Amoxicillin removal from an aqueous solution by adsorption using graphene oxide/calcium alginate biocomposite

A Kaur$^{1,2}$ and C Maity$^{1,2}$

$^1$Chemical Science Research Group, Division of Research and Development
$^2$School of Chemical Engineering and Physical Sciences
Lovely Professional University, Phagwara, Punjab, India – 144411

Email: amarjeet.25361@lpu.co.in

Abstract. Antibiotics have been used for the treatment of microbial infections over several decades for both human and animals. However, these antibiotics are classified as potent pollutants due to their increasing overuse. Amoxicillin is one of the most frequently used antibiotics and its bioaccumulation in the food chain can threaten the human health. Herein, we show the adsorption activity of amoxicillin from aqueous solution using graphene oxide/calcium alginate composite, which is easy to operate, low-cost material. The composite has been characterized by UV-Vis spectroscopy, IR, SEM. The adsorption properties of amoxicillin were investigated through several parameters including the solution pH, the absorbent does, the initial concentration. Finally, a mechanism of adsorption has been proposed in this study.

1. Introduction
Millions of patients are diagnosed and prescribed antibiotics every year for various bacterial infections ranging from simple infections to severe sepsis [1]. Antibiotics are being used worldwide for either killing bacteria or refrain them from reproducing in not only for human therapy, but also for farming industry [2]. However, with the increasing use of various antibiotics, their occurrence in soil and aquatic environment have attracted significant attention in recent years as an emerging issue. Humans and animals can only poorly metabolize or absorb the used antibiotics and, therefore, a large fractions are excreted as an unmodified parent compound through urine and faeces [3]. This makes antibiotics a potential pollutants as they caused various adverse effect such as lowering of human immunity, chronic toxicity to aquatic lives and detrimental effect on water quality [4]. Antibiotic pollution has serious impact on human health and ecological sustainability. Pharmaceutical residues enter in the resources of water and soil and thereby can enter into the biological species grown in these areas [5]. Therefore antibiotic removal is an important and critical issue in current scenario.

Amoxicillin, a β-lactam antibiotic of penicillin class organism, is one of the most commonly used therapeutic agents against the bacterial infections [6]. It has been reported that amoxicillin is hardly decomposable and persists as active substance within human excretion [7]. Their existence on the
environment results in ecological destruction and threatens human health via bioaccumulation in the food chain [8]. Therefore, amoxicillin must be removed from the environment on urgent basis [9].

In recent years, several techniques have been developed to remove amoxicillin from water. These include biodegradation [10], coagulation [11], biofilm [12], chemical oxidation [13], ionization [14], and adsorption [15]. Adsorption is an efficient technique as it associates easy handling procedure, economic and without formation of any toxic product. Adsorption involves non-covalent interactions between adsorbent and an adsorbate. The adsorption efficiency generally relates to the adsorbent’s surface area, functional groups, and porosity [16]. Several adsorbents such as granular activated carbon [17], bentonite clay [18], magnetic nanoparticle [19], and magnetic composite [20] have been utilized for amoxicillin adsorption from water. The reported adsorption processes are beneficial due to non-toxic nature, but lack recyclability and reusability. Herein, we describe amoxicillin adsorption from aqueous solution onto a potentially recyclable biocomposite consisting graphene oxide/biopolymer composite as easy operational, low cost and highly efficient technique.

As biopolymer we chose sodium alginate, a natural polysaccharide, which is biocompatible, nontoxic, hydrophilic and low-cost material. Sodium alginate consists chain structure of α-L-guluronic acid and β-D-mannuronic acid residues that provides three-dimensional fiber network upon exchange of multivalent cation such as Ca$^{2+}$ with monovalent sodium ions [21]. Alginates find application in the removal of metal ions, dyes form the environment, where it has been used as an adsorbent [22]. Improvement of alginate’s adsorption capacity is possible by nanoparticle addition to the alginate network to prepare a composites [23]. In this work, graphene oxide (GO) nanoparticle has been chosen as dopant in calcium-alginate (CA) fiber network. GO is an important precursor of graphene which has been used worldwide as adsorbent due to its high surface area, large π-electron system and good stability. The presence of hydrophilic groups, such as –OH, –C=O, and –CO$_2$H group at GO surface makes it hydrophilic and suitable for applications in aquatic and biological environment [24]. Lately, removal of several ions and dyes from aqueous solution have been achieved using GO [25]. However, to the best of our knowledge, amoxicillin removal from aqueous solution using GO/CA composite as adsorbent has not been reported. In this study, GO/CA biocomposite fibers were synthesized and amoxicillin adsorption onto the fibers were investigated varying parameters including solution pH, the adsorbant dose, the agitation time, the concentration of amoxicillin. Finally adsorption mechanism of amoxicillin on the composite fibers was proposed.

2. Experimental Methods

2.1. Materials

Sodium salt of alginic acid, calcium chloride, graphite fine powder, potassium permanganate, sodium nitrate, hydrogen peroxide, hydrochloric acid (37%), and sulphuric acid (concentrated) were obtained from commercial vendors and used as received. Amoxicillin was purchased from Abolt healthcare Pvt. Ltd. in the form of capsules. Deionized water was used throughout the experiments.

2.2. Graphene oxide preparation

A modified Hummer method was employed for the preparation of GO [26]. Briefly, to a mixture of graphite fine powder (1.50 g) and NaN$_3$ (0.75 g), conc. sulphuric acid (34.50 ml) was mixed slowly maintaining 0 ºC in an ice-bath. Thereafter, KMnO$_4$ (4.50 g) was added gently to the suspension of acidic graphite maintaining 20 ºC temperature. After complete the addition, the mixture was allowed to get ambient temperature. Then it was stirred for 30 minutes keeping the temperature at 35 ºC. The mixture became a thick pasty with a small amount of gas evolution. Thereafter, water (300.0 ml) was added slowly to the thick pasty (caution: an exothermic reaction with effervescence). Then the reaction mixture was allowed to get ambient temperature. The suspension was then further diluted with water (210.0 ml) and H$_2$O$_2$ (30 %, 1.5 ml). Finally, the mixture was allowed to get the ambient temperature, followed by filtration and washing with deionized water. After air dry, a black coloured GO powder was obtained.
2.3. Graphene oxide/alginate biocomposite fiber preparation

GO/CA biocomposite fibers were prepared by wet spinning technique. Briefly, a suspension of GO (0.04 g) in water (50.0 ml) was sonicated for ½ hour. Thereafter, sodium alginate (2.0 g) was taken in deionized water (100.0 ml). Air bubbles were removed from the obtained mixture after stirring for around 1 hour. The dispersed GO was, then, slowly added to the solution of sodium alginate with stirring to obtain a homogeneous solution. Thereafter, this solution was introduced into a solution of calcium chloride (5 %, 20.0 g in 400 ml deionized water) using a syringe. The obtained GO/CA composite was then washed with water for five times, followed by air dry at ambient condition (figure 1). The composite fibers were then characterized by infrared spectroscopy (IR), scanning electron microscope (SEM).

![Sequence of preparation of GO/CA biocomposite fibers for adsorption of amoxicillin](image.png)

Figure 1: Sequences of preparation of GO/CA biocomposite fibers for adsorption of amoxicillin (Inset: Chemical structure of amoxicillin).

2.4. Measurement

The UV-Vis spectra absorption spectra were obtained with a UV-Vis spectrometer (Shimadzu UV-1800) and IR spectra (Fourier transform infrared, FTIR) were obtained using FTIR-8400 Shimadzu spectrophotometer. Material surface was analysed using scanning electron microscope (SEM) and SEM micrograph was obtained using JEOL, JSM-7610F.

2.5. Adsorption experiments

The batch equilibrium techniques were used to carry out the adsorption experiments in a water bath shaker at ambient condition. The pH effect on amoxicillin adsorption was studied using GO/CA biocomposite (0.025 g) in an amoxicillin solution (50 ml, 600 mg/litre) and solution pH was rectified from pH = 2 to pH = 10 with appropriate amount of aqueous sodium hydroxide or hydrochloric acid solution. The mixture was allowed to mix for 5h at ambient temperature, followed by centrifugation at 15000 rpm for 10 minutes. Afterwards, filtrate having amoxicillin was analysed using a UV-Visible spectrophotometer (vide infra) at 270 nm. It was compared with the initial concentration of amoxicillin at that pH solution. The adsorption capacity was computed utilising following equation:

\[ q = \frac{m}{V} (C_0 - C_t) \]

where, \( q \) (mg/g) = adsorption capacity, \( C_0 \) and \( C_t \) (mg/l) = initial and final concentration in solution respectively, \( V \) (litre) = volume of solution, \( m \) = weight of the adsorbent (GO/CA composite in g). For the calculation of \( C_t \), we followed Lambert-Beer’s law.
Effect of adsorbent does was investigated by taking different amount of GO/CA composite fibers (5 mg to 30 mg) for an amoxicillin solution (50 ml, 600 mg/l) at pH = 6. The dynamic adsorption test was performed taking 25 mg composite was taken in an amoxicillin solution (50 ml, 600 mg/l) at pH 6 and then antibiotic concentration was studied at different time. For the adsorption equilibrium experiments, 25.0 mg composite was taken into different amoxicillin concentration (0.20 g/l, 0.40 g/l, 0.60 g/l, 0.80 g/l, 1.00 g/l) in 50 ml solution at pH 6.

3. Result and Discussion

3.1. GO, CA and GO/CA-biocomposite characterization

The GO commonly described as the sp² hybridized carbon coexisted with islands of sp³ hybridized carbons featuring oxygen-containing functionalities [27]. Dispersed GO at neutral pH is dark colored and in FTIR, it reveals the presence of vibrational modes of different functional groups such as epoxides (875 cm⁻¹), alkoxy (1028 cm⁻¹), epoxy (1132 cm⁻¹), C=C (aromatic, 1579 cm⁻¹), carboxyl C=O (1697 cm⁻¹), hydroxy (–OH, 3230 cm⁻¹) groups [28]. The FTIR spectra of CA showed the vibration bands of –OH bond (3265 cm⁻¹), carboxyl C=O (1658 cm⁻¹), carboxylate ion (1581 cm⁻¹) [29]. The FTIR of GO/CA composite exhibited changes due to incorporation of GO in CA (figure 2a). The morphology of GO, CA and GO/CA composite were examined by scanning electron microscope (SEM). Many wrinkles can be found in the SEM image of GO (Figure 2b). These wrinkles have several layers that could increase pharmaceutical adsorption. A lot of ravines and wire like network have been observed in the SEM image of CA (figure 2c), which accounted for large surface area and provided more adsorption sites [30]. The SEM image of GO/CA biocomposite showed many wrinkles with very rough surface area and many ravines (figure 2d – e).

![Figure 2: (a) IR spectra with vibrational mode of characteristic functional groups for CA, GO and GO/CA biocomposite, (b – e) SEM micrograph for of GO (b), CA (e), and GO/CA biocomposite (d) and zoom in image of GO/CA in d (e).](image)

3.2. Factor effecting amoxicillin adsorption via biocomposites

3.2.1. pH Effect. pH of the solution is an important factor of amoxicillin adsorption as it contains functional groups (e.g., –NH₂, –CO₂H, –C=O), amide bond, a phenol functionality. Adsorption process can greatly be affected via changing the surface charge of the molecule and its ionization behaviour. Adsorption behaviour of amoxicillin on GO/CA composite at different pH is shown at figure 3a. At a low pH, the adsorption of amoxicillin is less presumably due to competition for
adsorbant sites between protons and amoxicillin molecule with protonated amine group. The adsorption capacity increases with increasing pH. Amoxicillin is zwitterionic in nature at pH 5 (presence of negative (–CO$_2$) and positively (–NH$_3$) charged functional groups) [18]. Adsorption is maximum at this pH range due to enhanced electrostatic interaction between the adsorbate and charged amoxicillin molecule. Furthermore, amoxicillin molecules and GO/CA biocomposite fibers can lead to π-π stacking interaction between benzene ring and π-bonds (–C=C- & –C=O). All these factors leads significant adsorption of amoxicillin onto GO/CA fibers under nearly neutral condition. However, at higher pH, the adsorption decreases significantly due to creation of carboxylate anions and electrostatic repulsion.

3.2.2. Effect of adsorbent quantity. Amoxicillin adsorption on GO/CA fibers depends on the number of active sites of adsorbant. With increasing adsorbant dose, adsorbant capacity greatly increases. At pH 6 & $C_0 = 600$ mg/litre, the removal percentage increases with increasing GO/CA biocomposite fibers (figure 3b). This is due to more active sites available for amoxicillin by increasing adsorbant concentration.

3.2.3. Effect on initial concentration of amoxicillin. For constant amount of adsorbant dose, the adsorption rate would slow down with increasing concentration of amoxicillin due to the saturation of adsorbent sites. The adsorption isotherm of GO/CA composite fibers (figure 3c) at different initial amoxicillin concentration shows with increasing adsorbate amount (for 25 mg GO/CA composite) the adsorption capacity increases until the saturation of adsorbent sites.

3.2.4. Effect on the contact time. The adsorption of amoxicillin (0.60 g/litre) onto GO/CA fibers (25 mg) depends on available time for interaction (shown in figure 3d). The adsorption is rapid at first hour and then reaches equilibrium gradually. The initial adsorption capacity is high due to presence of many active sites on composite fibers. Thereafter, the available active surface is filled up by the adsorbate and adsorption process slow down.

Figure 3: Factors affecting amoxicillin adsorption on GO/CA biocomposite: (a) solution pH, (b) adsorbent quantity, (c) initial amount of amoxicillin and (d) agitation time. The lines are drawn to guide the eye.

3.3. Discussion on adsorption mechanism
The surface of GO provides many functional groups including epoxy, hydroxy, carbonyl, carboxylic group, whereas calcium alginate provides hydroxyl and carboxylic functional groups. The adsorption mechanism of amoxicillin includes ionic interaction, hydrogen bonding, π-π interactions. Amine and carboxylic functional group of amoxicillin provides the ionic interaction via zwitterion formation around neutral pH range, whereas aromatic ring and π-functional groups contributes to the affinity towards graphene-conjugated basal plane via π-π interactions.

4. Conclusions
In conclusion, we demonstrate the amoxicillin adsorption onto GO/CA biocomposite at different conditions. An environment-friendly, highly efficient and easily accessible adsorbent was obtained by simple mixing an aqueous suspension of GO, sodium alginate solution and calcium chloride at ambient temperature. The GO/CA biocomposite was characterized with IR spectroscopy and SEM microscopy. The adsorption capacity of the biocomposite was evaluated following the absorbance at 270 nm following UV-Vis spectroscopy. The adsorbent shows maximum adsorption property near neutral pH range and the adsorption capacity increases with active adsorbent areas until a saturation point reached. For the adsorption process, several non-covalent interactions such as hydrogen bonding, electrostatic, and π-π interactions take part a crucial role in adsorption capacity of GO/CA biocomposite. Currently, we are working on the model(s) of adsorption isotherm, reusability of the biocomposite adsorbent and, ease of adsorbate recovery.

Acknowledgement
The work was supported by the School of Chemical Engineering and Physical Sciences and Centre Instrument Facility of Lovely Professional University. The authors thank Dr. Jashanpreet Singh for fruitful discussions.

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