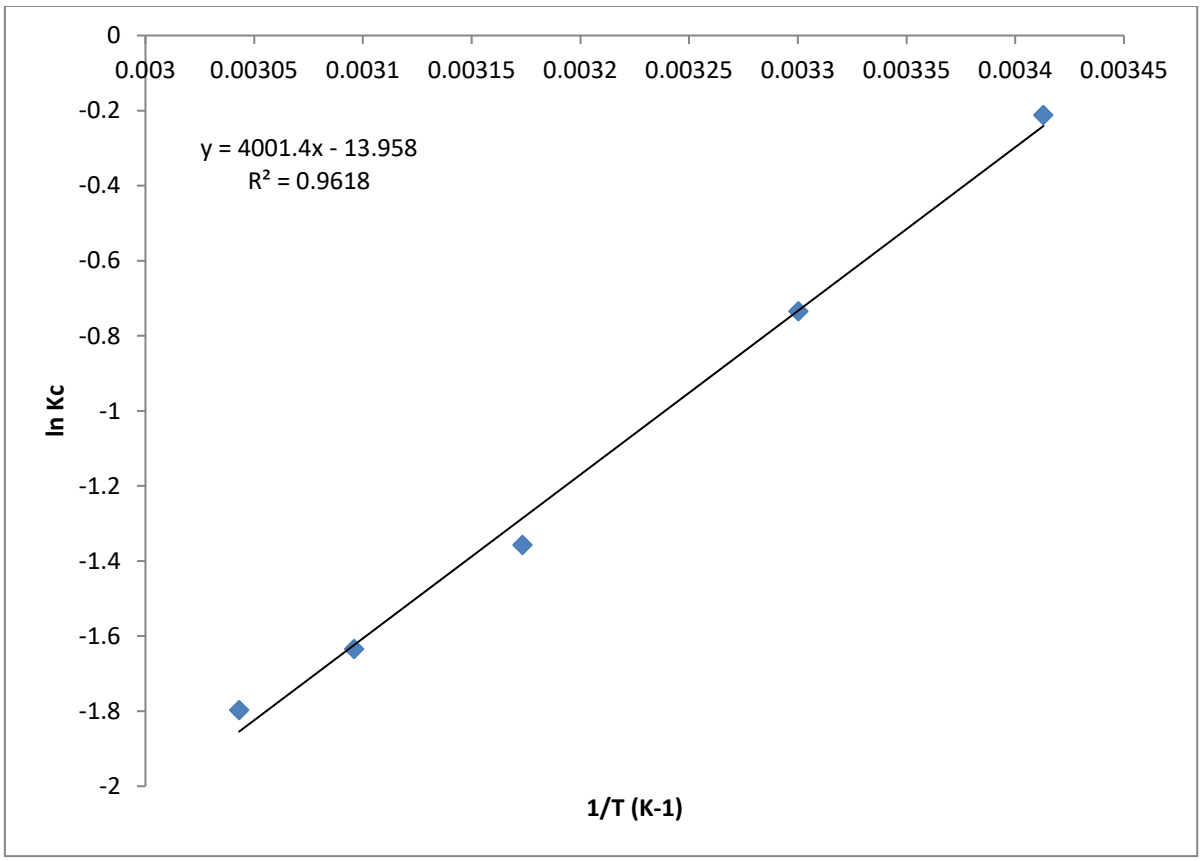


Figure 4.32: Van't Hoff plot for adsorption of AMP using GSB

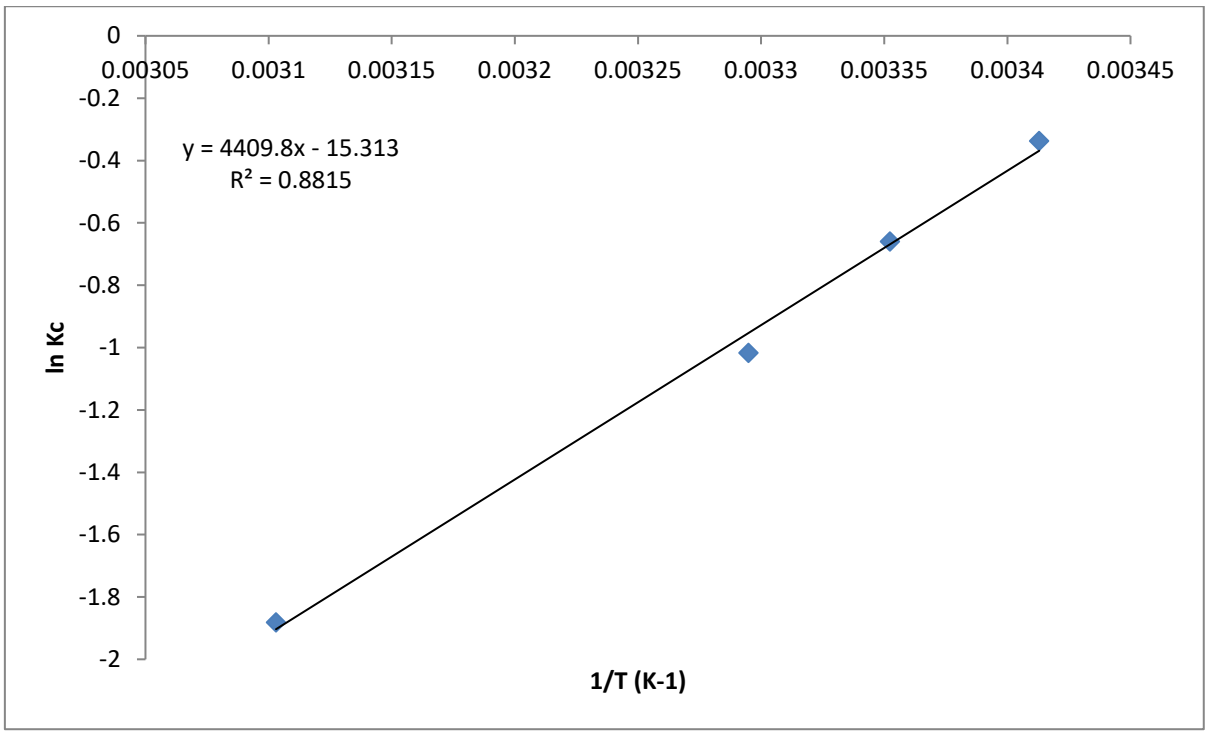


Figure 4.33:Van't Hoff plot for adsorption of CHLR using GSB

The calculated enthalpy values (ΔH^0) involved in the adsorption processes for AMX, AMP and CHLR by GSB were all negative (Table 4.24).

Table 4.24: ΔH^0, ΔS^0 and ΔG^0 Values of Sorption Studies of AMX, AMP and CHLR using GSB

Analyte	Conc (mg/L)	ΔH^0 (kJ/mol)	ΔS^0 (J/Kmol)	ΔG^0 (kJ/mol)			
				293 K	303 K	313 K	323 K
AMX	30	-56,693	-189,002	-1.3154	0.5746	2.4646	4.3546
AMP	30	-43,267	-116,047	0.7348	1.8952	3.0557	4.2162
CHLR	30	-46,663	-127,312	0.6394	1.9125	3.1857	4.4588

The results indicate the exothermic nature of the sorption processes for the adsorbates onto GSB. Adsorption processes for the antibiotics gave ΔH^0 values that were greater than 40 kJ/mol, hence sorption was highly influenced by chemisorption.

The values of entropy changes (ΔS^0) for the adsorption of the adsorbates with GSB were negative. This suggests that entropy of the adsorbates molecules on the surface of the adsorbent decreased with increase in temperature. Foo & Hameed (2011) and Bansal (2012) explained that the probable reason for a reduction in entropy is increase in temperature. They further noted that reactions such as association, fixation or immobilization of adsorbate molecules as a result of adsorption, could result in a loss of degrees of freedom for the adsorbate molecules, thereby producing negative entropy effects.

The values of ΔG^0 were positive for the adsorption of all adsorbates at all temperatures except for AMX at 293 K which was negative. According to Guler & Sarioglu (2014), low ΔG^0 values imply that the reaction/process is feasible though it may be spontaneous or non-spontaneous. Positive values of ΔG^0 indicate that the sorption processes were feasible and non-spontaneous. Hence the sorption of AMX at 293 K was both feasible and spontaneous. Since the Gibbs free energy values increased with temperature increase, the sorption processes were enhanced at lower temperatures (Tan *et al.,* 2009). The result implies that the sorption processes of the antibiotics onto GSB are exothermic.

The effect of temperature on the adsorption capacity of GS for the sorption of AMX, AMP and CHLR from solution is shown by data presented in Table 4.25.

Table 4.25: Effect of temperature on percentage removal of AMX, AMP and CHLR using GS

GS	% AMX	% AMP	% CHLR
20	18.36	30.47	30.41
30	16.70	22.81	26.04
40	12.41	18.38	19.92
50	11.47	12.65	12.20
60	2.69	5.72	3.46

Increasing the solution temperature resulted in a reduction in the percentage quantity of the antibiotics adsorbed similar to the observations obtained with GSAa, GSAb and GSB. The effect imposed on the adsorption efficiency of the adsorbent for AMX, AMP and CHLR suggests that adsorption is exothermic.

AMX was the least removed compound from solution by GS at all temperatures studied relative to AMP and CHLR. Adsorption was better at 20 ^oC giving the highest percentages of adsorbate sorbed from solution.

The Van't Hoff plots for the sorption of AMX, AMP and CHLR onto GS are presented from Figures 4.34 to 4.36. Linear regression equations obtained from the linear plots of the Van't Hoff plots were used to calculate ΔH^0, ΔS^0 and ΔG^0.

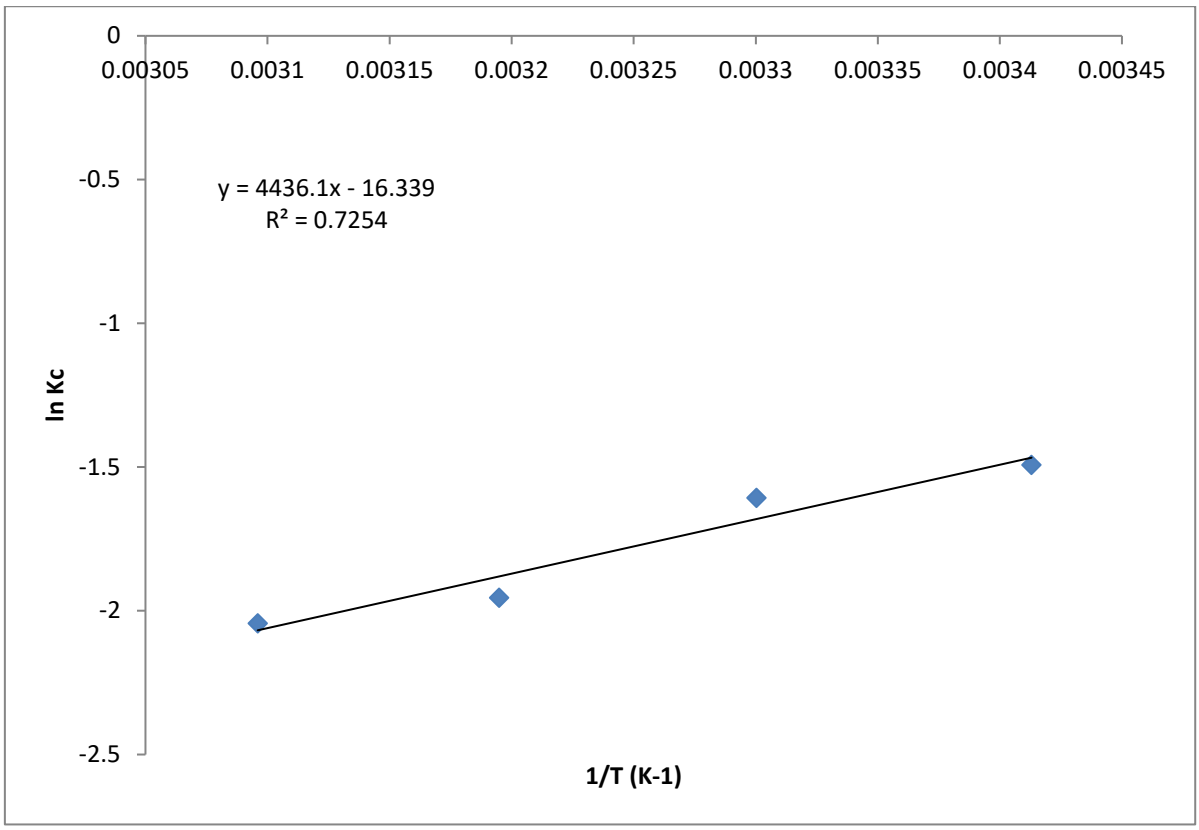


Figure 4.34: Van't Hoff plot for adsorption of AMX using GS

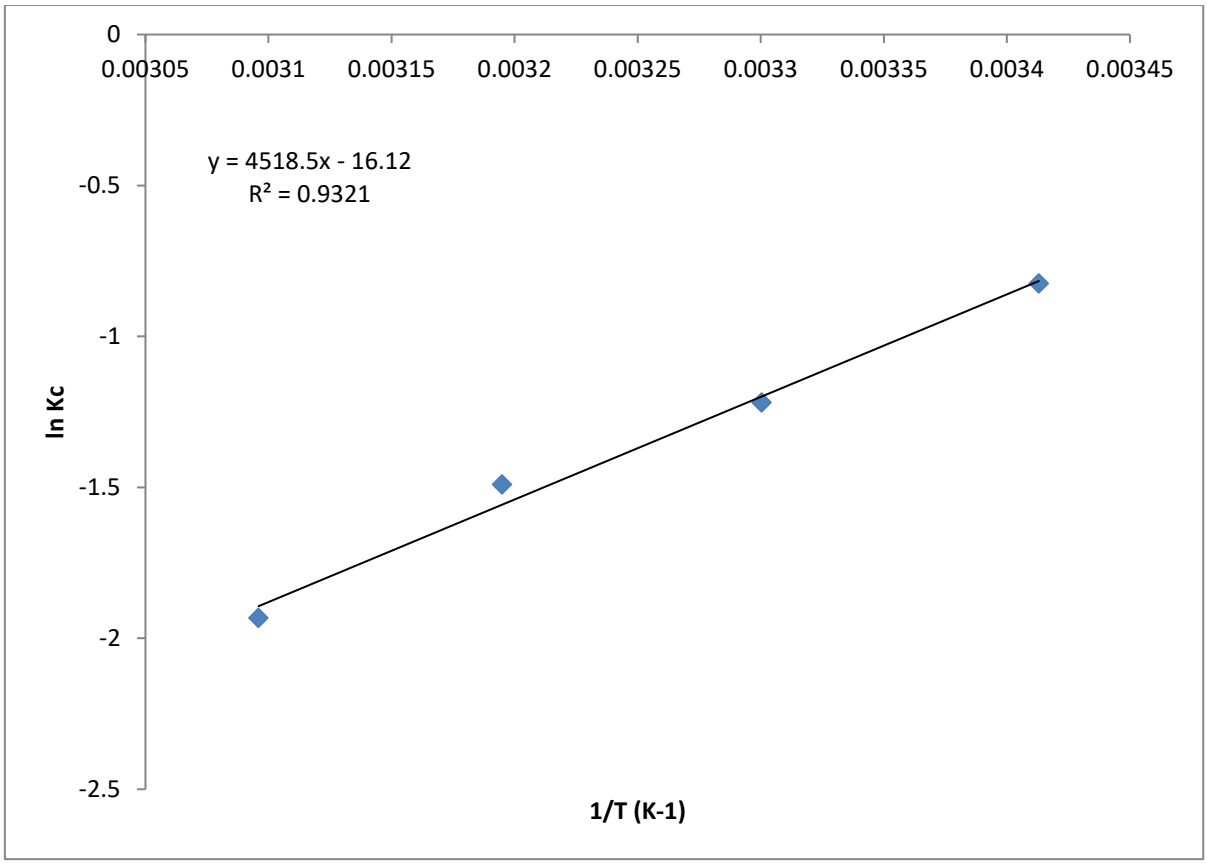


Figure 4.35: Van't Hoff plot for adsorption of AMP using GS

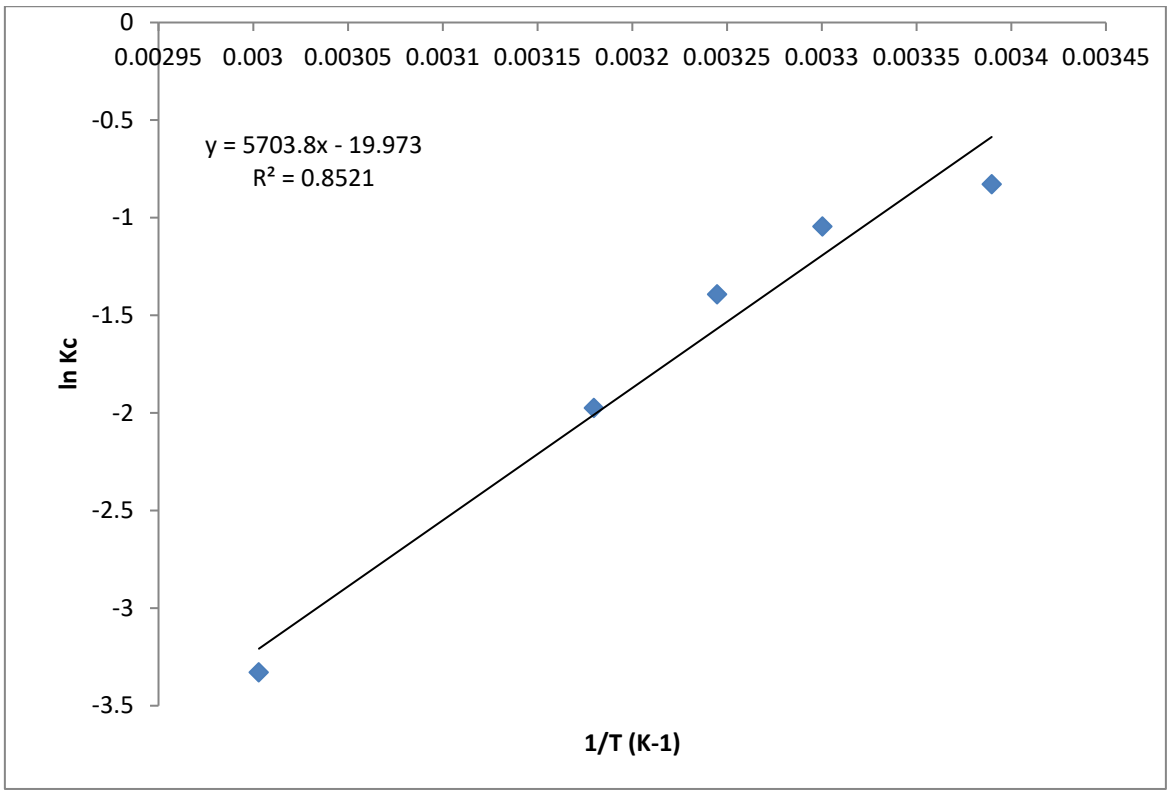


Figure 4.36: Van't Hoff plot for adsorption of CHLR using GS

ΔH^0 values calculated for the adsorption of AMX and AMP using GS were negative and lower than 40 kJ/mol (Table 4.26). The results suggested that the adsorption processes were exothermic and characterized by physisorption favoured by lower temperatures. The adsorption of CHLR proceeded via an exothermic chemisorption process since the ΔH^0 value was negative and above 40 kJ/mol. This explains the drop in the sorption capacity of the adsorbent for the adsorbates in solution as temperature increases from 20 ^oC to 60 ^oC.

Table 4.26: ΔH^0, ΔS^0 and ΔG^0 values of Sorption Studies of AMX, AMP and CHLR using GS

Analyte	Conc (mg/L)	ΔH^0 (kJ/mol)	ΔS^0 (J/Kmol)	ΔG^0 (kJ/mol)			
				293 K	303 K	313 K	323 K
AMX	30	-36,882	-135,842	2.9197	4.2781	5.6365	6.9950
AMP	30	-37,567	-134,022	1.7014	3.0417	4.3819	5.7221
CHLR	30	-47,421	-166,056	1.2334	2.8940	4.5545	6.2151

The entropy change (ΔS^0) values (all negative), indicated low degrees of freedom for the antibiotic molecules in solution as the temperature was increased. The reduced spontaneity of the antibiotic molecules suggest that there may be a decreasing randomness at the GS-solution interface during the adsorption of the antibiotics, as a result of the rising solution temperature. Yu *et al.* (2001) and Zha *et al.* (2013) noted that low degrees of freedom for

adsorbate molecules results in a reduction in spontaneity for the adsorbates at the adsorbent-solution interface.

The Gibbs free energy (ΔG^0) values for the sorption processes at the different temperatures were positive with values ranging from 1.2334 kJ/mol at 293 K to 6.9950 kJ/mol at 323 K. This means that the adsorption of antibiotics onto GS was a non-spontaneous process. The magnitude of the values of ΔG^0 increases with increasing temperature, demonstrating that higher temperatures do not facilitate the adsorption of the antibiotics onto GS as previously reported by Tan *et al.* (2009).

Increasing the reaction temperature affected the adsorption processes negatively leading to a decrease in the quantities of adsorbates adsorbed from solution as temperature increase. The ΔH^0 values for adsorption processes were all negative indicating exothermic reactions. Entropy change (ΔS^0) values were also negative suggesting reduced randomness at the adsorbent-solution interface. Hence, no significant changes occurred in the internal structure of the adsorbent resulting from adsorption of antibiotics onto the four adsorbents. The sorption of AMX and AMP with GSAa, GSAb and GSB were all controlled by chemisorption mechanisms while physisorption mechanisms influenced their sorption from solution using GS. On the contrary, the sorption of CHLR from solution using GSAa and GS proceeded via chemisorption mechanisms. Although the majority of the sorption processes were non-spontaneous, they were feasible and favored at low solution temperatures as observed from the trend of ΔG^0 values.

4.3.6 Scanning Electron Microscopy (SEM) of the Adsorbents

Scanning Electron Microscopic (SEM) analysis was conducted on adsorbents obtained before and after the adsorption process. The micrographs of the surface morphology assessment of the adsorbents are presented in Figures 4.37 – 4.40

Figures 4.37 (a) and (b) shows the SEM images of GSAa before and after sorption respectively.

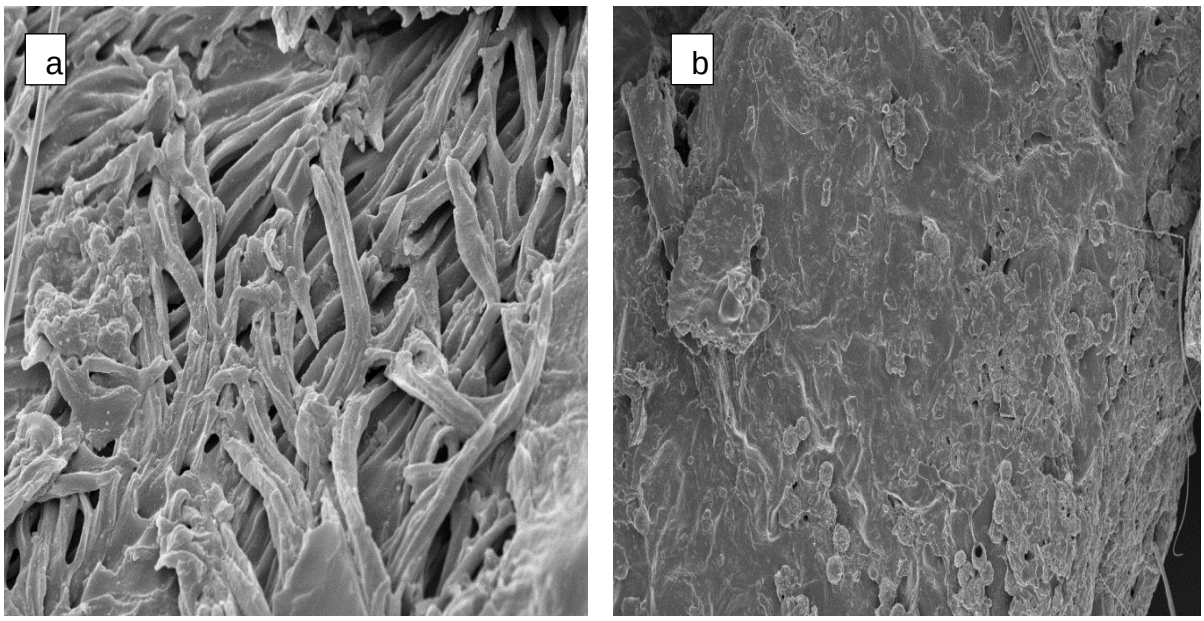


Fig 4.37: SEM images of GSAa before and after adsorption

The surface morphology of carbonized grape slurry GSAa before sorption appeared as a cluster of smooth needle/fibrous-like particulates with well-defined and open pore spaces of different size ranges (Figure 4.37(a)). The smooth surface of adsorbent materials may be the results of the transformation of the plant lignin-cellulose, which arises as a result of the deconstruction of the plant cells (Xie *et al.,* 2012). Similar morphologies have been previously reported severally for a number of adsorbents produced from plant biomasses (Ojha *et al.,* 2004; Karami & Rohani, 2009; Li *et al.,* 2012; Gougazeh & Buhl, 2014).

Sufficient and large open pore spaces have been reported to facilitate, and increase adsorption process via enhanced and efficient intra-particle diffusion into the inner surfaces of porous adsorbents (Sant'Anna & de Souza, 2012). Upon adsorption the adsorbent surface was completely covered, with the pore spaces filled by the adsorbates (Figure 4.37(b)).
The surface morphologies of GSAb are presented in Figures 4.38(a) and 4.38(b).

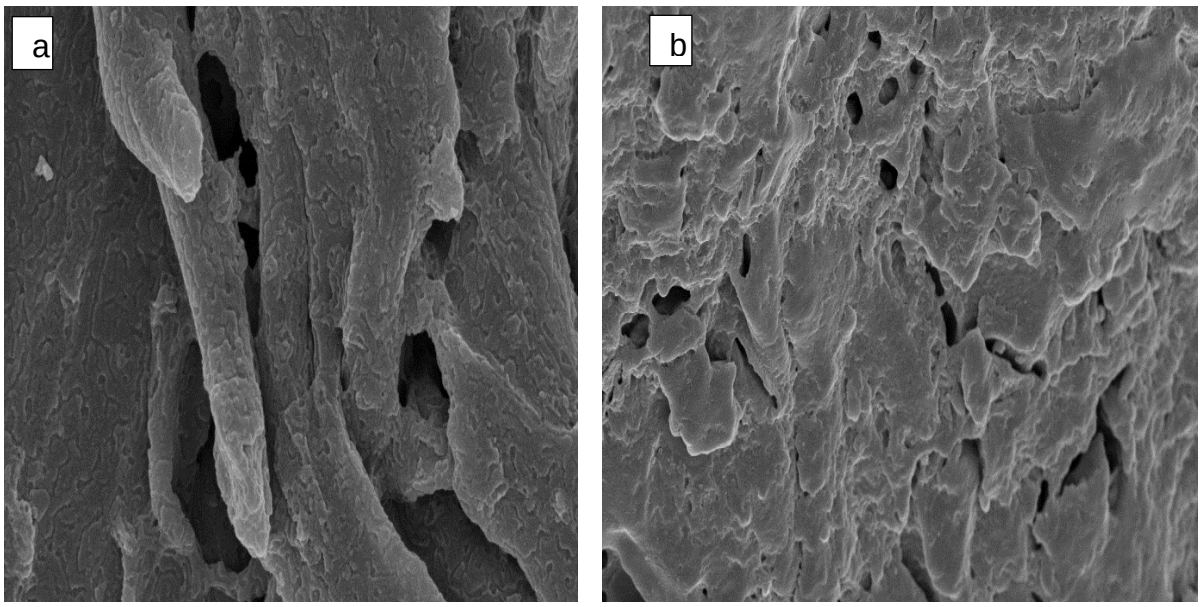


Fig 4.38: SEM images of GSAb before and after sorption

Figure 4.38(a) showed a smooth, broadened fibrous-like surface enriched with small diameter open pore spaces. Although the morphology of GSAb looks similar to that of GSAa, the broadened surface may not be unconnected with the effect of the degradation profile of the acid used for the modification of the biomass (Karami & Rohani, 2009; Rezende *et al.,* 2011). Also, large numbers of pore spaces were developed in the process, and this translates to the possibility of increased adsorption via internal diffusion into the inner structure of the porous adsorbent material. As observed with GSAa, the adsorbent surface of GSAb was covered up, with the pore spaces filled by the adsorbates after adsorption (Figure 4.38(b)).

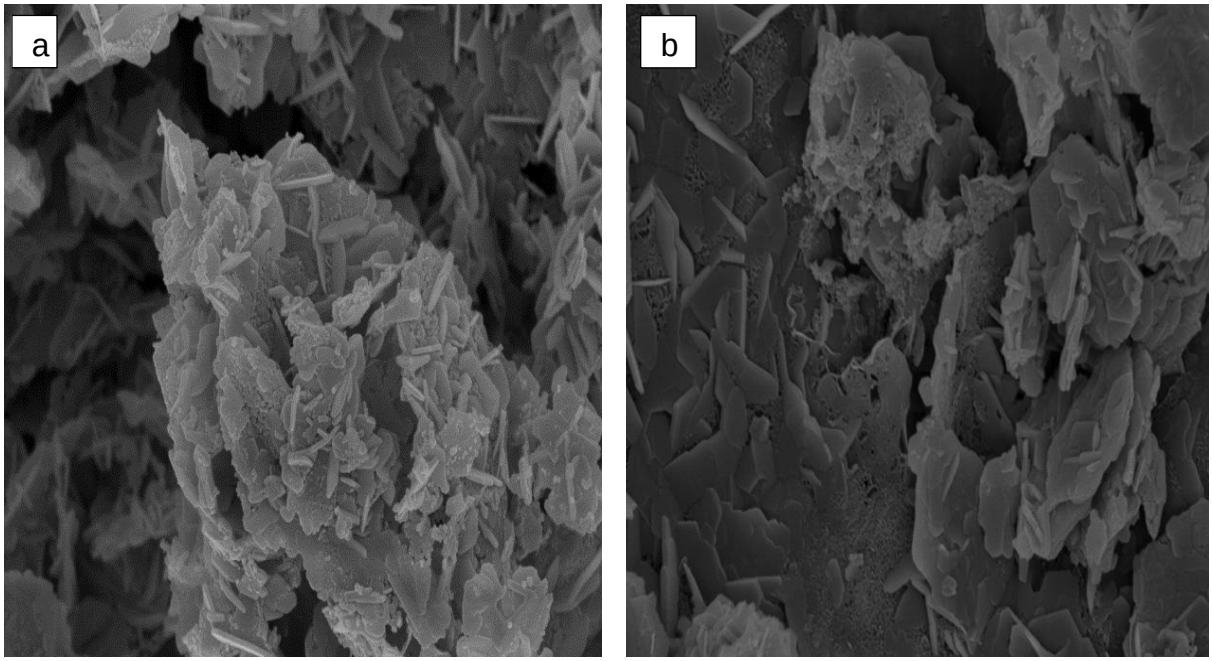


Fig 4.39: SEM images of GSB before and after sorption

Upon the modification of GSB, the crystalline morphology of clustered rhombohedral to other polygonal grain structures was achieved (Figure 4.39(a)). According to Sant'Anna & de Souza (2012), the use of strong alkaline solution such as KOH and NaOH may result in the detachment of plant fibres, cell wall collapse and porous formation on cell wall surfaces. Increased hydrolysis may result in increase in SEM roughness index which may correspond to enhancing adsorbent sorption capacity (Karimi & Taherzadeh, 2016). GSB surface and porous pores were filled up by the adsorbates after adsorption (Figure 4.39(b)).

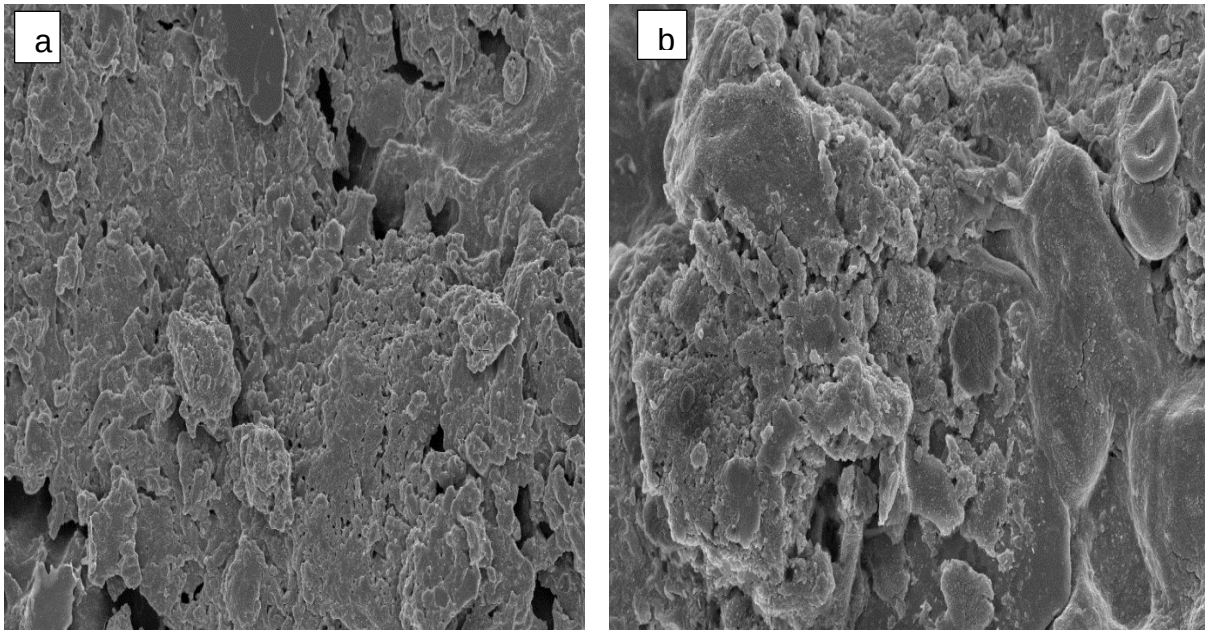


Fig 4.40: SEM images of the GS before and after sorption

The morphology of the un-carbonized and un-modified grape slurry biomass (GS) was mostly of distorted crystalline particles and was fussed together at the centre (Figure 4.40(a)). A closer examination of the surface indicates probable octahedral particles with smoothened surfaces. Pore spaces in the micrograph of GS were not clearly defined; hence the internal surfaces of the material might not be accessible for the diffusion of the fluid, due to closed porosity.

In general, biomass pre-treatment depends on a number of factors such as pH, temperature, plant material type, and this in-turn, may result in some variability in the carbonized and modified products. This includes structural changes which might have an effect on the surface functionalities in terms of sorption site density. Furthermore, the modification process may either tend to increase or decrease the number of activated sites available for sorption. Rezende *et al.* (2011) reported that pre-treatment decreases lignin content of plant cells, and change the surface texture, thereby making them rougher than the non-treated biomasses. This explains the enhanced sorption properties of carbonized and treated adsorbents compared to those of the untreated and un-carbonized adsorbents.

4.4 Column Study

A column study was conducted using GSB in order to determine the effect of time on the efficiency of the adsorption process in a continuous fixed bed column. GSB was used for the study because of its high sorption efficiency relative to other adsorbents for the three antibiotics investigated. 200 ml of spiked water containing of 30 mg/L AMX, AMP and CHLR, was passed through a fixed column packed with 4.50 g of GSB and filled to the 3cm mark, at a flow rate of 2 ml/min. The efficiency for the removal of the β-lactam antibiotics AMX, AMP and CHLR on a fixed bed mode is shown in Figure 4.41.

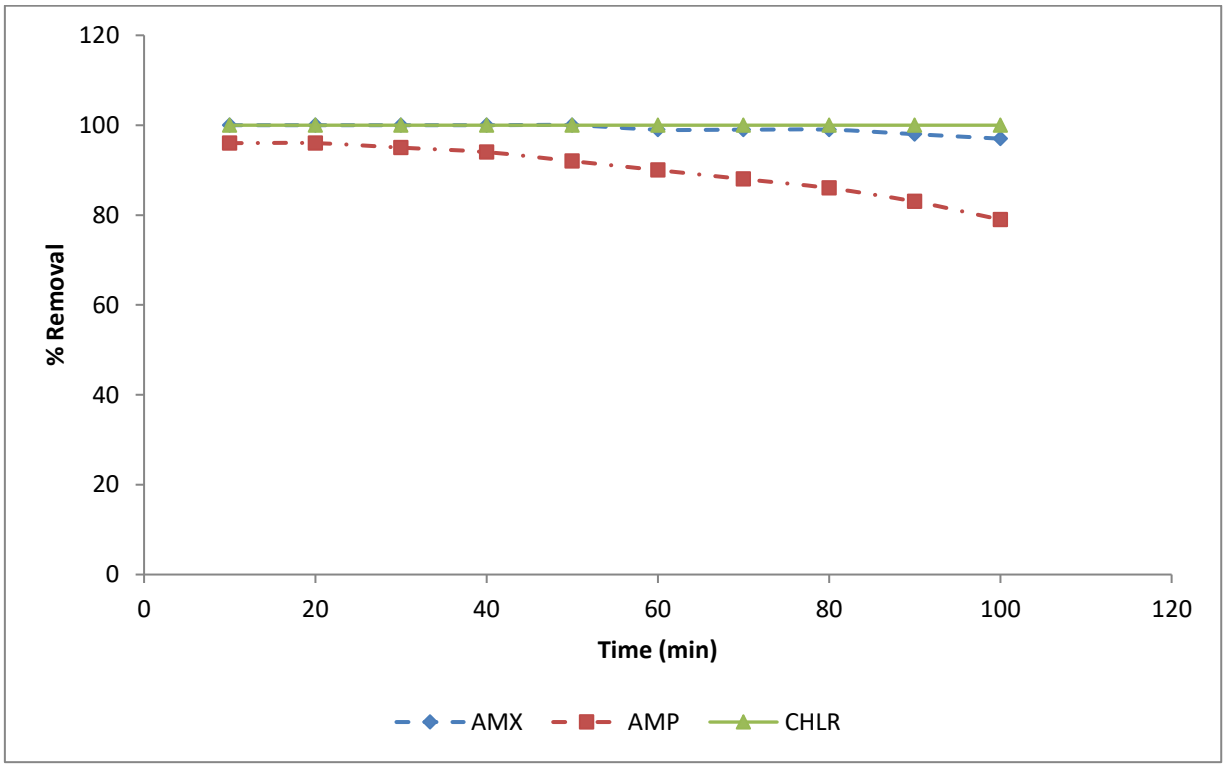


Figure 4.41: A fixed bed column sorption of AMX, AMP and CHLR onto GSB

The removal of CHLR was greater than 99.9 % with the passage of 200 ml of the spiked solution through the column (i.e over a total time period of 100 min). A percentage removal higher than 99 % was obtained for AMX, between a time lapse of 10 min and 80 min after the spiked solution was fed through the column. The sorption efficiency of the adsorbent for CHLR and AMX appeared to be the same for contact times between 10 – 50 min. However, as contact time increased above 80 min, the sorption of AMX onto GSB in the bed column reduced to about 97 % (Figure 4.41).

The efficiency removal for AMP was highest between 10 min and 30 min, giving percentage removal efficiencies between 96 % and 95 %. Thereafter, a steady drop in the efficiency for removal of the adsorbate from solution by GSB was observed (Figure 4.41), giving the lowest quantity adsorbed (79 %) after a contact time of 100 min.

It was reported that increase in inflow and flow rate beyond a certain time and adsorbate volume may cause adsorption of the adsorbate to decrease, because of the decrease in resident time of the adsorbate in the column (Goyal *et al.,* 2009). The results are consistent with the findings made in this study whereby the sorption efficiency reduced as the volume of the adsorbate solution passing through the column increased.

The column study showed that the sorption removal efficiency of CHLR was better than that of AMX followed by AMP, regardless of the experimental conditions. However, the overall result obtained for the sorption removal efficiencies for all adsorbates was satisfactory for the fixed bed effectiveness of adsorption in a continuous flow of adsorbate solution. This outcome implies the non-complexity and cheapness of the adsorption process and the high probability for the removal of β-lactam antibiotics from surface water using the carbonized grape slurry waste.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The occurrence of residues of amoxicillin (AMX), ampicillin (AMP) and chloramphenicol (CHLR) in the Diep River were variable and site dependent. AMX was detected in all surface water samples while AMP was only detected at sampling station P5, CHLR was however not detected in any of the collected water samples.

Sorption studies revealed that modified carbonized grape slurry biomasses had enhanced removal capability relative to the un-carbonized and unmodified grape slurry biomass for the abatement of the selected β-lactam antibiotics from simulated wastewaters. The morphologies of the adsorbents' surfaces revealed the advantages of carbonization and surface activation of the adsorbents over the un-carbonized and unmodified adsorbent. Carbonization and activation of the grape slurry facilitated large surface area, enhanced pore size distribution and availability of increased active sites, resulting in improved adsorption efficiencies of the adsorbents. The EDX chemical analysis of GSAa, GSAb, GSB and GS, indicated high carbon content, and the presence of surface oxygen on all adsorbents. Also, the adsorbents had low ash content, which is assumed to have facilitated their adsorption potential.

Fourier Transform Infra-Red (FT-IR) analysis of the GS adsorbent showed prominent characteristic bands of the asymmetric C-H and symmetric CH_2 bond vibrations. The FT-IR spectra of GSAa and GSAb suggest that activation of the adsorbents with acids resulted in the suppression of prominent absorption bands that were obtained for GS. However, FT-IR analysis of GSB revealed a shift for the absorption band obtained for GS producing a hydroxyl stretch.

The different adsorbents gave variable equilibrium sorption times for the selected antibiotics. Sorption kinetics of GSAa, GSAb, GSB and GS for the adsorbates were also analysed for the pseudo-first order, pseudo-second order and the Elovich kinetic model mechanisms. The pseudo-second order was the major rate controlling step for the sorption of AMX, AMP and CHLR on all adsorbents with the exception of GS for AMX and AMP. Also the Elovich model had some influence on the sorption processes of the adsorbates onto the respective adsorbents to a lesser extent since it gave correlation coefficient values > 0.50. Though, the sorption of the antibiotics was also influenced by physical mechanisms, the sorption processes were highly influenced by chemical processes. The sorption of AMX and AMP

onto GS was influenced more by physical mechanisms because data fitted best into the pseudo-first order kinetic model.

Freundlich, Langmuir and Temkin equilibrium isotherm sorption models were employed, and their parameters evaluated by fitting the data obtained from batch experiments into the model equations. The Freundlich and Langmuir isotherm models had better correlation coefficients to the experimental data. This suggests the possibility of both heterogeneous adsorption and monolayer coverage of the adsorbent surfaces. The sorption would reach saturation resulting in limited capacities of the adsorbent for sorption. Also through the influence of the Temkin isotherm model, the heat of adsorption was assumed to be lost linearly with enhanced sorbent surface coverage.

Enthalpy change (ΔH^0) values calculated for the sorption processes of AMX, AMP and CHLR were negative for the four adsorbents, hence sorption processes were exothermic. The sorption processes of AMX and AMP onto GSAa, GSAb and GSB were strongly influenced by chemical reactions while sorption of the same adsorbates onto GS was influenced by physical reactions based on the enthalpy change values obtained. However, the sorption of CHLR onto all adsorbates was influenced by chemical reactions according to the obtained values for enthalpy change. Results of the standard entropy (ΔS^0) and free energy change (ΔG^0) suggested that there were reduced interactions at the adsorbent surface/solution interface as the temperature was increased. Feasible non-spontaneous processes thus resulted for the sorption of the antibiotics. The order of removal efficiencies of the adsorbents for AMX, AMP and CHLR were GSB > GSAa > GSAb > GS.

Column studies suggested that removal efficiency of adsorbent was enhanced with increased contact time and sorbate volume. This study concludes that carbonized and modified grape slurry successfully remediated the β-lactam compounds from aqueous solutions.

5.2 Recommendation

Further extensive column, recovery and reuse studies of adsorbents are still needed to fully understand practical application of the study to societal needs. More investigations also need to be carried out on possible doping of adsorbents with stabilizers for improved sorption efficiencies and reuse potentials. It will also be necessary to identify and characterize metabolites of the antibiotics (since hydrolysis seems to be responsible for lack of detection in some locations) in future work. Metabolites or degradation products of different β-lactam antibiotic compounds may therefore serve as indicators of contamination of aqueous systems.

REFERENCES

- Abegglen, C. 2009. Eliminating micro-pollutants: wastewater treatment methods. *Eawag News,* **67e**: 25-27
- Abegglen, C., Joss, A., McArdell, C. S., Fink, G., Schlusener, M. P., Ternes, T. A. & Siegrist, H. 2009. The fate of selected micro-pollutants in a single-house MBR. *Water Research*, **43** (7): 2036 – 2046
- Abuin, S., Codony, R., Compano, R., Granados, M. & Prat, M. D. 2006. Analysis of macrolide antibiotics in river water by solid-phase extraction and liquid chromatography-mass spectrometry. *Journal of Chromatography A*, **1114** (1): 73 – 81
- Ademiluyi, F. T., Amadi, S. A. & Amakama, N. J. 2009. Adsorption and Treatment of Organic Contaminations using Activated Carbon from Waste Nigerian Bamboo. *Journal of Applied Science Environmental Management*, **13** (3): 39-47
- Adriano, W. S., Veredas, V., Santana, C. C. & Goncalves, L. R. B. 2005. Adsorption of amoxicillin on chitosan beads: Kinetics, equilibrium and validation of finite bath models. *Biochemical Engineering Journal*, **27** (2): 132 – 137
- Ahile, U. J., Adejo, S. O., Tughgba, M. S., Tyohemba, R. L. & Ama, S. O. 2015. Kinetic and Equilibrium Studies For the Adsorption of Amoxicllin from Aqeous Solution on Carbonized Groundnut Shells. *2nd International Conference on Chemical, Biological, and Environmental Sciences*, May 20 – 21, 2015, Dubai, pg 1 – 5
- Al-degs, Y. S., El-Barghouthi, M. I., El-Sheikh, A. H. & Walker, G. M. 2008. Effect of solution pH, ionic strength, and temperature on adsorption behavior of reactive dyes on activated carbon. *Dyes and Pigments*, **77** (1): 16 – 23
- Ali, I., Asim, M. & Khan, T. A. 2012. Low cost adsorbents for the removal of organic pollutants from wastewater. *Journal of Environmental Management*, **113**: 170 – 183
- Al-Qodah, Z. & Shawabkah, R. 2009. Production and characterization of granular activated carbon from activated sludge. *Brazilian Journal of Chemical Engineering*, **26** (1): 127 – 136.
- Aminu, A. S., Gimba, C. E., Kagbu, J., Turoti, M., Itodo, A. U. & Sariyya, I. A. 2010. Sorption Efficiency Study of Pesticide Adsorption on Granulated Activated Carbon from Groundnut Shell Using GC/MS. *World Rural Observations*, **2** (1): 18 – 24
- Asgari, G., Roshani, B. & Ghanizadeh, G. 2012. The investigation of kinetic and isotherm of fluoride adsorption onto pumice stone. *Journal of Hazardous Materials*, **217-218**: 123 – 132
- Ayanda, O. S., Adeyi, O., Durojaiye, B. & Olafisoye, O. 2012. Adsorption Kinetics and Intraparticulate Diffusivities of Congo Red onto Kola Nut Pod Carbon. *Pol. J. Environ. Stud.*, **21** (5): 1147 – 1152

- Ayanda, O. S., Fatoki, O. S., Adekola, F. A. & Ximba, B. J. 2013. Removal of tributyltin from shipyard process wastewater by fly ash, activated carbon and fly ash/activated carbon composite: adsorption models and kinetics. *Journal of Chemical Technology and Biotechnology*, **88** (12): 2201 – 2208
- Baker, D. R. & Kasprzyk-Hordern, B. 2011. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionization tandem mass spectrometry. *Journal of Chromatography A*, **1218** (12): 1620 – 1631
- Balasubramani, K. & Sivarajasekar, N. 2014. Adsorption Studies of Organic Pollutants onto Activated Carbon. *International Journal of Innovative Research in Science, Engineering and Technology*, **3** (3): 10575 – 10581
- Bansal, O. P. 2012. Thermodynamics of Equilibrium Adsorption of Antibiotics by Clay Minerals and Humic Acid-Clay Complexes. *Natl. Acad. Sci. Lett.*, **35** (2): 109 – 114
- Batt, A. L. & Aga, D. S. 2005. Simultaneous analysis of multiple classes of antibiotics by ion trap LC/MS/MS for assessing surface water and ground water contamination. *Analytical Chemistry*, **77**: 2940 – 2947
- Batt, A. L., Bruce, I. B. & Aga, D. S. 2006. Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. *Environmental Pollution*, **142** (2): 295 – 302
- Bendz, D., Paxeus, N. A., Ginn, T. R. & Loge, F. J. 2005. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hoje River in Sweden. *Journal of Hazardous Materials*, **122** (3**)**: 195 – 204
- Besse, J.P. & Garric, J. 2007. Human pharmaceuticals in surface waters Implementation of a prioritization methodology and application to the French situation. *Toxicology Letters*, **176**: 104 – 123
- Biswas, S. & Mishra, U. 2015. Continuous Fixed-Bed Column Study and Adsorption Modeling: Removal of Lead Ion from Aqueous Solution by Charcoal Originated from Chemical Carbonization of Rubber Wood Sawdust. *Journal of Engineering*, **34** (2): 1398-148
- Boparai, H. K., Joseph, M. & O'Carroll, D. M. et al. 2011. Kinetics and thermodynamics of cadmium ion removal by adsorption onto nano zerovalent iron particles. *Journal of Hazardous Materials*, **186**: 458 – 465
- Braschi, I., Blasioli, S., Fellet, C., Lorenzini, R., Garelli, A., Pori, M. & Giacomini, D. 2013. Persistence and degradation of new B-lactam antibiotics in the soil and water environment. *Chemosphere*, **93** (1): 152 – 159
- Budyanto, S., Soedjono, S., Irawaty, W. & Indraswati, N. 2008. Studies of Adsorption Equilibria and Kinetics of Amoxicillin from Simulated Wastewater using Activated Carbon and Natural Bentonite. *Journal of Environmental Protection Science*, **2**: 72 – 80

- Callahan, A. 1953. Effect of Sulfonamides and Antibiotics on Panophthalmitis Complicating Cataract Extraction. AMA Arch Ophthalmol., **49** (2): 212 – 219.
- Carballa, M., Omil, F., Lema, J. M., Llompart, M., Garcia-Jares, C., Rodriguez, I., Gomez, M. & Ternes, T. 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Research*, **38**: 2918 – 2926
- Caturala, F.,Martin-Martinez, J. M., Molina-Sabio, M.,Rodriguez-Reinso, F. & Torregrosa, R. 1988. Adsorption of substituted phenols on activated carbons. *Journal of Colloid and Interface Science*, **124**: 528 – 534
- Celiz, M. D., Tso, J. & Aga, D. S. 2009. Pharmaceutical metabolites in the environment: Analytical challenges and ecological risks. *Environmental Toxicology and Chemistry*, **28** (12): 2473 – 2484
- Chayid, M. A. & Ahmed, M. J. 2015. Amoxicillin adsorption on microwave prepared activated carbon from Arundo donax Linn: Isotherms, kinetics, and thermodynamics studies. *Journal of Environmental Chemical Engineering*, **3**: 1592 – 1601
- Chern, J. –M. & Wu, C. –Y. 2001. Desorption of dye from activated carbon beds: effects of temperature, pH and alcohol. *Water Research*, **35** (17): 4159 – 4165
- Chowdury, Z. Z., Hamid, S. B. A., Das, R., Hasan, M. R., Zain, S. M., Khalid, K. & Uddin, M. N. 2013. Preparation of Carbonaceous Adsorbents from Lignocellulosic Biomass and Their Use in Removal of Contaminants from Aqueous Solution. *BioResources*, **8** (4): 6523 – 6555
- Christian, T., Schneider, R. J., Farber, H. A., Skutlarek, D., Meyer, M. T. & Goldbach, H. E. 2003. Determination of Antibiotic Residues in Manure, Soil, and Surface Waters. *CLEAN Soil Air Water*, **31** (1): 36 – 44
- Cleuvers, M. 2002. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. *Toxicology Letters*, **142**: 185 – 194
- Comerton, A.M., Andrews, R.C. & Bagley, D.M. 2009. Practical overview of analytical methods for endocrine-disrupting compounds, pharmaceuticals and personal care products in water and waste water – a review. *Philosophical Transactions Royal Society*, **367**: 3923 – 3939
- Cunningham, V. L., Buzby, M., Hutchinson, T., Mastrocco, F., Parke, N. & Roden, N. 2006. Effects of Human Pharmaceuticals on Aquatic Life: Next Steps. *Environmental Science and Technology*, **3457**: np
- Dada, A. O., Olalekan, A. P., Olatunya, A. M. & Dada, O. 2012. Langmuir, Freundlich, Temkin and Dubinin-Radushkevich Isotherms Studies of Equilibrium Sorption of Zn^{2+} Unto Phosphoric Acid Modified Rice Husk. *Journal of Applied Chemistry*, **3** (1): 38 – 45
- Demirbas, A. 2004. Adsorption of lead and cadmium ions in aqueous solutions onto modified lignin from alkali glycerol delignication. *Journal of Hazardous Materials*, **109** (1-3): 221 – 226

- Demirbas, E., Kobya, M., Sunturk, E. & Ozkan, T. 2004. Adsorption kinetics for the removal of Chromium (IV) from aqueous solutions on the activated carbons prepared from Agricultural waste. *Water SA.*, **30** (4): 533 – 539
- Deschamps, E., Vasconcelos, O., Lange, L., Donnici, C. L., da Silva, M. C. & Sales, J.A. 2012. Management of effluents and waste from pharamaceutical industry in Minas Gerais, Brazil. *Brazilian Journal of Pharmaceutical Sciences*, **48** (4): np
- Diaz-Cruz, M. S. & Barcelo, D. 2005. LC-MS^2 trace analysis of antimicrobials in water, sediment and soil. *Trends Analytical Chemistry*, **24** (7): 645 – 657
- Do, D. D. 1998. *Adsorption Analysis: Equilibria and Kinetics*. World Scientific, pg 837.
- Dousa, M. & Hosmanova, R. 2005. Rapid determination of Amoxicillin in premixes by HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, **37** (2): 373 – 377
- Duong, H. A., Pham, N. H., Nguyen, H. T., Hoang, T. T., Pham, H. V., Pham, V. C., Berg, M., Giger, W. & Alder, A. C. 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. *Chemosphere*, **72** (6): 968 – 973
- Dutta, M., Baruah, R. & Dutta, N. N. 1997. Adsorption of 6-aminopenicillanic acid on activated carbon. *Separation and Purification Technology*, **12**: 99 – 108
- El-Guendi, M. 1991. Homogeneous surface diffusion model of basic dyestuffs onto natural clay in batch adsorbers. *Adsorpt. Sci. Technol.*, **8** (2): 217–225
- El-Shafey, E. –S. I., Al-Lawati, H. & Al-Sumri, A. S. 2012. Ciprofloxacin adsorption from aqueous solution onto chemically prepared carbon from date palm leaflets. *Journal of Environmental Sciences*, **24** (9): 1579 – 1586
- Fakhri, A. & Adami,S. 2014. Adsorption and thermodynamic study of Cephalosporins antibiotics from aqueous solution onto MgO nanoparticles. *Journal of the Taiwan Institute of Chemical Engineers*, **45**: 1001 – 1006
- Fan, H. –T., Shi, L. –Q., Shen, H., Chen. & Xie, K. –P. 2016. Equilibrium, isotherm, kinetic and thermodynamic studies for removal of tetracycline antibiotics by adsorption onto hazelnut shell derived activated carbons from aqueous media. *Royal Society of Chemistry*, **6**: 109983 – 109991
- Fan, S., Wang, Y., Wang, Z., Tang, J., Tang, J. & Li, X. 2017. Removal of methylene blue from aqueous solution by sewage sludge-derived biochar: Adsorption kinetics, equilibrium, thermodynamics and mechanism. *Journal of Environmental Chemical Engineering*, **5**: 601 – 611
- Fan, Y., Wang, B., Yuan, S., Wu, X., Chen, J. & Wang, L. 2010. Adsorptive removal of chloramphenicol from wastewater by NaOH modified bamboo charcoal. *Bioresource Technology*, **101**: 7661 – 7664
- Fatoki, O. S., Ayanda, O. S., Adekola, F. A., Ximba, B. J. & Opeolu, B. O. 2012. Preparation and Characterization of Activated Carbon – nFe_3O_4, Activated Carbon –

$nSiO_2$ and Activated Carbon – nZnO Hybrid Materials. *Particle and Particle Systems Characterization*, **29** (3): 178 – 191

- Fent, K., Weston, A. A. & Caminada, D. 2006. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*, **76**: 122 – 159
- Fernando, A., Monteiro, S., Pinto, F. & Mendes, B. 2009. Production of Biosorbents from Waste Olive Cake and Its Adsorption Characteristics for Zn^{2+} Ion. *Sustainability,* **2009** (1): 277 – 297
- Fierro, V., Torne-Fernandez, V., Montane, D. & Celzard, A. 2008. Adsorption of phenol onto activated carbons having different textural and surface properties. *Microporous and Mesoporous Materials*, **111** (1-3): 276 – 284
- Firdaous, L., Fertin, B., Khelissa, O., Dhainaut, M., Nedjar, N., Chataigne, G., Ouhoud, L., Lutin, F. & Dhulster, P. 2017. Adsorptive removal of polyphenols from an alfalfa white proteins concentrate: Adsorbent screening, adsorption kinetics and equilibrium study. *Separation and Purification Technology*, **178**: 29 – 39
- Florey, M. E. 1944. Clinical Uses of Penicillin. *British Medical Bulletin*, **2** (1): 9 – 13.
- Foo, K. Y. & Hameed, B. H. 2011. Preparation of activated carbon from date stones by microwave induced chemical activation: application for methylene blue adsorption. *Chemical Engineering Journal*, **170** (1): 338 – 341
- Fytianos, K., Voudrias, E. & Kokkalis, E. 2000. Sorption-desorption behaviour of 2,4-dichlorophenol by marine sediments. *Chemosphere*, **40** (1): 3 – 6
- Gaspard, S., Altenor, S., Passe-Countrin, N., Ouensanga, A. & Brouers, F. 2006. Parameters from a new kinetic equation to evaluate activated carbons efficiency for water treatment. *Water Research*, **40**: 3467-3477
- Ghasemi, M., Naushad, M., Ghasemi, N. & Khosravi-fard, Y. 2014. Adsorption of Pb(II) from aqueous solution using new adsorbents prepared from agricultural waste: adsorption isotherm and kinetic studies. *J. Ind. Eng. Chem.*, **20**: 2193 – 2199
- Glinka, T., Blais, J. & Dudley, M. 2005. A novel series of 3-heteroarylthio carbacephems with activity against resistant Gram-positive bacteria. *In: Abstracts of the 45th interscience conference on antimicrobial agents and chemotherapy*, Washington, DC, 16–19 Dec 2005.
- Gobel, A., McArdell, C. S., Suter, M. J. –F. & Giger, W. 2004. Trace Determination of Macrolide and Sulfonamide Antimicrobials, a Human Sulfonamide Metabolite, and Trimethoprim in Wastewater Using Liquid Chromatography Coupled to Electrospray Tandem Mass Spectrometry. *Analytical Chemistry*, **76** (16): 4756 - 4764
- Goddard, A. F., Jessa, M. J., Barrett, D. A., Shaw, P. N., Idstrom, J., Cederberg, C. & Spiller, R. C. 1996. Effect of omeprazole on the distribution of metronidazole, amoxicillin, and clarithromycin in human gastric juice. *Gastroenterology*, **111**: 358 – 367

- Gong, R., Ding, Y., Li, M., Yang, C., Liu, H. & Sun, Y. 2005. Utilization of powdered peanut hull as biosorbent for removal of anionic dyes from aqueous solution. *Dyes and Pigments*, **64**: 187 – 192
- Gougazeh, M. & Buhl, J. –Ch. 2014. Synthesis and characterization of zeolite A by hydrothermal transformation of natural Jordanian kaolin. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, **15**: 35 – 42
- Goyal, M., Bhagat, M. & Dhawan, R. 2009. Removal of mercury from water by fixed bed activated carbon columns. *Journal of Hazardous materials*. **171**: 1009-1015
- Gracia-Lor, E., Sancho, J. V., Serrano, R. & Hernandez, F. 2012. Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. *Chemosphere*, **87**: 453 – 462
- Gu, C. & Karthikeyan, K. G. 2005. Sorption of the Antimicrobial Ciprofloxacin to Aluminum and Iron Hydrous Oxides. *Environmental Science & Technology*, **39** (23): 9166 – 9173
- Guedidi, H., Reinert, L., Leveque, J. –M., Soneda, Y., Bellakhal, N. & Duclaux, L. 2013. The effects of the surface oxidation of activated carbon, the solution pH and the temperature on adsorption of ibuprofen. *Carbon*, **54**: 432 – 443
- Guler, U. A. & Sarioglu, M. 2014. Removal of tetracycline from wastewater using pumice stone: equilibrium, kinetic and thermodynamic studies. *Journal of Environmental Health Science & Engineering*, **12** (79): 1 – 11
- Gulkowska, A., Leung, H. W., So, M. K., Taniyasu, S., Yamashita, N., Yeung, L. W. Y., Richardson, B. J., Lei, A. P., Giesy, J. P. & Lam, P. K. S. 2008. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. *Water Research*, **42**: 395 – 403
- Guo, J. –Z., Li, B., Liu, L. & Lv, K. 2014. Removal of methylene blue from aqueous solutions by chemically modified bamboo. *Chemosphere*, **111**: 225 – 231
- Gupta, S. S. & Bhattacharyya, K. G. 2011. Kinetics of adsorption of metal ions on inorganic materials: A review. *Advances in Colloid and Interface Science*, **162** (1-2): 39 – 58
- Hameed, B. H. & Rahman, A. A. 2008. Removal of phenol from aqueous solutions by adsorption onto activated carbon prepared from biomass material. *Journal of Hazardous Materials*, **160**: 576 – 581
- Hameed, B.H., Mahmoud, D. K. & Ahmad, A. L. 2008. Equilibrium modeling and kinetic studies on the adsorption of basic dye by a low-cost adsorbent: Coconut (*Cocos nucifera*) bunch waste. *Journal of Hazardous Materials*, **158** (1): 65 – 72
- Han, X., Wang, W. & Ma, X. 2011.Adsorption characteristics of methylene blue onto low cost biomass material lotus leaf. *Chem. Engg. J.* 171: 1 – 8

- Heberer, T. 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology Letters*, **131** (1-2): 5 – 17
- Heidari, M., Kazemipour, M., Bina, B., Ebrahimi, A., Ansari, M., Ghasemian, M. & Amin, M. M. 2013. A qualitative survey of five antibiotics in a water treatment plant in Central Plateau of Iran. *Journal of Environmental and Public Health*, **2013**: 1 – 9
- Hernando, M. D., Mezcua, M., Fernandez-Alba, A. R. & Barcelo, D. 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, **69** (2): 334 – 342
- Herrell, W. E. 1945. *Penicillin and other antibiotic agents*. Philadelphia and London: W. B. Suanders & Co., pp 363.
- Hirsch, R., Ternes, T., Haberer, K. & Kratz, K. –L. 1999. Occurrence of antibiotics in the aquatic environment. *Science of The Total Environment*, **225** (1-2): 109 – 118
- Ho, Y. S. & McKay, G. 2002. Appliocation of Kinetic Models to the Sorption of Copper (II) on to Peat. *Adsorption Science & Technology*, **20** (8): 797 – 815
- Ho, Y. S. 2006. Review of second-order models for adsorption systems. *Journal of Hazardous Materials*, **136** (3): 681 – 689
- Ho, Y. S., Wase, J. D. A. & Forster, C. F. 1995. Batch nickel removal from aqueous solution by sphagnum moss peat. *Water Research*, **29** (5): 1327 – 1332
- Homem, V. & Santos, L. 2011. Degradation and removal methods of antibiotics from aqueous matrices – A review. *Journal of Environmental Management*, **92** (10): 2304 – 2347
- Hou, J. P. & Poole, J. W. 1969. Kinetics and mechanism of degradation of Ampicillin in solution. *Journal of Pharmaceutical Sciences*, **58** (4): 447 – 454
- Houndt, T. & Howard, O. 2000. Long-term Shifts in Patterns of Antibiotic Resistance in Enteric Bacteria. *Applied and Environmental Microbiology*, **66**: 5406 – 5409
- Huang, C. H. & Sedlak, D. L. 2001. Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry. *Environmental Toxicology and Chemistry*, **20** (1): 133 – 139
- Huang, C. H., Renew, J. E., Pinkston, K. & Sedlak, D. L. 2001. Occurrence And Fate Of Antibiotic Compounds In Municipal Wastewater And Animal Waste. *Water Environment Federation*, **12**: 686 - 697
- Idris, S., Iyaka, Y. A., Ndamitso, M. M., Mohammed, E. B. & Umar, M. T. 2011. Evaluation of Kinetic Models of Copper Lead Uptake from Dye Wastewater by Activated Pride od Barbados Shell. *American Journal of Chemistry*, **1** (2): 47 – 51

- Jiang, Y., Li, M., Guo, C., An, D., Xu, J., Zhang, Y. & Xi, B. 2014. Distribution and ecological risk of antibiotics in a typical effluent-receiving river (Wangyang River) in north China. *Chemosphere*, **112**: 267 – 274
- Jiwalak, N., Rattanaphani, S., Bremner, J. B. & Rattanaphani, V. 2010. Equilibrium and kinetic modeling of the adsorption of indigo carmine onto silk. *Fibers and Polymers*, **11** (4): 572 – 579
- Jorgensen, S.E. & Halling-Sorensen, B. 2000. Drugs in the environment. *Chemosphere*, **40**: 691-699
- Karadag, D., Koc, Y., Turan, M. & Armagan, B. 2006. Removal of ammonium ion from aqueous solution using natural Turkish clinoptilolite. *Journal of Hazardous Materials*, **B136**: 604 - 609
- Karami, D. & Rohani, S. 2009. A Noel Approach for the Synthesis of Zeolite Y. *Industrial & Engineering Chemistry Research*, **48** (10): 4837 – 4843
- Karimi, K. & Taherzadeh, M. J. 2016. A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. *Bioresource Technology*, **203**: 348 – 356
- Kennedy, K. J., Gorur, S. S., Elliott, C.A., Andras, E. & Guiot, S.R. 1989. Media effects on performance of anaerobic hybrid reactors. *Water Research*, **23**: 1397 – 1405
- Kolpin, D. W., Furlong, E. T., Meyer, M, T., Thurman, E. M., Zaugg, S. D., Barber, L. B. & Buxton, H. T. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaisance. *Environmental Science and Technology*, **36** (6): 1202 – 1211
- Kosmulski, M. & Saneluta, C. 2004. Point of zero charge/isoelectric point of exotic oxides: Tl_2O_3. *Journal of Colloid and Interface Science*. **280** (2): 544–545
- Kumar, P. S., Ramalingam, S., Senthamarai, C., Niranjanaa, M., Vijayalakshmi, P. & Sivanesan, S. 2010. Adsorption of dye from aqueous solution by cashew nut shell: studies on equilibrium isotherm, kinetics and thermodynamics of interactions. *Desalinations*, **261**: 52 – 60
- Kummerer, K. 2000. Drugs, diagnostic agents and disinfectants in wastewater and water – a review. *Schriftenreihe des Vereins fur Wasser, Boden und Lufthygiene*, **105**: 59 – 71
- Kummerer, K. 2009. The presence of pharmaceuticals in the environment due to human use – a present knowledge and future challenges. *Journal of Environmental Management*, **90** (8): 2354 – 2366
- Kummerer, K., Al-Ahmad, A. & Mersch-Sundermann, V. 2000. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere*, **40** (7): 701 – 710

- Lapworth, D. J., Baran, N., Stuart, M. E. & Ward, R. S. 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environmental Pollution*, **163**: 287 – 303
- Larsson, D.G.J., De Pedro, C. & Paxeus, N. 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *Journal of Hazardous Materials*, **148**: 751 – 755
- Le-Minh, N., Khan, S. J., Drewes, J. E. & Stuetz, R. M. 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water Research*, **44** (15): 4295 – 4323
- Le-Minh, N., Stuetz, R. M. & Khan, S. J. 2012. Determination of six sulfonamide antibiotics, two metabolites and trimethoprim in wastewater by isotope dilution liquid chromatography/tandem mass spectrometry. *Talanta*, **89**: 407 – 416
- Leng, L., Yuan, X., Huang, H., Shao, J., Wang, H., Chen, X. & Zeng, G. 2015. Bio-char derived from sewage sludge by liquefaction: characterization and application for dye adsorption. *Applied Surface Science*, **346**: 223 – 231
- Li, H., Zhang, D., Han, X. & Xing, B. 2014. Adsorption of antibiotic ciprofloxacin on carbon nanotubes: pH dependence and thermodynamics. *Chemosphere*, **95**: 150 – 155
- Li, L., Quinlivan, P. A. & Knappe, D. R. U. 2002. Effects of activated carbon surface chemistry and pore structure on the adsorption of organic contaminants from aqueous solution. *Carbon*, **40**: 2085 – 2100
- Li, W., Shi, Y., Gao, L., Liu, J. & Cai, Y. 2012. Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China. *Chemosphere*, **89** (11): 1307 – 1315
- Liao, P., Zhan, Z., Dai, J., Wu, X., Zhang, W., Wang, K. & Yuan,S. 2013. Adsorption of tetracycline and chloramphenicol in aqueous solutions by bamboo charcoal: a batch and fixed-bed column study. *Chemical Engineering Journal*, **228**: 496 – 505
- Lin, A. Y.-c., Yu, T.-h. & Lateef, S. K. 2009. Removal of Pharmaceuticals in secondary wastewater treatment processes in Taiwan. *Journal of Hazardous Materials*, **167**: 1163 – 1169
- Linares-Solano, A., Lopez-Gonzalez, J. de. D., Molina-Sabio, M. & Rodriguez-Reinoso, F. 1980. Active carbons from almond shells as adsorbents in gas and liquid phases. *J. Chem. Technol. Biotechnol.,* **30**: 65 – 72
- Lindberg, R. H., Wennberg, P., Johansson, M. I., Tysklind, M. & Andersson, B. A. V. 2005. Screening of Human Antibiotic Substances and Determination of Weekly Mass Flows in Five Sewage Treatment Plants in Sweden. *Environmental Science and Technology*, **39** (10): 3421 – 3429
- Lissemore, L., Hao, C., Yang, P., Sibley, P. K., Mabury, S. & Solomon, K. R. 2006. An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. *Chemosphere*, **64** (5): 717 – 729

- Lopez-Garzon, F. J., Moreno – Castilla, C., Guerrero-Ruiz, A., Rodriguez – Reinoso, F. & Lopez-Gonzalez, J. de. D. 1984. High temperature adsorption of hydrocarbons by activated carbons prepared from olive stones. *Adv. Sci. Technol.,* **1**: 103 – 109
- Ma, W., Dai, J., Dai, X., Da, Z. & Yan, Y. 2015. Core-shell molecularly imprinted polymers based on magnetic chitosan microspheres for chloramphenicol selective adsorption. *Monatsh Chem*, **146** (3): 465 – 474
- Mahmoodi, N. M., Hayati, B., Arami, M. & Bahrami, H. 2011. Preparation, characterization and dye adsorption properties of biocompatible composite (alginate/titania nanoparticle). *Desalination*, **275**: 93 – 101
- Malcolm, G. P. 2011. Beta-Lactam Antibiotics. *Antibiotic Discovery and Development*, pp 79 – 117.
- Manzetti, S. & Ghisi, R. 2014. The environmental release and fate of antibiotics – A review. *Marine Pollution Bulletin*, **79** (1-2): 7 – 15
- Manzetti, S. & Stenersen, J. H. V. 2010. A critical view of the environmental condition of the Sognefjord – A review. *Marine Pollution Bulletin*, **60** (12): 2167 – 2174
- Martins, A. C., Pezoti, O., Cazetta, A. L., Bedin, K. C., Yamazaki, D. A. S., Bandoch, G. F. G., Asefa, T., Visentainer, J. V. & Almeida, V. C. 2015. Removal of tetracycline by NaOH-activated carbon produced from macadamia nut shells: Kinetic and equilibrium studies. *Chemical Engineering Journal*, **260**: 291 – 299
- Mestre, A. S., Pires, J., Nogueira, J. M. F. & Carvalho, A. P. 2007. Activated carbons for the adsorption of ibuprofen. *Carbon*, **45** (10): 1979 – 1988
- Miao, X. S., Bishay, F., Chen, M. & Metcalfe, C. D. 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environmental Science and Technology*, **38**: 3533 -3541
- Mitchell, S. M., Ullman, J. L., Teel, A.L. & Watts, R. J. 2014. pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin. *Science of The Total Environment*, **466 – 467**: 547 – 555
- Mohammad, Y. S., Shaibu-Imodagbe, E. M., Igboro, S. B., Giwa, A. & Okuofu, C. A. 2014. Modeling and Optimization for Production of Rice Husk Activated Carbon and Adsorption of Phenol. *Journal of Engineering*, **2014** (10): 1-10
- Mohd-Din, A. T., Ahmad, M. A. & Hameed, B. H. 2015. Ordered mesoporous carbons originated from non-edible polyethylene glycol 400 (PEG-400) for chloramphenicol antibiotic recovery from liquid phase. *Chemical Engineering Journal*, **260**: 730 – 739
- Molstad, S., Lundborg, C. S., Karlsson, A. –K. & Cars, O. 2002. Antibiotic Prescription Rates Vary Markedly Between 13 European Countries. *Scandinavian Journal of Infectious Diseases*, **34** (5): 366 – 371

- Mompelat, S., Le Bot, B. & Thomas, O. 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. *Environment International*, **35** (5): 803 – 814
- Moussavi, G. & Khosravi, R. 2010. Removal of cyanide from wastewater by adsorption onto pistachio hull wastes: parametric experiments, kinetics and equilibrium analysis. *Journal of Hazardous Materials*, **183**: 724 – 730
- Moussavi, G., Alahabadi, A., Yaghmaeian, K. & Eskandari, M. 2013. Preparation, characterization and adsorption potential of the NH4Cl-induced carbon for the removal of amoxicillin antibiotic from water. *Chemical Engineering Journal*, **217**: 119 – 128
- Mozgawa, W., Handke, M. & Jastrzebski, W. 2004. Vibrationa spectra of aluminosilicate structrural clusters. *Journal of Molecular Structure*, **704** (1-3): 247 – 257
- Mozgawa, W., Jastrzebski, W. & Handke, M. 2005. Vibrational spectra D4R and D6R structural units. *Journal of Molecular Structure*, 744-747:663 – 670
- Mozgawa, W., Krol, M. & Bajda, T. 2011. IR spectra in the studies of anion sorption on natural sorbents. *Journal of Molecular Structure*, **993** (1-3): 109 – 114
- Munagapati, V. S. & Kim, D. 2017. Equilibrium isotherms, kinetics, and thermodynamics studies for congo red adsorption using calcium alginate beads impregnated with nano-geothite. *Ecotoxicology and Environmental Safety*, **141**: 226 – 234
- Nandi, B. K., Uppaluri, R. & Purkait, M. K. 2008. Preparation and Characterization of low cost ceramic membranes for micro-filtration applications. *Appl. Clay Sci.*, **42** (1–2): 102–110
- Nasser, M. M., Hamoda, M. F.& Radwan, G. H. 1996. Utilization of palm-fruit bunch particles for the adsorption of dye stuff wastes. *Adsorp. Sci. Technol.,* **13**: 1 – 6
- Nawar, S. S. & Doma, H. S., 1989. Removal of dyes from effluents using low-cost agricultural by-products. *Sci. Total. Environ.* **79**: 271 – 279
- Nebot, C., Gibb, S. W. & Boyd, K. G. 2007. Quantification of human pharmaceuticals in water samples by high performance liquid chromatography-tandem mass spectrometry. *Analytica Chimica Acta*, **598**: 87 – 94
- Nie, M., Yang, Y., Zhang, Z., Yan, C., Wang, X., Li, H. & Dong, W. 2014. Degradation of chloramphenicol by thermally activated persulfate in aqueous solution. *Chemical Engineering Journal*, **246**: 373 – 382
- Nikolaou, A., Meric, S. & Fatta, D. 2007. Occurrence patterns of pharmaceuticals in water and wastewater environments. *Anal Bioanal Chem*, **387**: 1225 – 1234
- Ojha, K., Pradhan, N. C. & Samanta, A. N. 2004. Zeolite from fly ash: synthesis and characterization. *Indian Academy of Sciences*, **27** (6): 555 – 564
- Olatunji, M. A., Khandaker, M. U., Amin, Y. M., Nabi, H. & Mahmud, M. E. 2016. Cadmium-109 Radioisotope Adsorption onto Polypyrrole Coated Sawdust of

Dryobalanops aromatic: Kinetics and Adsorption Isotherms Modelling. *RSC Advances*, **11**(10): np

- Ozkaya, B. 2006. Adsorption and desorption of phenol on activated carbon and a comparison of isotherm models. *Journal of Hazardous Materials*, B129 (1-3): 158 – 163
- Pal, A., He, Y., Jekel, M., RE=einhard, M. & Gin, K. Y. –H. 2014. Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. *Environment International*, **71**: 46 – 62
- Pan, B. & Xing,B. 2008. Adsorption Mechanisms of Organic Chemicals on Carbon Nanotubes. *Environmental Science & Technology*, **42** (24): 9005 – 9013
- Peak, D., Luther, G. W. & Sparks, D.L. 2003. ATR-FTIR spectroscopic studies of boric acid adsorption on hydrous ferric oxide. *Geochimica et Cosmochimica Acta*, **67** (14): 2551 – 2560
- Peng, X., Tan, C., Yu, Y. & Wamg, Z. 2008. Multiresidue determination of fluoroquinolone, sulfonamide, trimethoprim, and chloramphenicol antibiotics in urban waters in China. *Environmental Toxicology and Chemistry*, **27** (1): 73 – 79
- Petrovic, M., Hernando, M. D., Diaz-Cruz, M. S. & Barcelo, D. 2005. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: A review. *Journal of Chromatography A*, **1067**: 1 – 14
- Pezoti, O., Cazetta, A. L., Bedin, K. C., Souza, L. S., Martins, A. C., Silva, T. L., Santos Jr, O. O., Visentainer, J. V. & Almeida, V. C. 2016. NaOH-activated carbon of high surface area produced from guava seeds as a high-efficiency adsorbent for amoxicillin removal: Kinetic, isotherm and thermodynamic studies. *Chemical Engineering Journal*, **288**: 778 – 788
- Pietrzak, R. & Bandosz, T. J. 2007. Activated carbons modified with sewage sludge derived phase and their application in the process of NO_2 removal**.** *Carbon*, **45**: 2537–2546
- Putra, E. K., Pranowo, R., Sunarso, J., Indraswati, N. & Ismadji, S. 2009. Performance of activated carbon and bentonite for adsorption of amoxicillin from wastewater: Mechanisms, isotherms and kinetics. *Water Research*, **43** (9): 2419 – 2430
- Queiroz, A. C., Santos, J. D., Monteiro, F. J., Gibson, I. R. & Knowles, J. C. 2001. Adsorption and release studies of sodium ampicillin from hydroxyapatite and glass-reinforced hydroxyapatite composites. *Biomaterials*, **22**: 1393 – 1400
- Rahardjo, A. K., Susanto, M. J. J., Kurniawan, A., Indraswati, N. & Ismadji, S. 2011. Modified Ponorogo bentonite for the removal of ampicillin from wastewater. *Journal of Hazardous Materials*, **190**: 1001 – 1008
- Ramesh, A., Lee, D. J. & Wong, J. W. C. 2005. Thermodynamic parameters for adsorption equilibrium of heavy metals and dyes from wastewater with low-cost adsorbents. *Journal of Colloid and Interface Science*, **291**: 588 – 592

- Renew, J. E. & Huang, C. -H. 2004. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A*, **1042**: 113 – 121
- Reverte, S., Borrull, F., Pocurull, E. & Marce, R. M. 2003. Determination of antibiotic compounds in water by solid-phase extraction-high-performance liquid chromatography-(electrospray) mass spectrometry. *Journal of Chromatography A*, **1010**: 225 – 232
- Rezende, C. A., Maziero, P., Azevedo, E. R., Garcia, W. & Polikarpov, I. 2011. Chemical and morphological characterization of sugarcane bagasse submitted to delignification process for enhanced enzymatic digestibility. *Biotech Biofuels*, **4**:54 - 59
- Roggo, Y., Chalus, P., Maurer, L. Lema-Martinez, C., Edmond, A. & Jent, N. 2007. A review of near infrared spectroscopy and chemometrics in pharmaceutical technologies. *Journal of Pharmaceutical and Biomedical Analysis,* **44** (3): 683 – 700.
- Roosta, M., Ghaedi, M., Daneshfar, A., Sahraei, R. & Asghari, A. 2014. Optimization of the ultrasonic assisted removal of methylene blue by gold nanoparticles loaded on activated carbon using experimental design methodology. *Ultrason. Sonochem.*, **21**: 242 – 252
- Sanchez-Avila, J., Tauler, R. & Lacorte, S. 2012. Organic micro-pollutants in coastal waters from NW Mediterranean Sea: Sources distribution and potential risk. *Environment International*, **46**: 50 – 62
- Sant'Anna, C. & de Souza, W. 2012. Microscopy as a tool to follow deconstruction of lignocellulosic biomass. *Current Microscopy Contributions to Advances in Science and Technology (A. Méndez-Vilas, Ed.):* np
- Schwarzenbach, R. P., Escher, B. I., Fenner, C., Hofstetter, T. B., Johnson, A. C., Gunten, U. V. & Wehrli, B. 2006. The Challenge of Micropollutants in Aquatic Systems. *Science*, **313**: 1072 – 1077
- Shaibu, S. E., Adekola, F. A., Adegoke, H. I. & Ayanda, O. S. 2014. A Comparative Study of the Adsorption of Methylene Blue onto Synthesized Nanoscale Zero-Valent Iron-Bamboo and Manganese-Bamboo Composites. *Materials*, **7** (6): 4493 – 4507
- Sikarwar, S. & Jain, R. 2016. Adsorption kinetics studies of an anti-inflammatory drug Mesalamine using Unsaturated Polyester Resin (UPR). *Journal of Molecular Liquids*, **224**: 219 – 226.
- Simon, D. C. & John, B. 2005. Ecosystem response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin*, **51**: 218 – 223
- Snow, D. D., Bartelt-Hunt, S. L., Saunders, S. E. & Cassada, D. A. 2007. Detection, Occurrence, and Fate of Emerging Contaminants in Agricultural Environments. *Water Environment Research*, **79** (10); 1061 – 1064

- Stuart, B. 2004. *Infrared Spectroscopy: Fundamentals and Applications*. John Wiley & Sons, Ltd: pg 71-88
- Stuart, M., Lapworth, D., Crane, E. & Hart, A. 2012. Review of risk from potential emerging contaminants in UK groundwater. *Science of the Total Environment*, **416**: 1 -21
- Subha, R. & Namasivayam, C. 2008. Kinetics and isotherm studies for the adsorption of phenol using low cost micro porous ZnCl2 activates coir pith carbon. *Canadian Journal of Civil Engineering*, **36** (1): 148 – 159
- Subramanyam, B. & Das, A. 2009. Study of the adsorption of phenol by two soils based on kinetic and isotherm modeling analyses. *Desalination*, 249(3): 914 – 921
- Sun, G. & Xu, X. 1997. Sunflower stalk as adsorbents for color removal from textile waste water. *Ind. Eng. Chem. Res.,* **36**: 808 – 812
- Sun, Y., Ding, C., Cheng, W. & Wang, X. 2014. Simultaneous adsorption and reduction of U(VI) on reduced graphene oxide-supported nanoscale zerovalent iron. *Journal of Hazardous Materials*, **280**: 399 – 408
- Tan, I. A. W., Ahmad, A. L. & Hameed, B. H. 2009. Adsorption isotherms, kinetics, thermodynamics and desorption studies of 2,4,6-trichlorophenol on oil palm empty fruit bunch-based activated carbon. *Journal of Hazardous Materials*, **164**: 473 – 482
- Tan, I. A. W., Hameed, B. H. & Ahmad, A. L. 2007. Equilibrium and kinetic studies on basic dye adsorption by oil palm fibre activated carbon. *Chemical Engineering Journal*, **127**(1-3): 111 – 119
- Tannin, S. & Gurgey, I. 1988. Bleaching of cotton seed and sunflower oils by active carbons from rice hulls. *Chim. Acta. Turica.,* **16**: 209 – 219
- Thiele-Bruhn, S. & Beck, I, -C. 2005. Effects of sulfonamide and tetracycline antibiotics on soil microbial activity and microbial biomass. *Chemosphere*, **59** (4): 457 – 465.
- Unuabonah, E. I., Adebowale, K. O. & Olu-Owolabi, B. I. 2007. Kinetic and thermodynamic studies of the adsorption of lead (II) ions onto phosphate-modified kaolinite clay. *Journal of Hazardous Materials*, **144**: 386 – 395
- Vadivelan, V. & Kumar, K. V. 2005. Equilibrium, kinetics, mechanism, and process design for the sorption of methylene blue onto rice husk. *Journal of Colloid and Interface Science*, **286** (1): 90 – 100
- Vieno, N. M., Harkki, H., Tuhkanen, T. & Kronberg, L. 2007. Occurrence of Pharmaceuticals in River Water and Their Elimination in a Pilot-Scale Drinking Water Treatment Plant. *Environmental Science & Technology*, **41** (14): 5077 – 5084
- Wahab, R., Mishra, A., Yun, S., Hwang, I. H., Mussarat, J., Al-Khedhairy, A. A., Kim, Y. – S. & Shin, H. 2012. Fabrication, growth mechanism and antibacterial activity of ZnO micro-spheres prepared via solution process. *Biomass and Bioenergy*, **39**: 227 – 236

- Wang, L., Zhang, J., Zhao, R., Li, Y., Li, C. & Zhang, C. 2010. Adsorption of Pb(II) on activated carbon prepared from *Polygonum orientele* Linn.: Kinetics, isotherms, pH, and ionic strength studies. *Bioresource technology*, **101** (15): 5808 – 5814
- Watkinson, A. J., Murby, E. J. & Constanzo, S. D. 2007. Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Research*, **41** (18):4164 – 4176
- Weber, T. W. & Chakkravorti. 1974. Pore and solid diffusion models for fixed-bed adsorbers. *AlChE J.*, **20**: 228
- Xia, M., Li, A., Zhu, Z., Zhou, Q. & Yang, W. 2013. Factors influencing antibiotics adsorption onto engineered adsorbents. *Journal of Environmental Sciences*, **25** (7): 1291 – 1299.
- Xie, M., Nghiem, L. D., Price, W. E. & Elimelech, M. 2012. Comparison of the removal of hydrophobic trace organic contaminants by forward osmosis and reverse osmosis. *Water Research*, **46** (8): 2683 – 2692.
- Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z. & Li, J. 2007. Occurrence and elimination of antibiotics at four sewage treatment pplants in the Pearl River Delta (PRD), South China. *Water Research*, **41** (19): 4526 – 4534.
- Xu, W., Zhang, G., Zou, S.-C., Li, X.-D. & Liu, Y.-C. 2006. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Environmental Pollution*, **145**: 672 – 679, May.
- Yang, J., Yu, M. & Chen, W. 2015. Adsorption of hexavalent chromium from aqueous solution by activated carbon prepared from longan seed: kinetics, equilibrium and thermodynamics. *J. Ind. Eng. Chem.*, 21: 414 – 422.
- Yang, K. & Xing, B. S. 2010. Adsorption of organic compounds by carbon nanomaterials in aqueous phase: Polanyi theory and its application. *Chem. Rev.*, **110**: 5989 – 6008.
- Yang, K., Wu, W. H., Jing, Q. F. & Zhu, L. Z. 2008. Adsorption of aniline, phenol, and their substitutes by multi-walled carbon nanotubes. *Environmental Science & Technology*, **42**: 7931 – 7936.
- Yang, S., Li, J., Shao, D., Hu, J. & Wang, X. 2009. Adsorption of Ni(II) on oxidized multi-walled carbon nanotubes: Effect of contact time, pH, foreign ions and PAA. *Journal of Hazardous Materials*, **166** (1): 109 – 116.
- Yoon, J., Cao, X., Zhou, Q & Ma, L. Q. 2006. Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, **368** (2-3): 456 – 464.
- Yu, B., Shukla, A., Shukla, S. S. & Dorris, K. L. 2001. The removal of heavy metals from aqueous solutions by sawdust adsorption – removal of lead and comparison of its adsorption with copper. *Journal of Hazardous Materials*, **B84**: 83 – 94.

- Zha, S. -x., Zhou, Y., Jin, X. & Chen, Z. 2013. The removal of amoxicillin from wastewater using organobentonite. *Journal of Environmental Management*, **129**: 569 – 576.
- Zhang, B., Zhang, H., Li, X., Lei, X., Li, C., Yin, D., Fan, X. & Zhang, Q. 2013. Synthesis of BSA/Fe3O4 magnetic composite microspheres for adsorption of antibiotics. *Material Science Engineering*, **33**: 4401 – 4408.
- Zhang, D., Pan, B., Zhang, H., Ning, P. & Xing, B. S. 2010. Contribution of different sulfamethoxazole species to their overall adsorption on functionalized carbon nanotubes. *Environmental Science and Technology*, **44**: 3805 – 3811.
- Zhang, J., Zhou, Q. & Ou, L. 2012. Kinetic, Isotherm, and Thermodynamic Studies of the Adsorption of Methyl Orange from Aqueous Solution by Chitosan/Alumina Composite. *Journal of Chemical & Engineering Data*, **57**: 412 – 419.
- Zhang, Q. & Chuang, K. T. 2001. Adsorption of organic pollutants from effluents of a Kraft pulp mill on activated carbon and polymer resin. *Advances in Environmental Research*, **3**: 251 – 258.
- Zhu, X. Y., Lee, S. L., Lee, Y. H. & Frauenheim, T. 2000. Adsorption and Desorption of an O2 Molecule on Carbon Nanotubes. *Physical Review Letters*, **85**(13): 2757
- Zorita, S., Martensson, L. & Mathiasson, L. 2009. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Science of the Total Environment*, **407** (8): 2760 – 2770.
- Zvinowanda, C. M., Okonkwo, J. O., Agyei, N. M. & Shabalala, P. N. 2008. Preparation and Characterization of Biosorbents Made From Maize Tassel. *Canadian Journal of Pure & Applied Sciences*, **2** (3): 483 – 488.
- Zwiener, C & Frimmel, F. H. 2000. Oxidative treatment of pharmaceuticals in water. *Water Research*, **34** (6): 1881 – 1885.
- Zwiener, C., Glauner, T. & Frimmel, F. H. 2000. Biodegradation of Pharmaceutical Residues Investigated by SPE-GC/ITD-MS and On-Line Derivatization. *Journal of Separation Science*, **23** (7-8): 474 – 478.

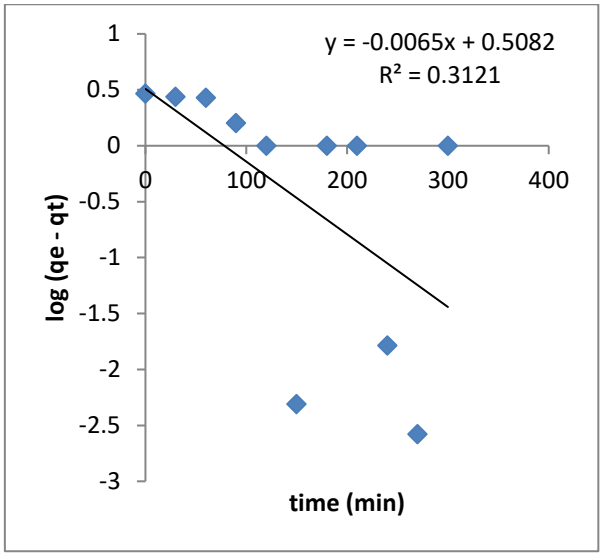
APPENDICES

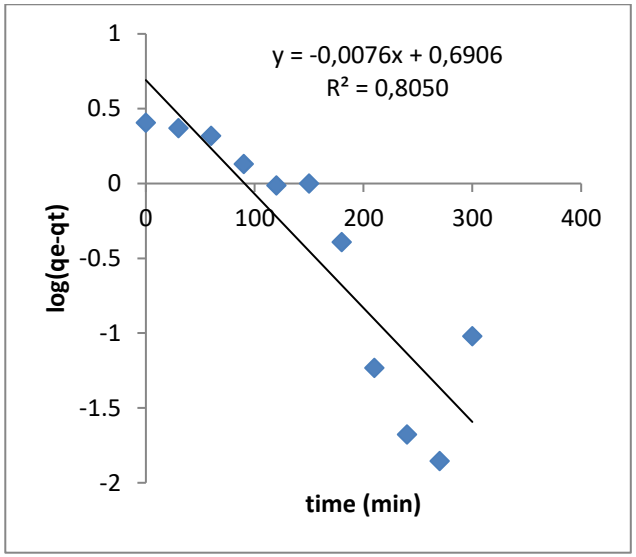
APPENDIX A: Effect of contact time on sorption quantities, q_t (mg/g) of AMX and AMP and CHLR

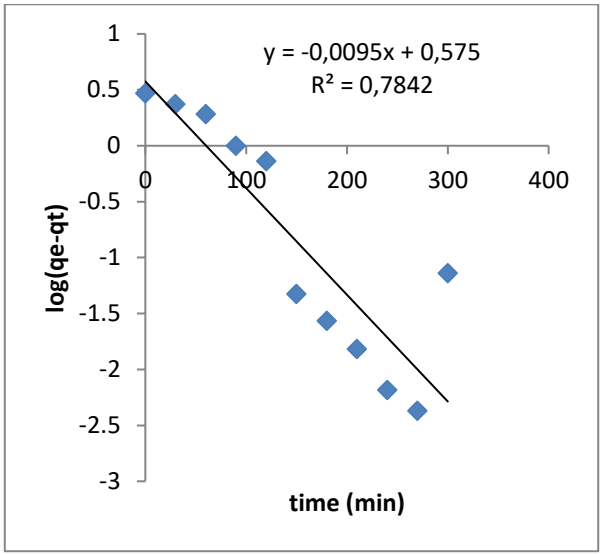
Time	*AMX*				*AMP*				*CHLR*			
(min)	*GSAa*	*GSAb*	*GSB*	*GS*	*GSAa*	*GSAb*	*GSB*	*GS*	*GSAa*	*GSAb*	*GSB*	*GS*
0	0.036	0.040	0.120	0.103	0.062	0.235	0.031	0.087	0.057	0.012	0.109	0.112
30	0.219	0.059	0.345	0.583	0.264	0.214	1.173	0.214	0.657	0.864	0.598	0.269
60	0.269	0.362	1.199	1.253	0.526	0.520	2.625	0.380	1.085	1.913	1.466	1.545
90	1.371	1.823	2.346	2.488	1.258	0.783	2.838	0.349	3.007	2.798	2.203	1.650
120	2.964	2.992	2.971	2.856	1.638	1.951	2.830	0.730	3.000	2.770	2.870	1.604
150	2.959	2.981	2.953	2.940	2.608	2.700	2.734	1.724	3.005	2.773	3.104	1.624
180	3.044	2.981	2.959	2.925	2.604	2.695	2.821	1.713	2.990	2.769	3.030	1.635
210	2.976	2.959	2.918	2.916	2.550	2.699	2.799	1.688	3.002	2.719	3.080	1.591
240	2.948	2.979	2.932	2.917	2.587	2.698	2.771	1.707	2.982	2.748	3.076	1.499
270	2.961	2.957	2.952	2.929	2.469	2.700	2.728	1.719	3.003	2.790	2.979	1.457
300	3.007	2.961	2.966	2.937	2.513	2.697	2.811	1.671	2.778	2.758	3.065	1.646

APPENDIX B: Linearized form of the pseudo-first order for AMX, AMP and CHLR by (a) GSAa, (b) GSAb, (c) GSB and (d) GS, respectively

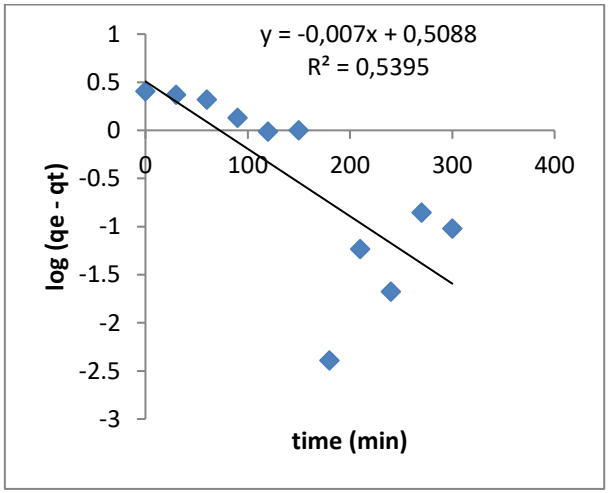
(a)

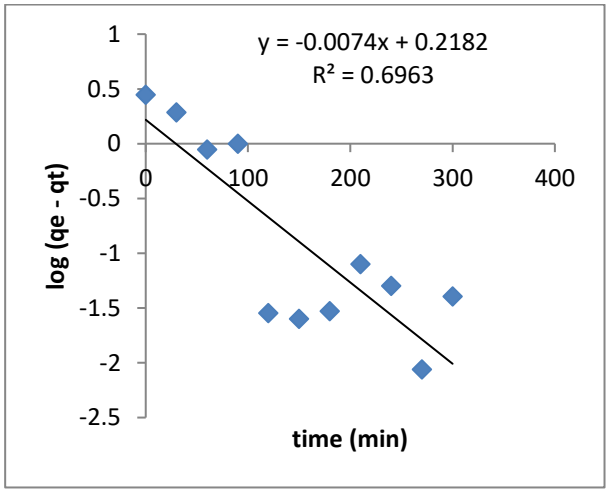


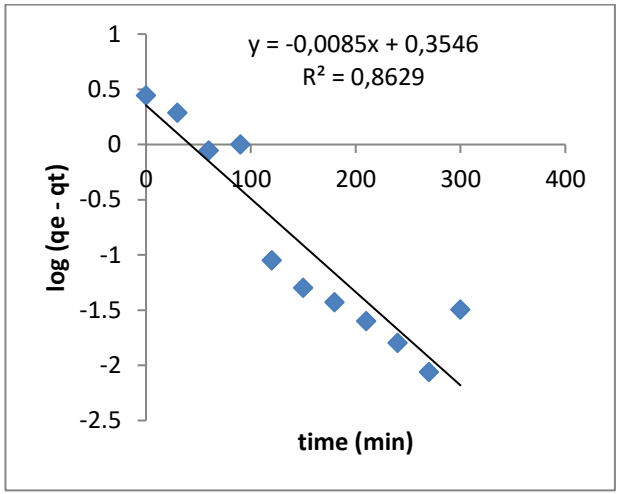




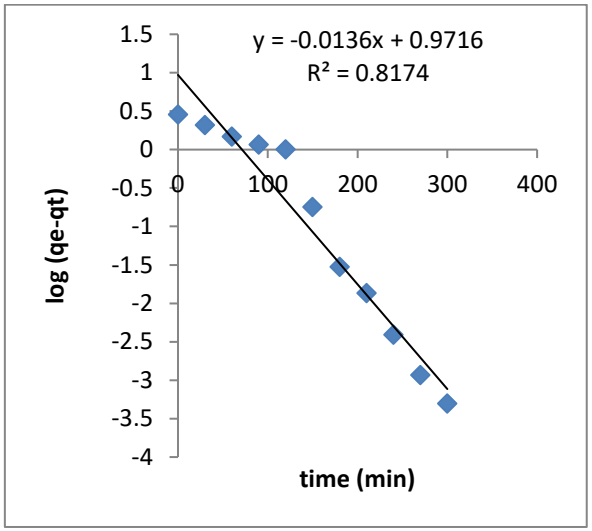
(b)

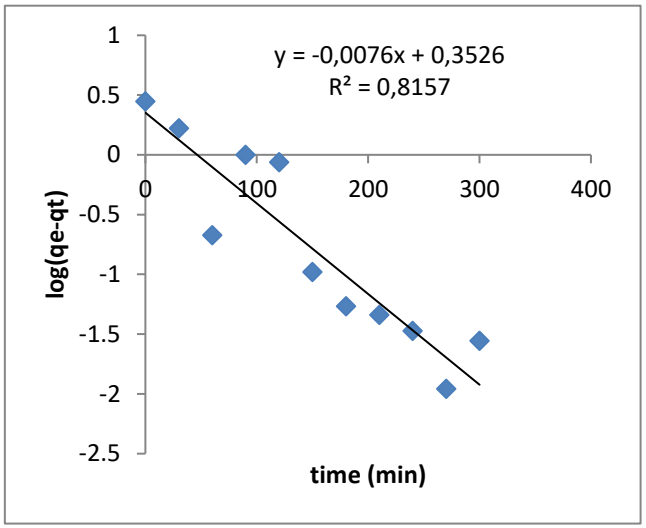


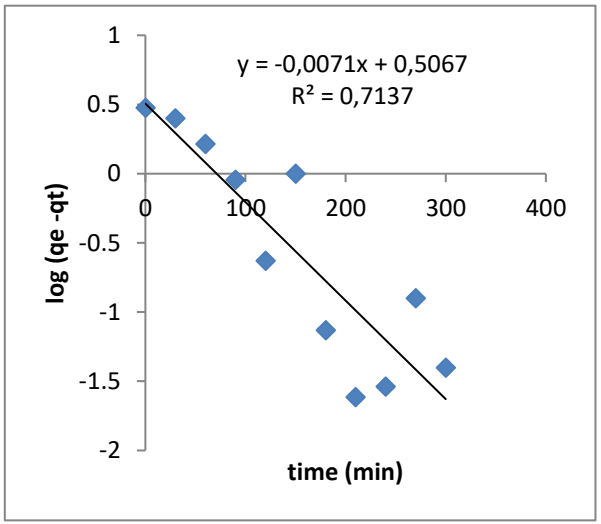




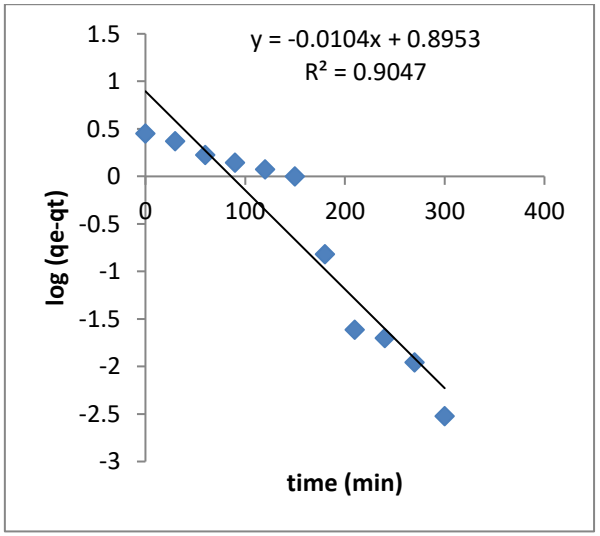
(c)

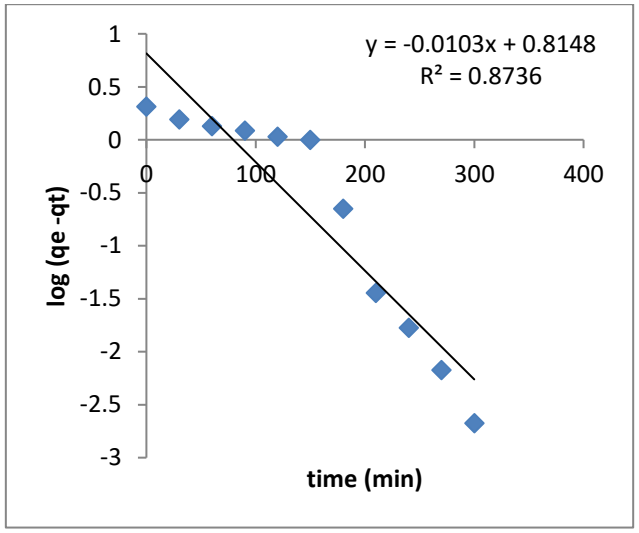

y = -0.0136x + 0.9716
R² = 0.8174
log (qe-qt)
time (min)

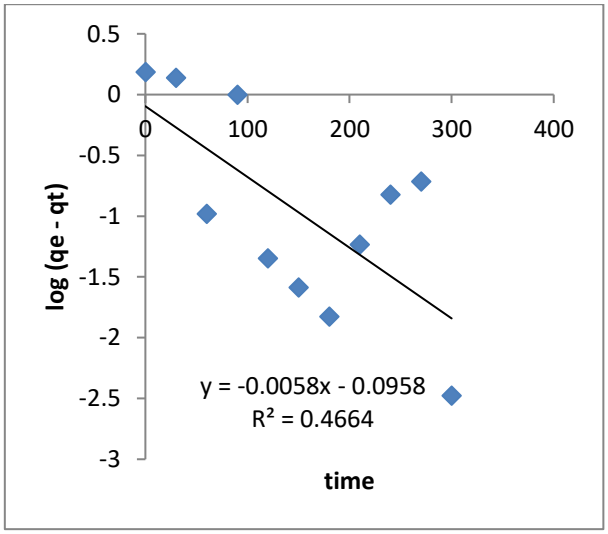

y = -0,0076x + 0,3526
R² = 0,8157
log(qe-qt)
time (min)


y = -0,0071x + 0,5067
R² = 0,7137
log (qe -qt)
time (min)

(d)

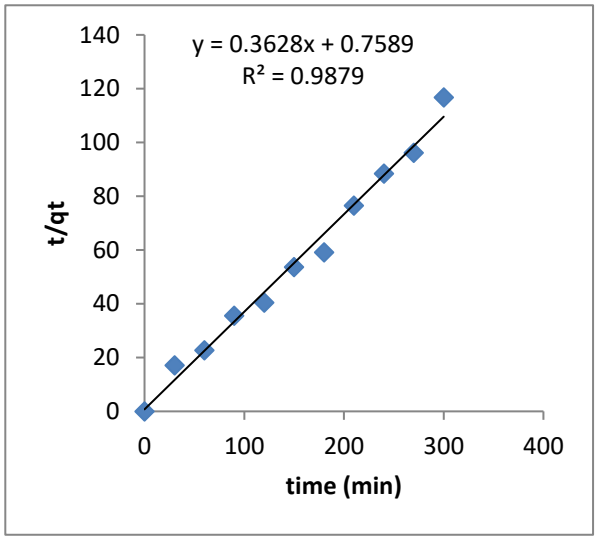

y = -0.0104x + 0.8953
R² = 0.9047
log (qe-qt)
time (min)

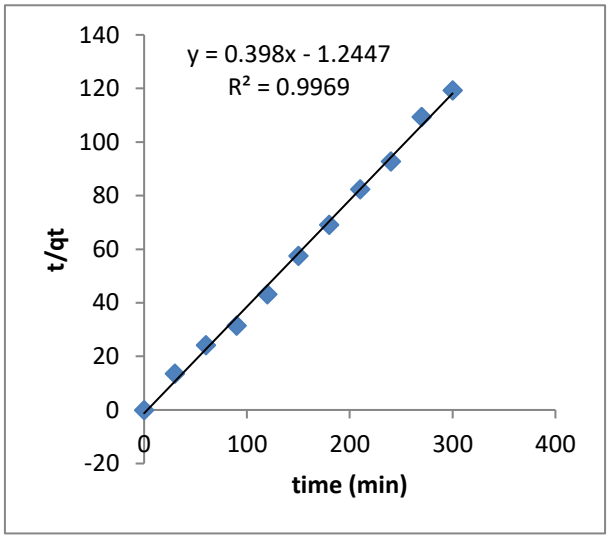

y = -0.0103x + 0.8148
R² = 0.8736
log (qe -qt)
time (min)

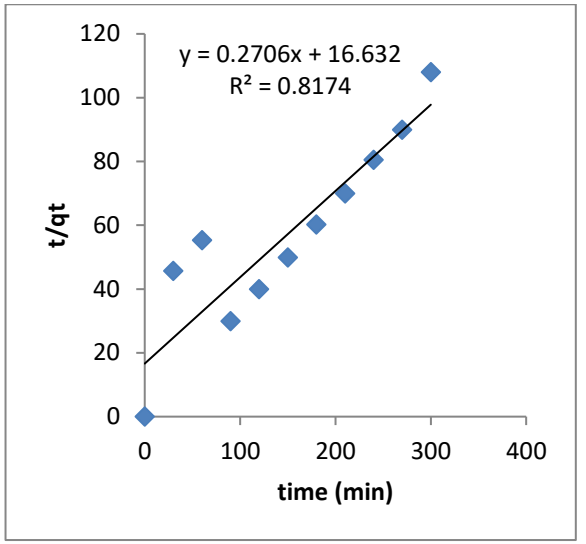

y = -0.0058x - 0.0958
R² = 0.4664
log (qe - qt)
time

APPENDIX C: Linearized form of the pseudo-second order for AMX, AMP and CHLR by (a) GSAa, (b) GSAb, (c) GSB and (d) GS, respectively

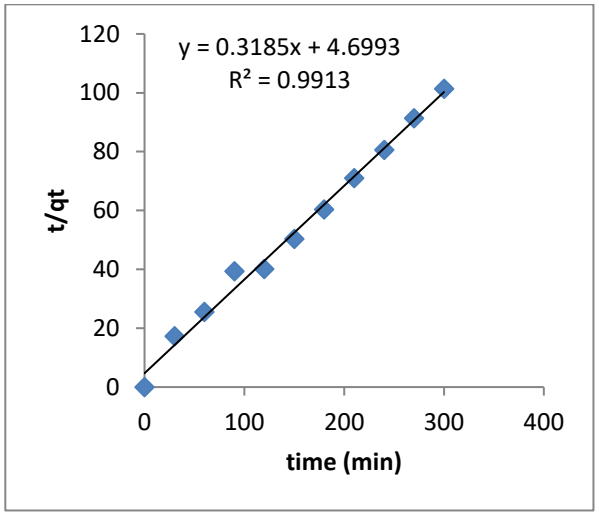
(a)

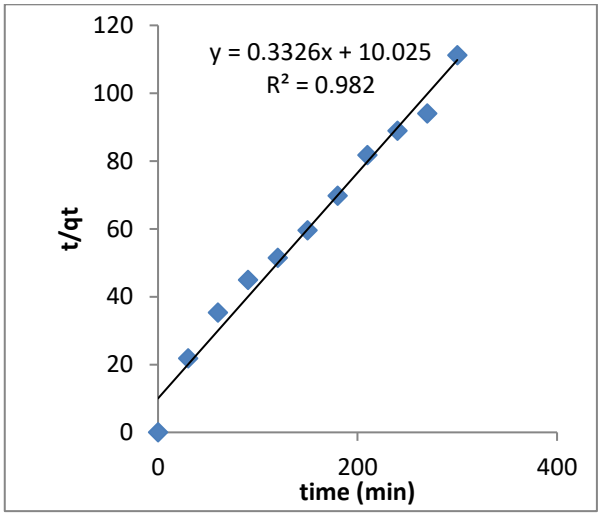


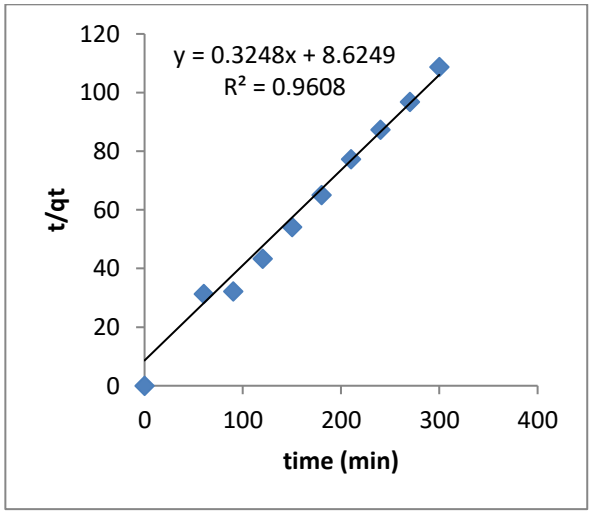




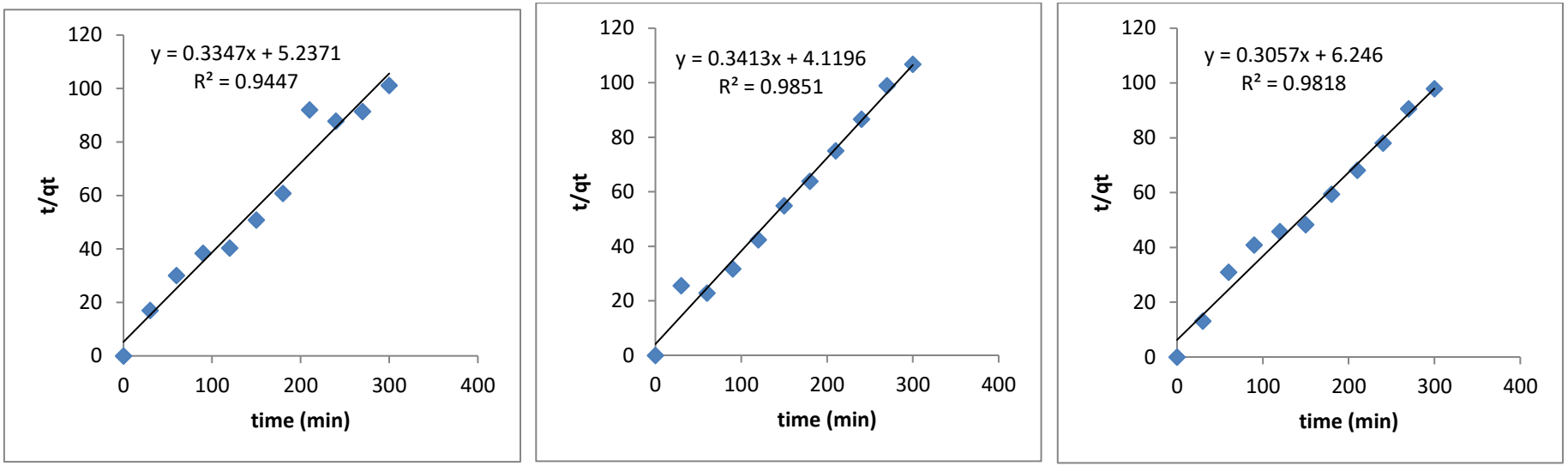
(b)



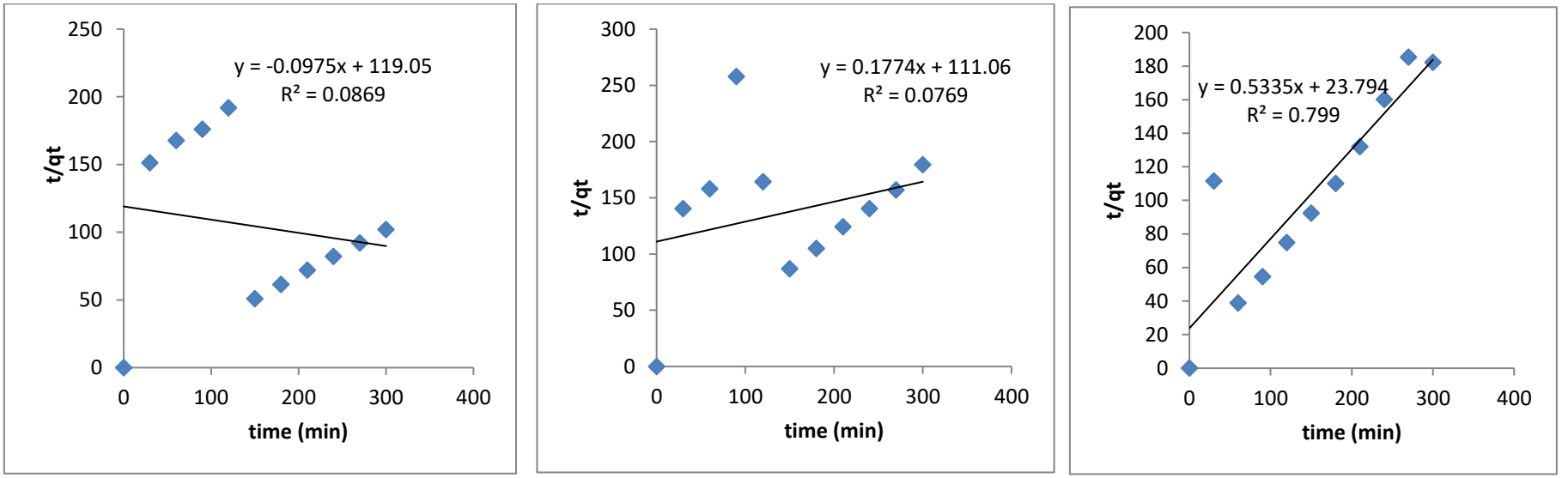




(c)

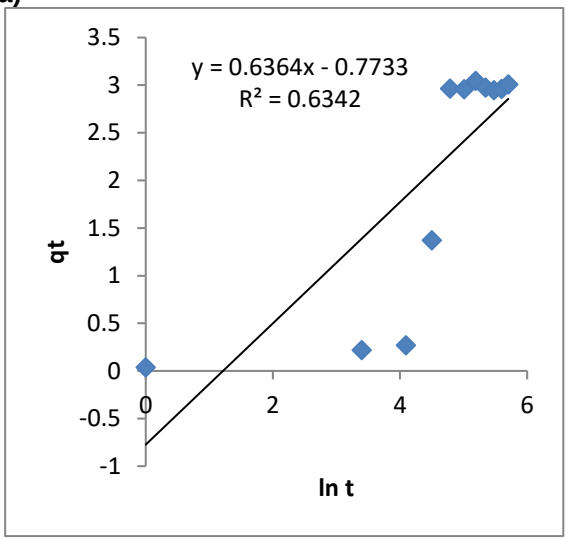

y = 0.3347x + 5.2371
R² = 0.9447
t/qt
time (min)
y = 0.3413x + 4.1196
R² = 0.9851
t/qt
time (min)
y = 0.3057x + 6.246
R² = 0.9818
t/qt
time (min)

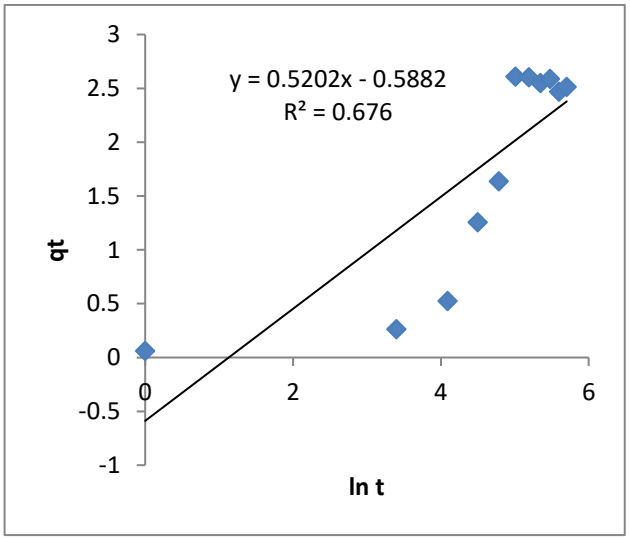
(d)

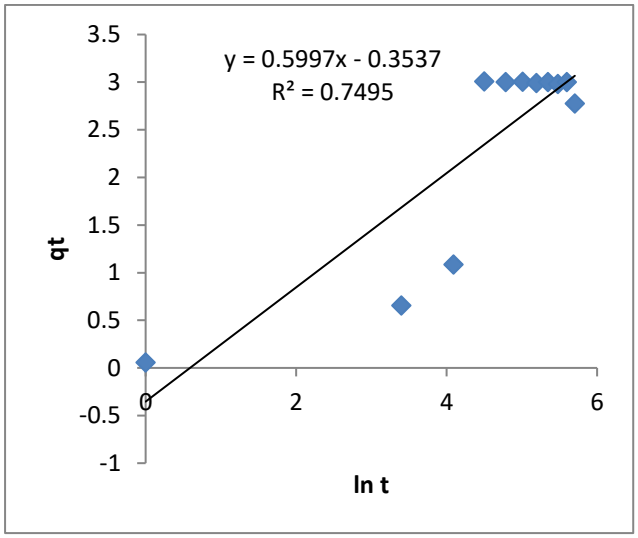

y = -0.0975x + 119.05
R² = 0.0869
t/qt
time (min)
y = 0.1774x + 111.06
R² = 0.0769
t/qt
time (min)
y = 0.5335x + 23.794
R² = 0.799
t/qt
time (min)

APPENDIX D: Linearized form of the Elovich model for AMX, AMP and CHLR by (a) GSAa, (b) GSAb, (c) GSB and (d) GS, respectively

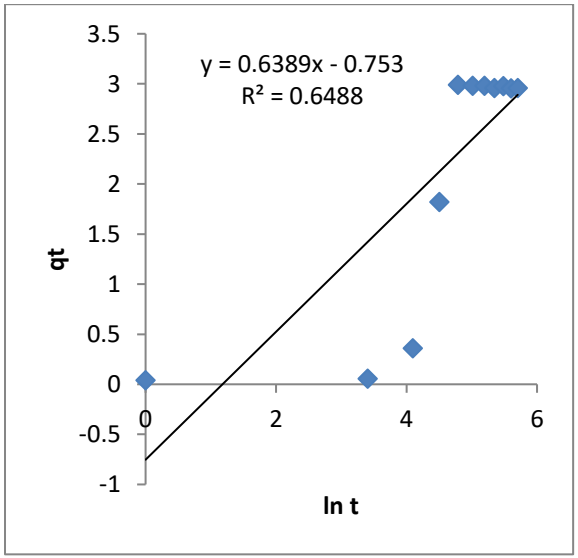
(a)

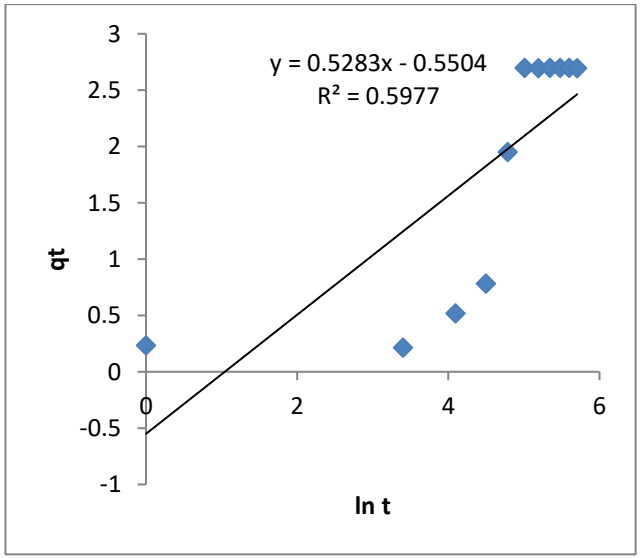


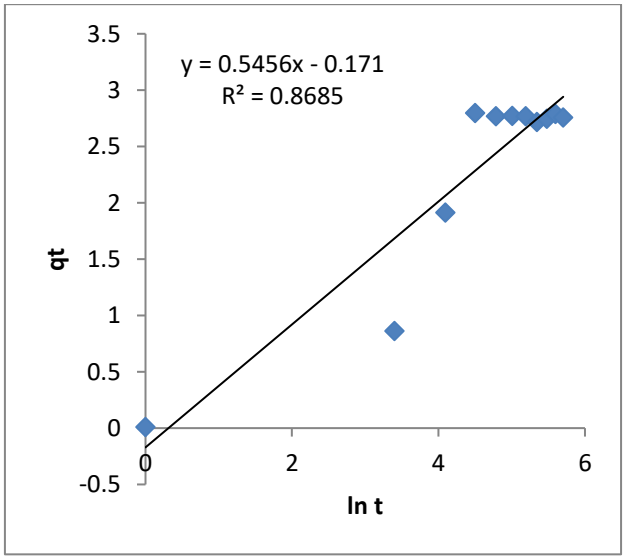




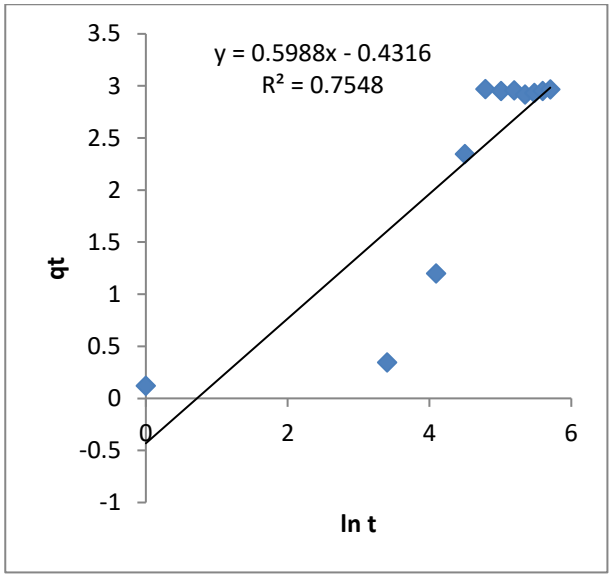
(b)

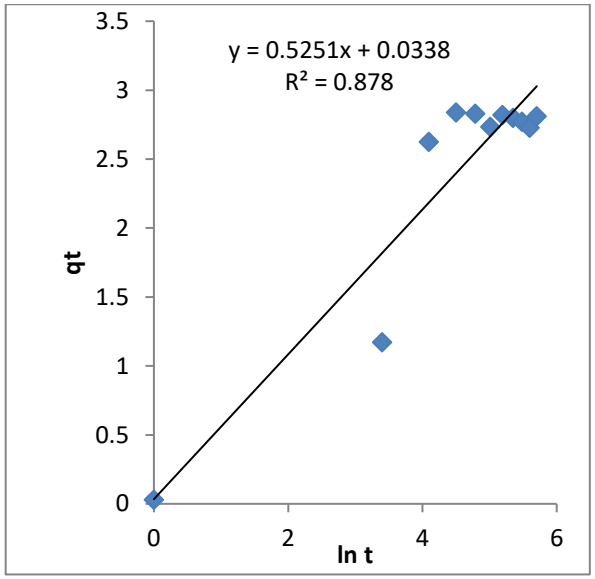


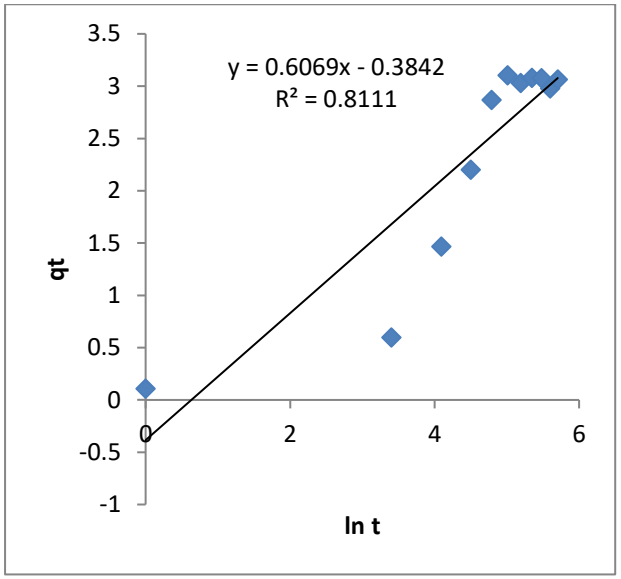




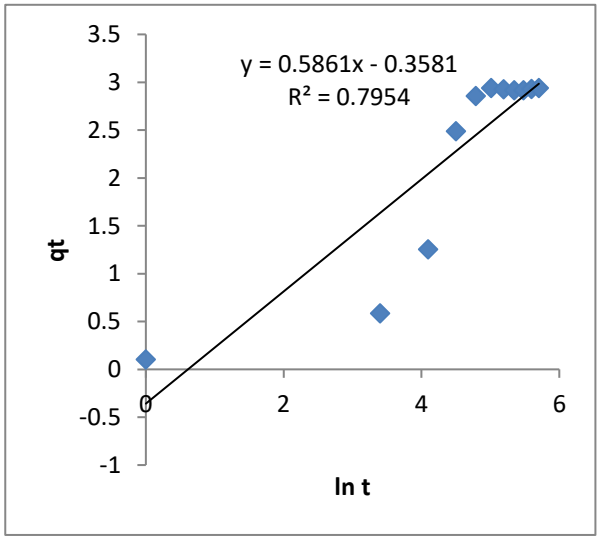
(c)

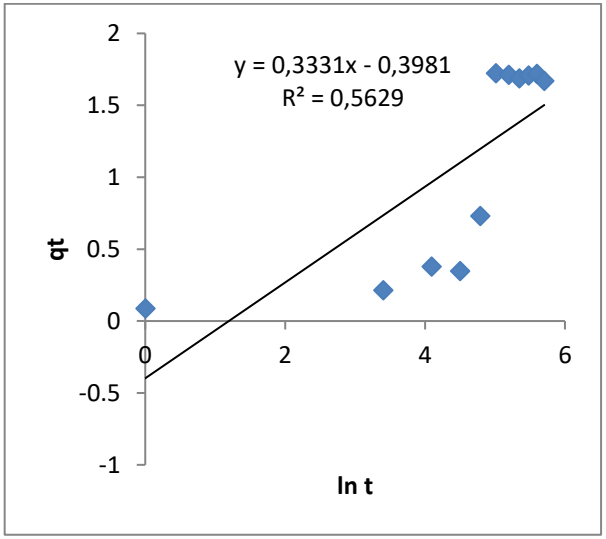

y = 0.5988x - 0.4316
R² = 0.7548
qt
ln t

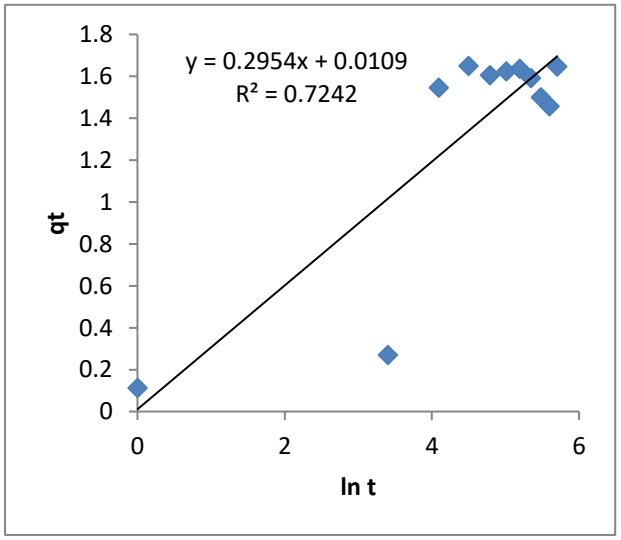

y = 0.5251x + 0.0338
R² = 0.878
qt
ln t


y = 0.6069x - 0.3842
R² = 0.8111
qt
ln t

(d)


y = 0.5861x - 0.3581
R² = 0.7954
qt
ln t


y = 0,3331x - 0,3981
R² = 0,5629
qt
ln t

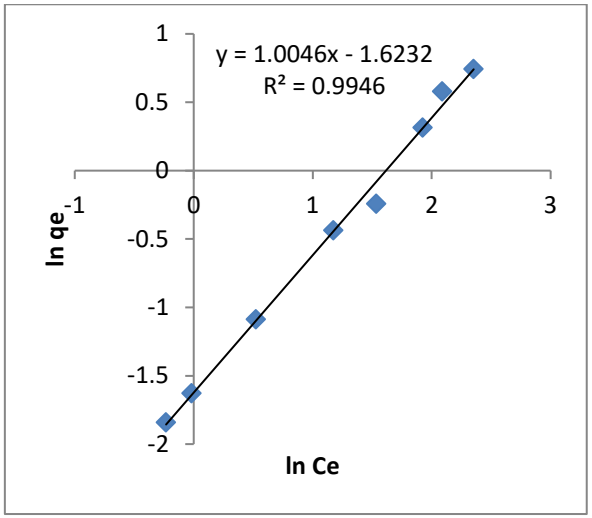

y = 0.2954x + 0.0109
R² = 0.7242
qt
ln t

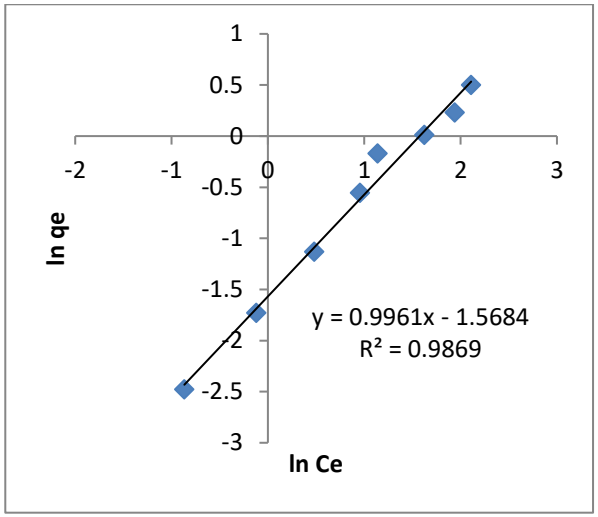
APPENDIX E: Effect of adsorbent dose on percent adsorbate removed

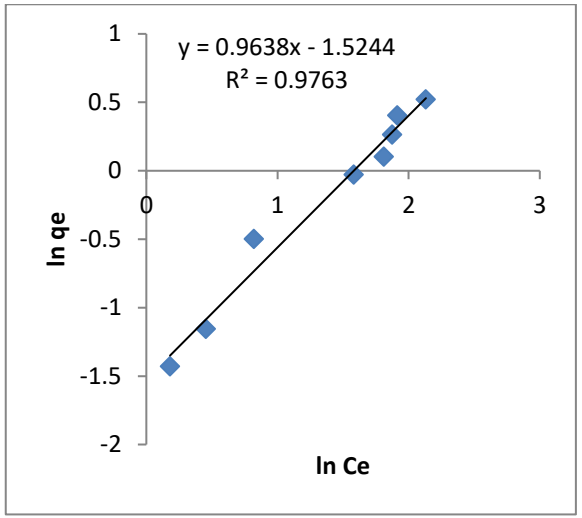
mass	AMX				AMP				CHLR			
(g)	GSAa	GSAb	GSB	GS	GSAa	GSAb	GSB	GS	GSAa	GSAb	GSB	GS
0,10	17,05	19,48	18,50	16,14	20,77	21,66	34,58	13,29	24,46	20,63	24,46	10,89
0,15	32,34	33,05	31,80	20,99	24,63	24,67	44,35	19,83	34,40	32,69	42,38	16,55
0,20	52,22	48,14	57,95	25,42	29,05	35,95	55,49	32,13	48,25	45,32	60,34	18,86
0,25	61,11	54,95	68,62	29,90	48,99	49,25	63,86	37,29	65,13	50,78	69,88	29,46
0,30	72,66	57,54	82,70	32,91	69,75	61,44	70,84	41,23	68,45	55,77	86,94	36,85
0,40	75,46	59,46	87,10	35,33	72,51	62,09	73,77	43,09	72,69	58,99	91,08	39,25

APPENDIX F: Linearized form of the Freundlich isotherm models for the adsorbates with (a) GSAa, (b) GSAb, (c) GSB and (d) GS

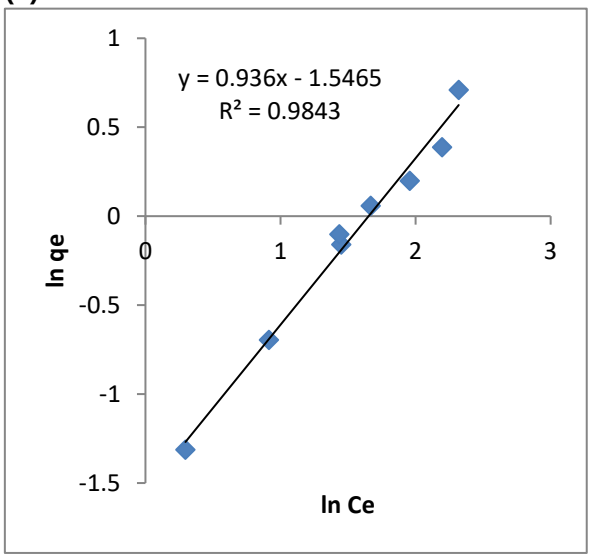
(a)

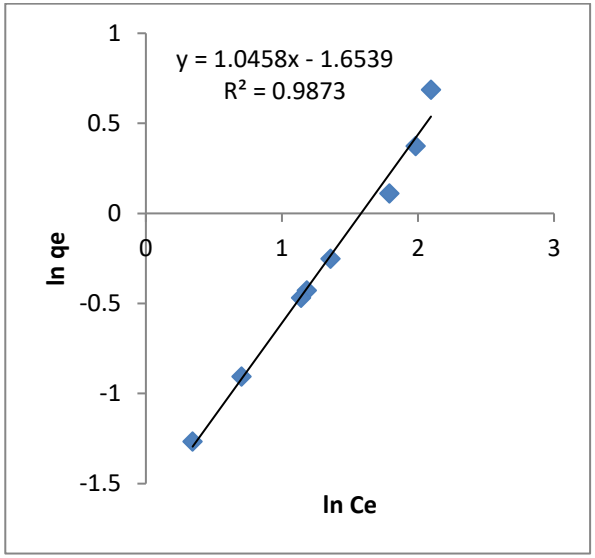


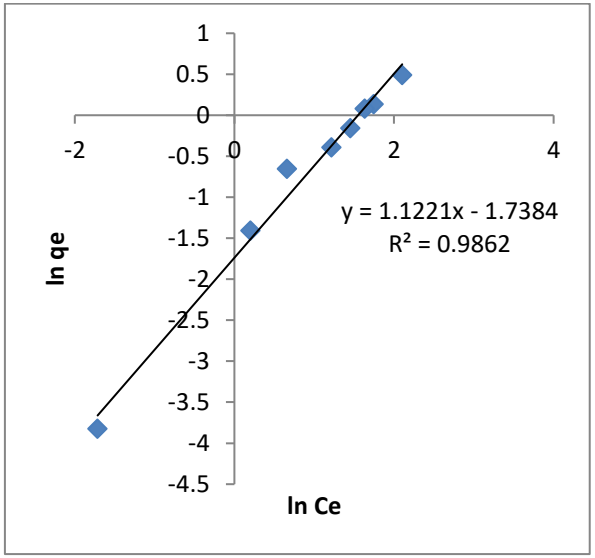




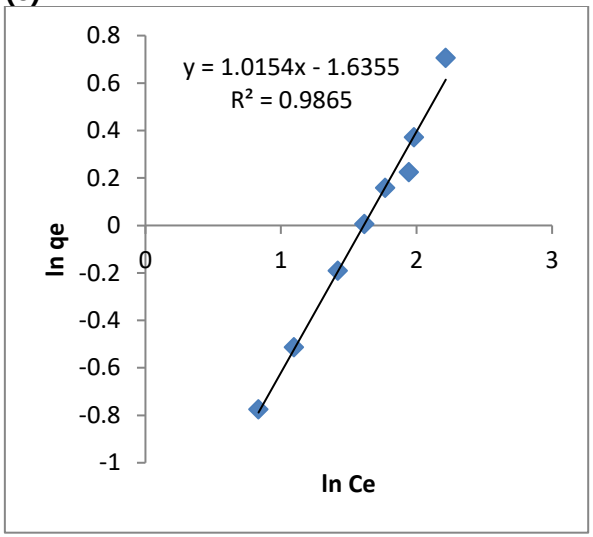
(b)

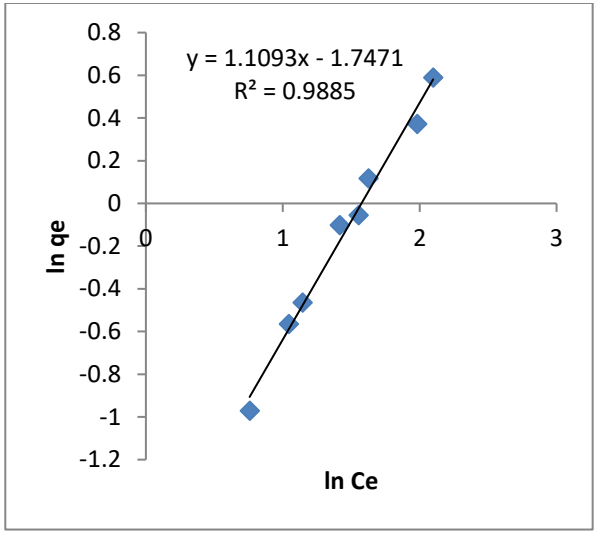


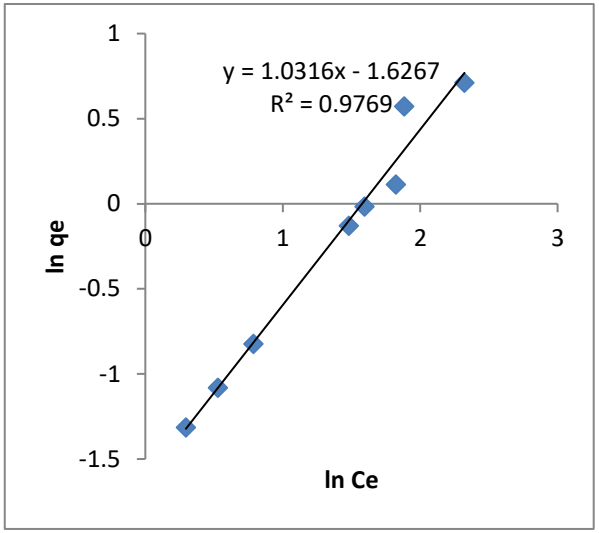




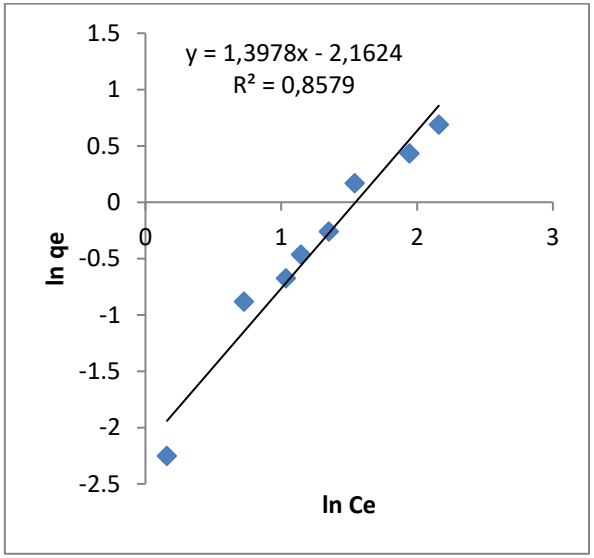
(c)

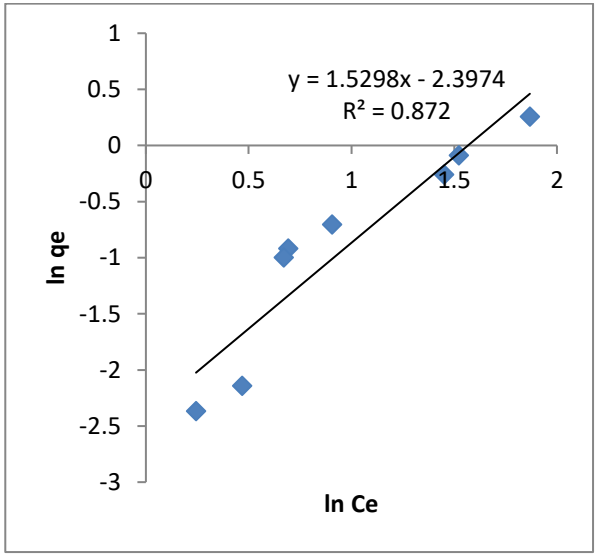

y = 1.0154x - 1.6355
R² = 0.9865
ln qe
ln Ce

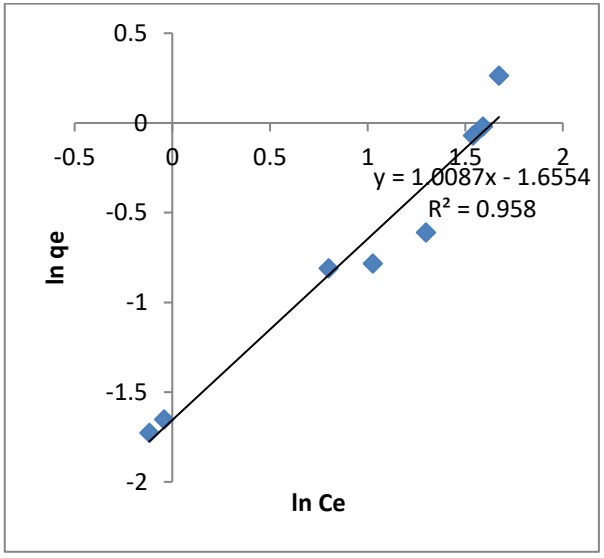

y = 1.1093x - 1.7471
R² = 0.9885
ln qe
ln Ce


y = 1.0316x - 1.6267
R² = 0.9769
ln qe
ln Ce

(d)

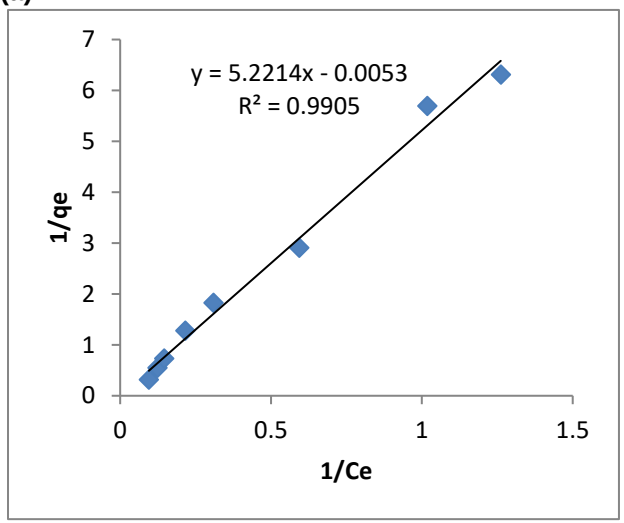

y = 1,3978x - 2,1624
R² = 0,8579
ln qe
ln Ce

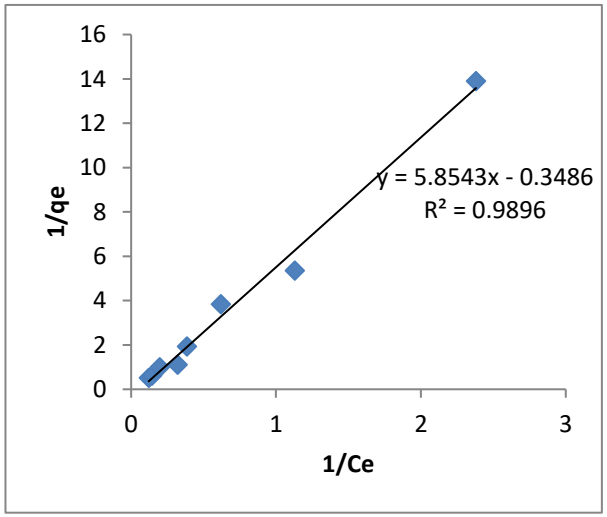

y = 1.5298x - 2.3974
R² = 0.872
ln qe
ln Ce

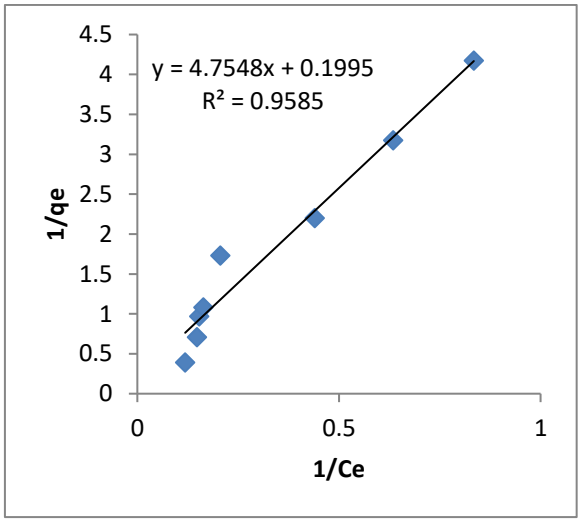

y = 1.0087x - 1.6554
R² = 0.958
ln qe
ln Ce

APPENDIX G: Linearized form of the Langmuir isotherm models for the adsorbates with (a) GSAa, (b) GSAb, (c) GSB and (d) GS

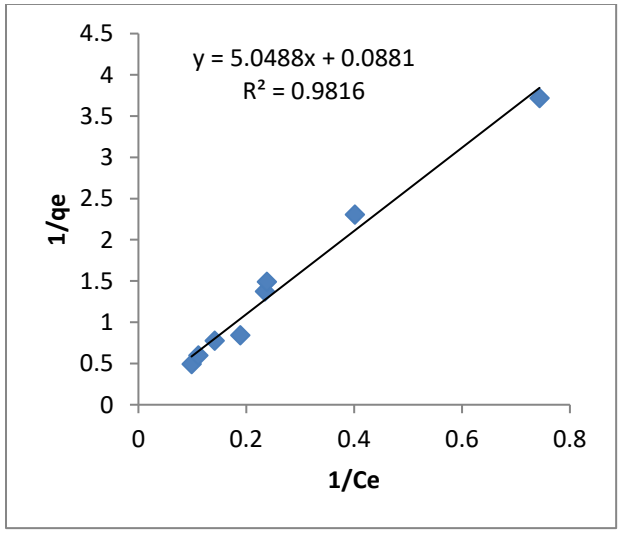
(a)

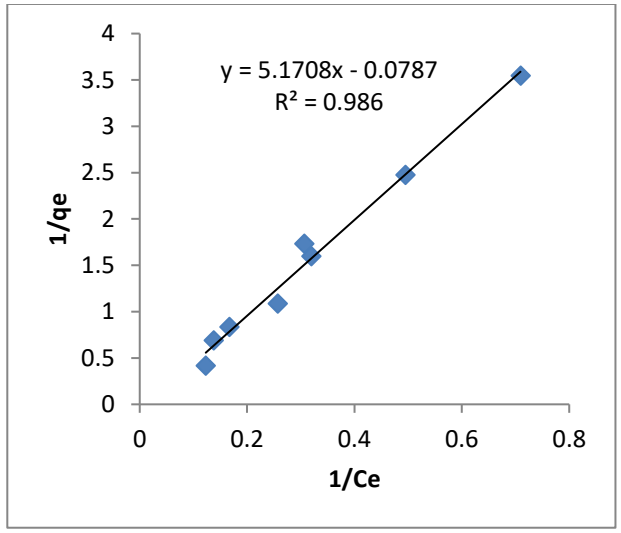


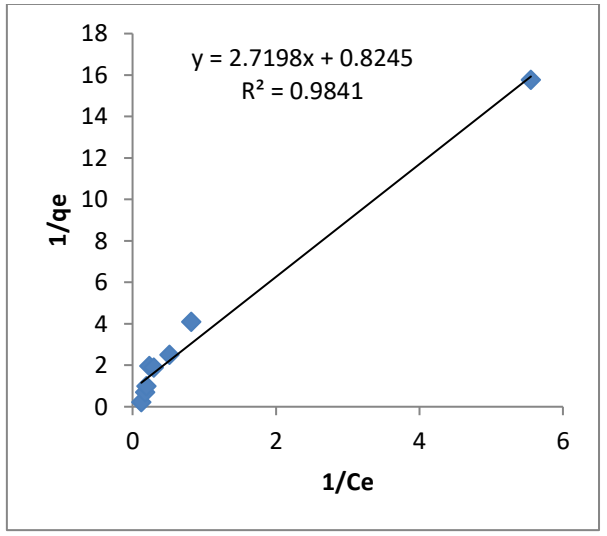




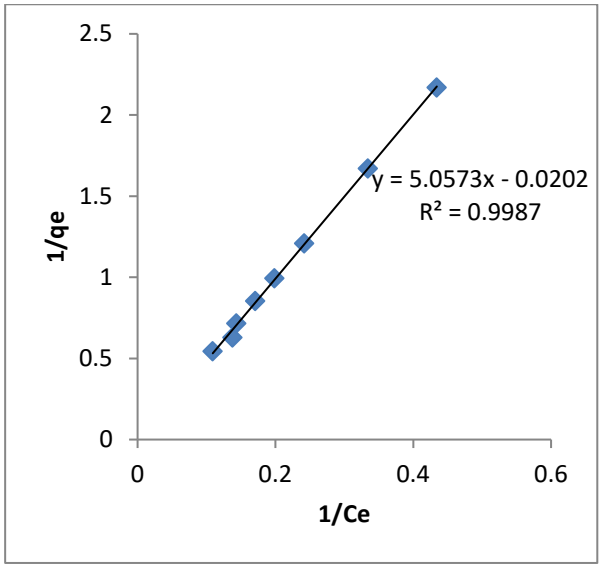
(b)

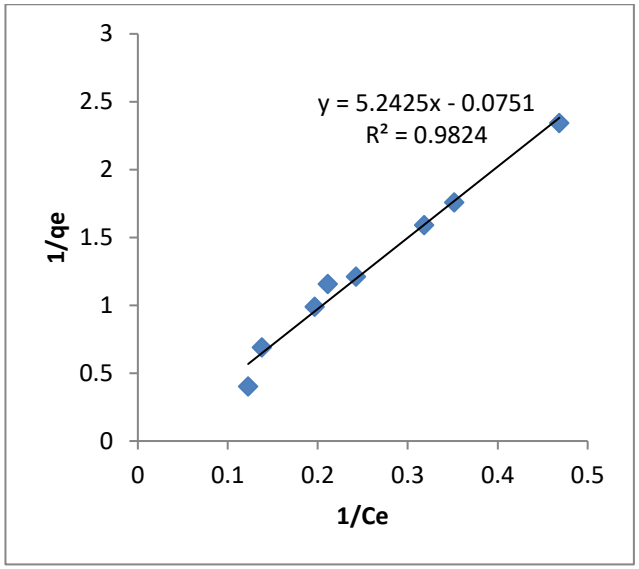


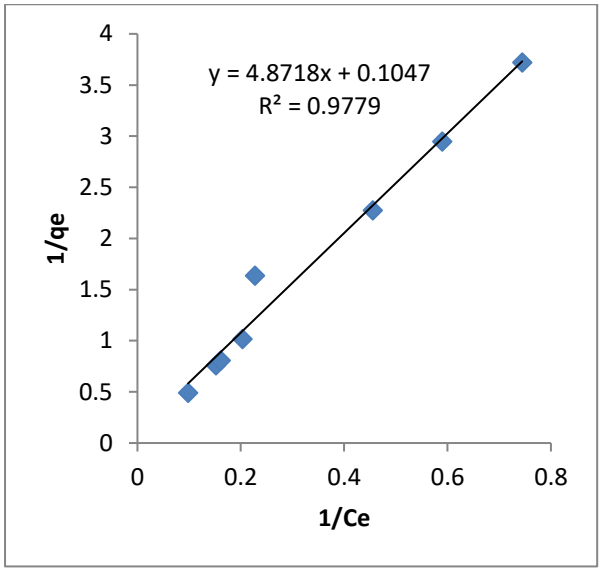




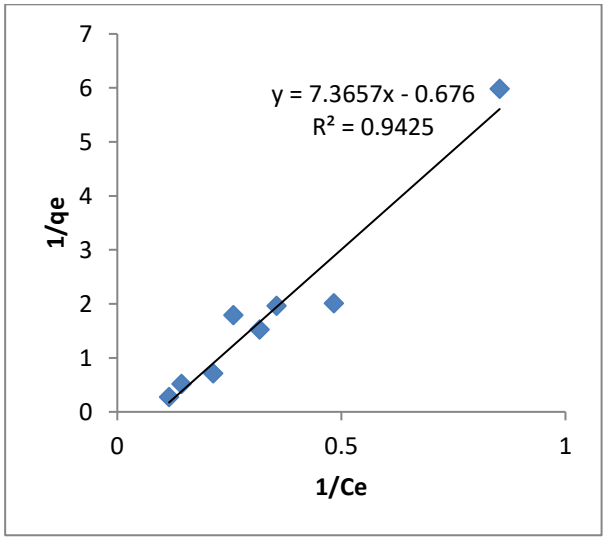
(c)

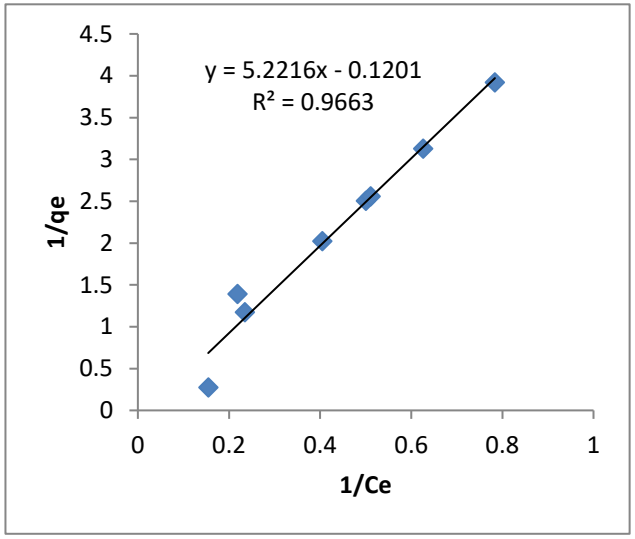

y = 5.0573x - 0.0202
R² = 0.9987
1/qe
1/Ce

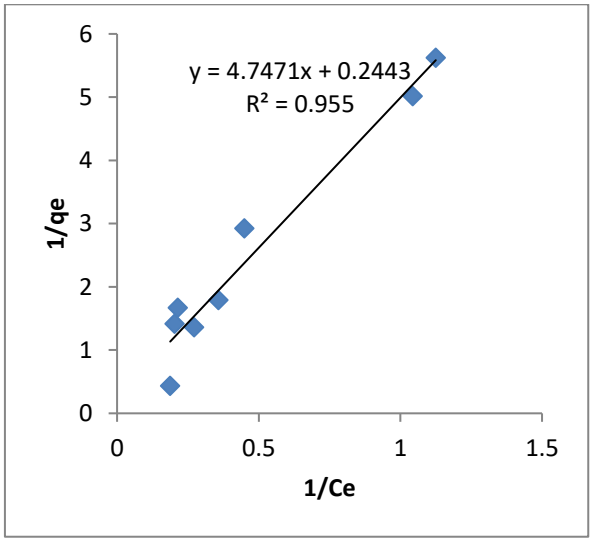

y = 5.2425x - 0.0751
R² = 0.9824
1/qe
1/Ce


y = 4.8718x + 0.1047
R² = 0.9779
1/qe
1/Ce

(d)


y = 7.3657x - 0.676
R² = 0.9425
1/qe
1/Ce


y = 5.2216x - 0.1201
R² = 0.9663
1/qe
1/Ce


y = 4.7471x + 0.2443
R² = 0.955
1/qe
1/Ce

APPENDIX H: Equilibrium adsorbed quantities, qe (mg/g) of adsorbates obtained with GSAa, GSAb, GSB and GS for all varying solution pHs

pH	AMX				AMP				CHLR			
	GSAa	GSAb	GSB	GS	GSAa	GSAb	GSB	GS	GSAa	GSAb	GSB	GS
4	1,452	1,052	2,257	1,158	1,245	1,212	1,142	1,035	2,067	1,585	1,687	1,032
6	1,314	1,266	2,326	1,361	1,378	1,515	1,424	1,503	2,354	1,741	1,842	1,224
8	1,417	1,433	2,399	1,510	1,632	1,566	1,589	1,676	2,414	1,794	1,862	1,036
10	1,599	1,522	2,356	1,611	1,899	1,718	1,721	1,687	2,501	1,831	1,964	1,058
12	1,752	1,570	2,202	1,686	2,251	2,378	1,763	1,746				