Removal of amprolium from water by roots and seeds ash of *Salvadora persica*

Samah Ali a,b and Abeer Abdelhalim a

aChemistry Department, College of Science, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia; bThe National Organization for Drug Control and Research, Giza, Egypt

ABSTRACT

Amprolium is one of the veterinary pharmaceuticals that has been widely used as antibacterial agents and as a growth promoter in livestock, it can produce chronic adverse actions that could affect many organisms and humans. In this study, Salvadora persica stem ash and roots were used as biosorbent for the removal of Amprolium hydrochloride (AMP) from aqueous systems. The effect of different parameters such as the initial concentration of AMP, pH, contact time, and adsorbent dosage were investigated. Maximum removal efficiency reached 98% and 96% for *S. persica* roots and seeds ash, respectively at pH = 7 and contact time of 40 min. Isothermal studies indicated that the AMP removal correlates more with the Freundlich isotherm model. *S. persica* roots exhibit better removal efficiency than *S. persica* seeds ash under all the studied parameters. This study shows that *S. persica* is efficient in removing AMP from water.

1. Introduction

Amprolium is one of many veterinary pharmaceuticals that has been widely used as antibacterial agents and as a growth promoter in livestock [1]. It is a pyrimidine derivative used in chicken feed to inhibit the growth of protozoan coccidian and exists as a cation in aqueous solution [2]. Many studies have reported that amprolium hydrochloride (AMP) inhibited the sporation of coccidial oocysts of different *Eimeria* species [3–6]. AMP was also found to reduce the severity of infection caused by *Sarcocystis cruzi* in cattle and by *Sarcocystis tenella* in sheep [7]. AMP is thiamine antagonist that prevents the synthesis of carbohydrate by blocking the thiamine receptors of the *Eimeria* species.

Most of the used veterinary pharmaceuticals including amprolium are poorly adsorbed in the gut of the animal, and therefore, the majority of the compound is being excreted unchanged in feces and urine [8,9]. The use of animal waste as fertilizer causes the release of these substances into the environment which may produce chronic adverse actions that could affect many organisms and humans. A study by Elmund et al. suggested that the existence of these pharmaceuticals in the waste changes the way it decomposes and alters the stabilization process of the waste resulting in a change in the biochemical reactions in the soil environment and therefore, increase their effect on soil pollution [10]. The contamination of these veterinary pharmaceuticals could not only affect the soil but also may affect the groundwater as well as the surface water. In many countries, groundwater is the major water recourse and is hard to remediate once polluted with pharmaceuticals [11]. Water is the main carrier of these pharmaceuticals to the environment and is considered bioavailable...
to the surrounding plants, aquatic organisms as well as bacteria [12].

Several studies have investigated the adverse biological effects of AMP on aquatic organisms. In a study by Hu et al., amprolium at a concentration of equal or above 5 mg/L was found to cause an acute toxicity effect and an acceleration in the hatching process on Zebrafish embryo while at low concentration (less than 0.05 mg/L) amprolium was found to suppress the hatching [13]. In the same study, amprolium at high concentration induced the superoxide dismutase expression and increased malondialdehyde content (a marker for oxidative stress). In another study, amprolium caused early mortality syndrome in broodfish fry and decreased the level of thiamin in the heart, liver, and muscle in Atlantic Salmon [14]. The daily administration of amprolium (280 mg/kg body weight) in pre-ruminant lambs caused severe diarrhea and extensive hemorrhages in most organs and lesions in the brain after four weeks [15]. Other adverse effects of amprolium have been reported, for example, a high concentration of amprolium caused urine backflow from cloaca to ceca in chicken [16], had acute death, lesions in the brain, swollen endothelial cells, and edema in sheep [17].

Several studies have concentrated on the developing analytical methods for the determination of trace levels of antibiotics in the environment. However, most of these studies only targeted a few classes of antibiotics and therefore it may not be relevant to environmental media with the presence of multiple classes of antibiotics such as AMP [12].

The determination of amprolium was achieved using different methods such as HPLC [12,18–20], GC [21], TLC [22], atomic spectrometry [23], spectrophotometric methods [24,25], capillary electrophoresis [21] and electrochemical method [26]. However, most of the reported studies performed were for the determination of amprolium in chicken feedstuff, meat, eggs, plasma, and tissues [21,27], but few studies were established for the analysis of amprolium in aqueous media.

Adsorption is considered to be the most efficient procedure for the removal of veterinary pharmaceutical contaminants from aquatic media due to the low cost, design simplicity, and ease of operation. Different biosorbent such as palm bark [28], Aspergillus fumigatus [29], rice straw [30], corn cobs [31], grape stalk, chitosan beads [32], and cork bark [33] are a few of the biosorbent materials that have been evaluated for the wastewaters treatment.

Salvadora persica (Miswak), presents in many parts of Saudi Arabia, belonging to the Salvadoraceae family. The roots of S. persica have been used for oral hygiene and was reported to reduce dental plaque. The sticks of S. persica have been used as toothpicks [34]. It has been shown that extracts of S. persica exhibit many biological activates including anticonvulsant, antibacterial, and anti-fungal effects [35]. Phytochemical studies on S. persica have resulted in the identification of different constituents, such as salvadourea, salvadorine, saponins, tannins, and alkaloids [35,36]. Salvadora persica has been used as biosorbent for heavy metals removal from the aqueous environment as a cleaning tool. The branches and leaves of this plant were used for the removal of uranium, thorium [37], lead, copper, nickel, cadmium [38], and chromium [39]. The stem ash of S. persica was also been used for arsenic removal [40]. The roots of S. persica have been used for the removal of toxic metals from aqueous solutions such as copper, lead, nickel, barium, and strontium [41,42].

The current study aims to determine the feasibility of using the roots of S. persica and the seeds ash as an adsorbent for the removal of AMP from aqueous solutions and to describe the optimization of a new method for AMP determination in aqueous solutions. The effects of different conditions such as solution pH, temperature, adsorbent dosage, and contact time were investigated. Furthermore, the sorption isotherm and kinetics studies were also evaluated to describe the experimental data.

2. Materials and reagents
Amprolium hydrochloride (C14H19N4Cl.HCl) > 98% from Sigma-Aldrich was provided by the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt). All reagents used were of analytical grade and were purchased from Sigma Chemical Co. Acetic acid, sodium acetate, sodium phosphate mono, and dibasic saponins, tannins, and alkaloids [35,36]. Several studies have resulted in the identification of different constituents, such as salvadourea, salvadorine, saponins, tannins, and alkaloids [35,36]. Salvadora persica has been used as biosorbent for heavy metals removal from the aqueous environment as a cleaning tool. The branches and leaves of this plant were used for the removal of uranium, thorium [37], lead, copper, nickel, cadmium [38], and chromium [39]. The stem ash of S. persica was also been used for arsenic removal [40]. The roots of S. persica have been used for the removal of toxic metals from aqueous solutions such as copper, lead, nickel, barium, and strontium [41,42].

The current study was performed at the chemistry department, Taibah University. The dried roots of S. persica were obtained from herbal markets located in Al-Madinah Al-Munawarah, Saudi Arabia and washed several times with distilled water and dried again, ground well, and sieved. The seeds of S. persica were firstly washed several times with distilled water and pre-ruminant lambs caused severe diarrhea and extensive hemorrhages in most organs and lesions in the brain after four weeks [15]. Other adverse effects of amprolium have been reported, for example, a high concentration of amprolium caused urine backflow from cloaca to ceca in chicken [16], had acute death, lesions in the brain, swollen endothelial cells, and edema in sheep [17].

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The current study was performed at the chemistry department, Taibah University. The dried roots of S. persica were obtained from herbal markets located in Al-Madinah Al-Munawarah, Saudi Arabia and washed several times with distilled water and dried again, ground well, and sieved. The seeds of S. persica were firstly washed several times with distilled water and
Figure 1. *S. persica* roots (a), roots powder (b), seeds (c) and seeds ash (d).

then dried, ground, burned at 300-500°C, crushed, and sieved (Figure 1).

5. Amprolium removal experiments

Amprolium removal experiments were performed by agitating 0.05 g of *S. persica* roots and seeds ash with 25 ml of AMP solution of desired concentration and pH by diluting the stock solution with distilled water. The mixture was then shaken at room temperature for 1 h. (200 rpm shaking speed). The effect of different parameters was investigated. Each measurement was done in triplicate. AMP solution was measured spectrophotometrically by monitoring the absorbance at $\lambda_{\text{max}}$ of 265 nm using UV–Vis spectrophotometer.

5.1. Effect of solution pH

The effect of pH on the sorption of AMP on *S. persica* roots and seeds ash were studied in pH ranging between 3.0 and 8.0 with 10 ppm fixed initial AMP concentrations and sorbents dosage of 0.05 g for 40 min.

5.2. Effect of contact time

The effect of contact time on the sorption of AMP on *S. persica* roots and seeds ash was performed at different shaking times (0, 15, 30, 40, 60, 80, and 120 min) by adding 0.05 g of sorbents to 10 ppm AMP at pH 7.

5.3. Effect of sorbents dosage

The effect of sorbent dose is investigated by shaking the solution containing 10 ppm of AMP with a variable amount of *S. persica* roots and seeds ash in the range between 0.02–0.2 mg/L for 40 min.

5.4. Effect of initial drug concentration

The effects of initial AMP concentration on adsorption were studied using different concentrations of AMP (5-100 ppm) at pH 7 for 40 min.

6. Analytical methods

The AMP solution was isolated from the adsorbent by filtration. Then, the amount of AMP adsorbed, $q_e$ (mg/g), was obtained according to Equation (1):

$$q_e = \frac{(C_0 - C_e) \cdot V}{M}$$  (1)

AMP removal efficiency was determined by calculating the sorption percentage using the following formula:

$$\% \text{ Sorption} = \frac{(C_0 - C_e)}{C_0} \cdot 100$$  (2)

where $C_0$: initial concentrations of AMP in solution; $C_e$: remain concentrations of AMP in solution; $V$: volume of solution; $M$: mass of sorbent in gram.

7. Results and discussion

7.1. Fourier Transfer Infrared (FTIR)

The FT-IR spectrum of *S. persica* ash exhibit several absorption peaks at different locations including at, 3459 cm$^{-1}$ (O-H stretching), 3350 cm$^{-1}$ (N-H stretching), 3084 cm$^{-1}$ (C–H stretching for alkene), 2960 (C–H stretching for alkane), $\sim$1685 cm$^{-1}$ (C=O stretch for conjugated ketone), 1388 cm$^{-1}$ (C–O stretch) $\sim$ 939 cm$^{-1}$ (C–O) which correspond to various oxygen containing functional groups as were reported for *S. persica* [43]. On the other hand, FT-IR spectrum of *S. persica* roots displayed strong broadband at 2521 cm$^{-1}$ corresponding to (O-H stretching), 1685 cm$^{-1}$ (C = O stretch for ketones), and lacked the stretching band for NH group. All other bands appear in low intensity comparing with *S. persica* ash (Figure 2).

7.2. Thermogravimetric analysis

Figure 3 shows the thermogravimetric analysis (TGA) curves for *S. persica* roots (A) and *S. persica* seeds ash (B). TGA investigates the thermal behaviour and variation in weight loss versus temperature resulting in vaporization of volatiles, degradation, decomposition, and oxidation of *S. persica* roots and seeds ash. TGA showed two main steps of weight loss for both samples. For the *S. persica* roots sample, the first step (100-350°C) shows a rapid increase in weight loss due to both degradation and decomposition at 200°C with 3% weight loss which increases to 50% at 380°C. The second step (350-500°C) in which the weight loss increase up to 78%. For the *S. persica* seeds ash sample, the first step (180-400°C) a weight loss of approximately 55% was observed at
Figure 2. FTIR spectra of *S. persica* roots and seeds ash.

Figure 3. TGA for *S. persica* roots and *S. persica* seeds ash.

400°C which increases up to 85% on the second step (400-600°C). The decomposition of both samples ends at about 800°C.

7.3. Effect of pH

The pH of the solution plays a crucial role in the adsorption of pollutants from aqueous solutions as it may affect the degree of ionization of adsorbate as well as the adsorbent surface charge. To determine the preferred pH for adsorption of AMP over *S. persica* seeds ash and *S. persica* roots, the percentage removal of AMP as a function of pH was studied. Figure 2 shows that the AMP removal efficiency increased gradually with increasing the pH. AMP exists as a cation in aqueous solution, at low pH, there is a high concentration of the positively charged hydronium ions that might compete with the cationic AMP molecules on the sorbents site. Accordingly, excess hydronium ions would cause the sorbents to become more positively charged due to the protonation of the functional groups which cause an electrostatic repulsion between the positively charged AMP and the sorbent’s surface. On the other hand, increasing the pH cause the sorbents surface to became more negatively charged which improved the electrostatic interaction between the sorbents and the cationic AMP molecules. The results showed that *S. persica* roots exhibit better removal efficiency than *S. persica* seeds ash over the studied pH range. The maximum adsorption of *S. persica* seeds ash and *S. persica* roots are 45% and 77%, respectively at pH 7. Figure 4 summarizes the percentage removal behaviour at various pH for both sorbents.

7.4. Effect of contact time

The effect of contact time on the sorption of AMP on *S. persica* roots and seeds ash was performed at different shaking times (0-120 min) by adding 0.05 g of sorbents to 10 ppm AMP at pH 7. Figure 5 reveals that the rate of AMP removal increased with time increasing to 40 min for both sorbents with maximum removal of 77% and 40% for *S. persica* roots and seeds ash respectively. And then, the adsorption rate became practically constant. That is because effective sites on the surface of the sorbent are covered with AMP as time passes and hence
no further adsorption occurs. The results showed that *S. persica* roots exhibit better removal efficiency than *S. persica* seeds ash.

### 7.5. Effect of sorbents dosage

The effect of sorbent dosage is investigated by shaking the solution containing 10 ppm of AMP with a variable amount of *S. persica* roots and seeds ash in the range between 0.02–0.2 mg/L for 40 min. As sorbents dosage increases the removal efficiency decreases (Figure 6). At a low concentration of sorbents, the number of active sites is high. With the increase in sorbents dosage there may be an aggregation of particles, as a result, the removal efficiency of AMP decreased. The results indicated that the best rate of removal was achieved with 0.02 mg of *S. persica* roots and seeds ash with a value of 76% and 35% respectively.

### 7.6. Effect of initial drug concentration

The effects of initial AMP concentration on adsorption were studied using different concentrations of AMP (5-100 ppm) at pH 7 for 40 min. When the initial concentration of AMP increased, the amounts of AMP removal also increased with both sorbents (Figure 7). These results indicate that the removal of AMP depends on its concentration, for instance, when the initial concentration of AMP increases from 5 to 100 ppm, the removal efficiency of *S. persica* roots and seeds ash increase from 64% to 98% and from 30% to 96% respectively.

### 7.7. Adsorption isotherm

The experimental data were analysed using Langmuir and Freundlich isotherm. The Langmuir adsorption isotherm describes the surface as homogeneous, was applied to interpret adsorption of AMP. The linear form of the Langmuir Equation (3) [44]:

$$\frac{C_e}{q_e} = \frac{1}{Q_0b} + \frac{1}{Q_0}C_e$$

where $q_e$: equilibrium adsorbent phase concentration of adsorbate (mg/L); $C_e$: equilibrium aqueous phase concentration of adsorbate (mg/L); $Q_0$: the monolayer adsorption capacity (mg/g); $b$: constant related to the free adsorption energy and the reciprocal of the concentration at which half-saturation of the adsorbent is reached.

Therefore, the plot of $C_e/q_e$ versus $C_e$ gives a straight line with slope equal to $1/Q_0$ and intercept equal to $1/Q_0b$. The curve could be illustrated by the equation above, as shown in Figure 8. The result of the application
of the Langmuir isotherm gives a linear relationship. Similarly, both sorbents have shown similar straight-line plots with positive slopes. The linear correlation coefficients for the S. persica roots and seeds ash sorbents are 0.860 and 0.991, respectively. The values for Q and b are 5.23 mg/g and 0.956 L/mg for S. persica roots and 4.09 mg/g and 1.1 L/mg for seeds ash, respectively.

Freundlich adsorption isotherm describes the surface as heterogeneous [45] and gives an expression that defines heterogeneity of the surface and the exponential distribution of active sites and their energies. Equation (4) represents the linear relationship of Freundlich adsorption isotherm [46].

\[
\log q_e = \log K_f + \frac{1}{n} \log C_e \tag{4}
\]

where \(K_f\): adsorption capacity and \(1/n\): adsorption intensity

The plot of \(\log C_e\) against \(\log q_e\) gives a straight line as shown in Figure 8. It was found that the isotherm data fits the Freundlich equation well with good correlation coefficients: \(R^2 = 0.9218\) and 0.9966 for S. persica roots and seeds ash respectively, showing that the sorption data correlates more to the Freundlich Isotherm model [47,48]. The data is presented in Table 1. Freundlich isotherms equation usually fits solute adsorption onto rough surfaces better than Langmuir isotherms [49], taking into account the heterogeneity of the solid surface and the variable energy distribution of the adsorption sites [50]. The S. persica roots showed higher adsorption capacity and affinity for AMP, as the sorbent surface is readily available for attracting AMP.

7.8. Sorption kinetic

In order to analyse the sorption kinetic of AMP on the surface of S. persica roots and seeds ash, the pseudo first and second order kinetic models were applied [51]. Pseudo-first-order kinetic model has been determined using the following equation:

\[
\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \tag{5}
\]

where \(q_t\): adsorption capacity at time t (mg/g); \(q_e\): adsorption capacity at equilibrium; \(k_1\): the pseudo-first-order rate constant of the adsorption process

\ | Langmuir Isotherm | Freundlich Isotherm |
|-----------------|---------------------|
| Sorbents        | \(q_m\) (mg g\(^{-1}\)) | \(b\) (L mg\(^{-1}\)) | \(R^2\) | \(K_f\) | \(n\) | \(R^2\) |
| Roots           | 5.23               | 0.9569               | 0.8607  | 2767.57 | 0.07496 | 0.9127  |
| Seeds ash       | 4.09               | 1.1                  | 0.9911  | 3.32E-05 | 0.095021 | 0.9966  |
Figure 9. Pseudo first order and pseudo second order kinetic models for the removal of AMP by: S. persica roots (A) and S. persica seeds ash (B).

Table 2. Kinetic parameters for the removal of AMP by S. persica roots and S. persica seeds ash.

| Sorbents      | First order kinetic model | Second order kinetic model |
|---------------|---------------------------|---------------------------|
|               | q_e (mg/g) | k_1 (min\(^{-1}\)) | R^2    | q_e (mg/g) | k_2 (g/mg·min\(^{-1}\)) | R^2    |
| Roots         | 0.017124 | 0.02303 | 0.8042 | 1.708817 | 0.176162 | 0.9969 |
| Seeds ash     | 0.095874 | 0.004376 | 0.8793 | 0.289494 | 0.26712 | 0.966  |

Pseudo-second-order kinetic model have been determined using the following equation:

\[
\frac{t}{q_t} = \frac{1}{k_2q_e^2} + \frac{t}{q_e}
\]

where \(K_2\) is the pseudo-second-order adsorption rate constant (g/mg min\(^{-1}\)). Figure 9 shows the linear fit of the experimental data to pseudo-first-order and pseudo-second-order kinetics. From Table 2 it is clearly observed that the AMP adsorption is better fitted on the pseudo-second-order kinetic \(R^2 = 0.9969\) and 0.966\) model than the pseudo first-order model \(R^2 = 0.8042\) and 0.8793\). The pseudo-second-order kinetics reflects that the concentrations of AMP and both sorbents are involved in the rate-determining step, which may be a chemical adsorption or and physisorption.

8. Conclusions

This study shows that the stem ash and roots of S. persica, low-cost biosorbent, is efficient in removing AMP from aqueous solution. The effect of initial concentration, adsorbent dosage, pH, and time was studied. Maximum removal efficiency reached 98% and 96% for S. persica roots and seeds ash, respectively at pH = 7 and a contact time of 40 min. Isothermal studies indicated that the AMP removal correlates more with the Freundlich isotherm model. S. persica roots exhibit better removal efficiency than S. persica seeds ash under all the studied parameters.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Samah Ali http://orcid.org/0000-0002-0081-2901
Abeer Abdelhalim http://orcid.org/0000-0002-4446-8372

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