Efficacy of Attapulgite Clay as Adsorbent for Metronidazole Drug Overdose *in vitro*.

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Abstract

Surface active materials have many applications in medicine. Attapulgite clay used in medicine as antidiarrheal, and as additives in some pharmaceutical formulations. Metronidazole drug is widely used as antiprotozoal drug in the treatment of amoebic dysentery and amoebic liver abscess. Drug overdose may treated by adsorbents especially by activated charcoal suspension to prevent further absorption, but usually it is unacceptable to drink. Hence, in this work a study was carried out to estimate the ability of attapulgite clay as adsorbent for metronidazole overdose in vitro as a possible alternative for activated charcoal.

The change in the concentration of metronidazole after incubation of known concentration of aqueous metronidazole solutions with attapulgite was measured as the adsorbed quantity. UV-Visible spectrophotometry technique was used to follow the quantity of the adsorbed drug. The adsorption also occurred in 0.1N HCl solution to simulate the acidity of the stomach. The adsorption experiments were repeated at (12, 25, 37.5, and 50ºC) to measure the thermodynamical parameters (ΔH^o, ΔG^o, ΔS^o).

Adsorption of metronidazole on attapulgite obeyed Freundlich adsorption isotherm of S2 type according to Giles classification of adsorption isotherm in solution. The maximum quantity adsorbed tends to be slightly increased in acidic medium (0.1N HCl) especially at low concentration of the drug. The thermodynamic parameters values were (ΔH^o=-19.86 KJ.mol^{-1}, ΔG^o=-17.994 KJ.mol^{-1}, and ΔS^o=6.25 J.mol^{-1}ºK^{-1}).

Metronidazole drug can be adsorbed by attapulgite clay surface at low concentration of the drug and the quantity of the adsorbed drug increased with increasing the initial concentration of the drug. The adsorption quantities were decreased with increasing temperature indicating an exothermic adsorption process which also reinforced by the values of thermodynamic parameters. Acidity has a slight effect on adsorption.

Keywords: Metronidazole, attapulgite, adsorption, drug overdose, ΔH^o, ΔG^o, ΔS^o.

الخلاصة

توجد تطبيقات عديدة للمواد الفعالة سطحيا في المجالات الطبية. يستعمل طين الاتابلغايت كمضاد للاسهال وفي تركيب بعض المستحضرات الطبية. دواء الميترونيدازول كمضاد للاوليات في علاج الدزنتري الاميبي وخراج الكبد الاميبي. إن التسمم بفرط الجرعة الدوائية يعالج بمعلق الفحم المنشط الذي يمتاز على سطحه الأدوية ويمنع امتصاصها لكن هذا المعلق غير مستساغ عادة من قبل المرضى. لذلك أجريت هذه الدراسة لتقدير قابلية طين الاتابلغايت للعمل كمادة مازة لدواء الميترونيدازول كبديل محتمل لمادة الفحم المنشط.

ان الاختلاف بين تركيز الدواء بعد مزج تراكيز مختلفة منه مع التابلغايت قد تم قياسه باعتباره المادة الممتزة. استخدمت تقنية المطيافية فوق البنفسجية لمتابعة الكميات الممتزة بعد مزج محلول الدواء مع طين البنتونايت. أعيدت التجربة عند أربعة درجات حرارية (12 و 25 و37.5 و50 درجة مئوية) وذلك لقياس الدوال الثرموداينمكية (ΔH^o, ΔG^o, ΔS^o) *لعملية الامتزاز*.

إن عملية امتزاز الميترونيدازول على سطح البنتونايت تجري حسب معادلة فرندلش للامتزاز ومن نوع S2 حسب تصنيف جيلز لايزوثرمات المتزاز وهي عملية باعثة للحرارة مع قيم دوال ثرموديناميكية صغيرة. إن عملية الامتزاز تحفز بزيادة درجة الحرارة و زيادة حامضية المحلول. كانت القيم الثرموديناميكية كالاتي:

(ΔH=-19.86 KJ.mol^{-1}, ΔG^o=-17.994 KJ.mol^{-1}, and ΔS^o=6.25 J.mol^{-1}$^oK^{-1}$).

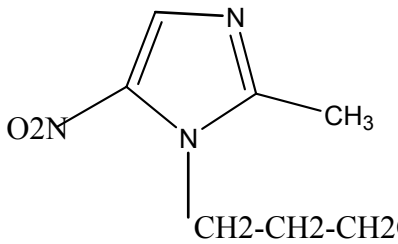
يستطيع طين الاتابلغايت امتزاز دواء الميترونيدازول عند التراكيز الواطئة وتزداد الكمية الممتزة بزيادة التراكيز الابتدائية للدواء. تقل كمية الامتزاز بزيادة درجة الحرارة مما يدل على أن التفاعل باعث للحرارة والتي تم تاكيدها من القيم الثرموديناميكية. كما وجد أن لتغيير الحامضية تأثير قليل على عملية الامتزاز.

مفاتيح الكلمات: الميترونيدازول - طين البنتونايت - التسمم – الامتزاز- ΔS^o - ΔG^o - ΔH^o.

Introduction

The term adsorption refers to the accumulation of atoms, ions or molecules (adsorbate) on a surface of a solid substance (adsorbent) [1]. There are a number of factors can influence the process of adsorption, the concentration of drug molecule, surface area of the clay, temperature, PH, ionic strength, solubility, chemical state of both adsorbent and adsorbate molecules and the kinetic effect. Details of these factors are available in textbooks and references [2-4].

Metronidazole drug has the following structural formula:



It has antiprotozoal and antibacterial actions. It is used in the treatment of amoebic dysentery and amoebic liver abscess as well as for the eradication of *E.histolytica* from patients passing cysts [3]. It is still a standard anti protozoal in the estimation of the activity of new antiprotozoal [5]. The poisoning by metronidazole may occur deliberately or accidentally and the treatment is needed

to prevent further absorption by using different active surface substances.

Solids have the property of holding molecules at their surfaces either from the gas phase or from solution; this property is quite marked in the case of porous and finely divided materials [2]. The medical significance of some active surface materials arises from their high adsorption capability. One of the uses of these active substances is in the utilization of it as a drug carrier [6].

The most important application of these materials in medicine is their use as physical antidotes in the treatment of acute poisoning by toxic substances and drug over dosages [7-8]. Activated charcoal was the most widely used solid surface as an antidote and to prevent further absorption of the drug, but it is unacceptable by patients because its color and taste [9-10]. Some clay materials were studied and found to possess similar characters to that of charcoal in the treatment of drug poisoning. Examples are kaolin [11-12], bentonite [13-14], and attapulgite [15]. Clays also used to prevent absorption of aflatoxin toxin in animals by inclusion of clays to the animal diet [16].

Attapulgite used in medicine as pharmaceutical excipients [17] and as antidiarrheal drug in the treatment of different types of diarrhea [18-19].

A modified preparation based on attapulgite clay were synthesized and found to be active adsorbent for drugs (phenothiazines) and in hemoperfusion columns as adsorbent materials for toxic substances like urea and poisons [20-21].

The term adsorption isotherm refers to the relation between the extent of adsorption (Qe) or (X/M) with the equilibrium concentration of the adsorbate in solution (Ce) at constant temperature. (X) is the amount of drug adsorbed in milligrams by (M) grams of the adsorbent [22]. Two main theories have been adopted to describe adsorption isotherms. The first, Langmuir adsorption isotherms which represented by the equation:

$$\frac{Ce}{Qe} = \frac{1}{ab} + \frac{Ce}{a} \quad \text{.....(1)}$$

Where (a) represents a practical limiting adsorption capacity when the surface is fully covered with a monolayer of adsorbate, and allows the comparison of the adsorption performance, particularly in the cases where the adsorbent did not reach its full saturation. The constant b is the equilibrium adsorption constant which related to the affinity of the binding sites is constants [23].

The applicability of these equations on the adsorbent-adsorbate (solute) system assume the formation of one layer of adsorbate molecules on the surface while the Freundlich adsorption isotherm (equation) consider heterogeneity of the surface and the formation of more than one layer is probable. The linear form of Freundlich isotherm is:

$$\log Qe = \log k + \frac{1}{n} \log Ce \quad \text{...(2)}$$

where k and n are Freundlich constants characteristics of the system, including the adsorption capacity and the adsorption intensity, respectively [24].

The process of adsorption from solution is more complicated than the corresponding process of gas adsorption on solid surface. The solvent effect and the complicated interaction between solvent molecules and drug molecules to be adsorbed have to be taken into account.

This work is concerned with the study of locally available attapulgite clay as adsorbent for the metronidazole drug from solution in vitro as a possible mean for the treatment of metronidazole poisoning.

Experimental

(a) Clay Treatment:

The attapulgite clay consists mainly of palygorskite mineral was obtained from geological survey enterprise clay was washed with excessive amounts of distilled water to remove any soluble materials, filtered and dried at 160° C for three hours and kept in an airtight container. The clay was grinded and sieved to a particle size of 75μm and then used in all adsorption experiments.

(b) Estimation of λ_{max} and Calibration Curve:

The wavelength at which maximum absorbance occur (λ_{max}) was recorded for the aqueous solution of drug and found to be 320 nm using (Cintra-5) UV-Visible spectrophotometer and 1cm quartz cell (Figure 1). This value was utilized for quantitative estimation through the course of this research. Solutions of different concentrations of the drug were prepared by serial dilution. Calibration curve of the absorbance versus concentration of drug solutions was plotted.

(c) Estimation of Equilibrium Time:

The time required for full saturation of attapulgite surface at 37.5ºC by metronidazole was determined by shaking 10ml of 100mg/L of drug solution with 0.5g of attapulgite. The concentration of drug solution was determined spectrophotometrically at different intervals of time till no further uptake of adsorbate by the adsorbent. The results showed that the time needed to attain equilibrium was 2 hours and this time was fixed for the following experiments.

(d) Systematic procedure:

A volume of 10ml of eight different concentrations of metronidazole drug (5, 10, 20, 30, 40, 50, 80, and 100 mg/L) was shaken with 0.5 g of attapulgite adsorbent at a certain temperature in a thermostated shaker bath at shaking speed 60cycles/minute. After the equilibrium time is elapsed, the mixtures were allowed to settle and the clear liquids were centrifuged at a speed of 3000rpm for 20 minutes. Absorbance was measured at the 320nm after making suitable dilution in order to fit Beer–Lambert's limitation and then converted into absolute concentration readings through the calibration curve. These experiments were also repeated using 0.1NHCl as a solvent for drug at 37.5ºC to simulate the acidity of stomach.

(e) Calculations:

The adsorption experiments were performed at different temperatures (12, 25, 37.5, and 50ºC) to determine the thermodynamic parameters (ΔH, ΔG, and ΔS).

These experiments were also repeated using 0.1NHCl as a solvent of drug at 37.5ºC to simulate the acidity of stomach and human temperature.

The equilibrium constant (K) for the adsorption process at each temperature is calculated from division of the drug adsorbed on the attapulgite surface on the quantity of drug present in solution:-

$$K = \frac{Qe * 0.5}{Ce * 0.01} \quad \text{.....(3)}$$

Where Qe is the amount adsorbed in (mg) per one gram of adsorbent, sometimes called (x/m) where (x) is the quantity in milligrams adsorbed by (m) grams of adsorbent. Ce is equilibrium concentration of the adsorbate expressed in mg/L. (0.5g) represent the weight of the clay that has been used and (0.01) represents the volume of the drug solution used in the adsorption process.

The change in free energy (ΔG) could be determined form the equation:-

$$\Delta G^\circ = -RT \ln K \quad \text{.....(4)}$$

Where R is the gas constant (8.314 $J.mole^{-1}.^{o}K^{-1}$) and T is the absolute temperature.

The heat of adsorption (Δ H) may be obtained from the equation:-

$$\ln Xm = \frac{-\Delta H^{\circ}}{RT} + cons\tan t \ \ldots\ldots.(5)$$

Where Xm is the maximum uptake of adsorption at a certain value of equilibrium concentration (Ce) that was fixed all temperatures. Plotting (InXm) versus (1/T) should produce a straight line with

a slope = ($-\Delta H^{o}/R$) as shown in Figure (4).

The change in entropy (ΔS°) was calculated from Gibbs equation:

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ} \ \ldots\ldots\ldots(6)$$

Results and Discussion

The results showed the applicability of Freundlich adsorption isotherm for the adsorption process of metronidazole on attapulgite clay surface as indicated by plotting the linear form of Freundlich equation (equation 2) as shown in Figure (2).

The correlation coefficient (r) of the linear form of Freundlich isotherm is 0.949; hence the Freundlich equation is the applied equation for the adsorption of the drug on attapulgite surface.

Adsorption isotherm of metronidazole on attapulgite surface was of S2 type according to Giles classification of adsorption in solution [25]. This type of adsorption isotherm found in the adsorption of the various antibiotics on the different antacids and other adsorbents in most cases obeyed the Freundlich adsorption isotherm [26].

In recent paper carried out to study the mechanism of adsorption of some drugs on clays, involves sorption interactions of some antibiotics with montmorillonite clay. Sorption mechanism was best described with a model that included cation exchange plus surface complexation of zwitterion forms of these compounds. These results indicated that soil and sediment sorption models for tetracyclines, and other pharmaceuticals with similar chemistry, must account for solution speciation and the presence of other competitor ions in clay pore waters [27].

The process of adsorption from solution is more complicated than the corresponding process of gas adsorption on solid surface. The solvent effect and the complicated between solvent molecules and drug molecules to be adsorbed has to be taken into account. Generally, adsorption is a natural process and usually accompanied by a decrease in free energy change and entropy of the system [24]. The study of adsorption process on attapulgite requires taking the nature of the surface into consideration. Clay surface in general consists of small patches of various kinds of active sites which are different in physical and chemical nature or in the steric orientation of molecules towards the surface [28]. According to the Giles interpretation for the adsorption isotherm shapes [25], the metronidazole molecules could be oriented in the direction parallel to the surface and the area of connection will be great, leading to a high attraction between metronidazole molecules and the attapulgite surface.

The clay structure, the nature of the exchangeable cation, and the presence of nonclay components are important factors affecting the drug-clay interaction. In general, clay structures with a high surface charge lead to a greater interaction with the drug. In addition to the presence of multivalent, exchangeable cations on the clay surface diminish interaction with the protonated form of drugs like tetracycline. Nonclay components such as calcite and dolomite increase the interactions of the zwitterionic and

anionic forms of tetracycline with the clay [29].

Metronidazole molecules might replace the water molecules on the attapulgite surface active sites. Although the attapulgite is able to remove heavy metals from aqueous [30], even there is no decisive reason to suppose that the ionic exchange is taking place with sodium or magnesium ions because these ions are natural components of attapulgite.

In the context of the potential usefulness of clays in retarding the rate of release of adsorbed drugs, dissolution dialysis studies of the release of metronidazole from other clay montmorillonite adsorbates have been conducted. The goal was to develop a means for improving local gastrointestinal therapy of amoebiasis while concurrently maintaining efficacy in treating hepatic amoebiasis.

The data related to the effect of pH on adsorption isotherms of metronidazole is shown in Figure (3) where a pH = 1 was chosen to simulate the pH of stomach fluid. The maximum quantity adsorbed tends to increase slightly in acidic medium (0.1NHCl) especially at low concentration of drug. The equilibrium concentrations (Ce) of the drug in solution are reduced as shown in the Figure (3) and one can explain this fact according to the change in the drug solubility at low pH value. The change in pH affects the solubility of adsorbate molecules which, in turn, affects its affinity towards the surface [24].

Figure (4) showed adsorption isotherms of metronidazole at four different temperatures. From this figure it can be concluded graphically the maximum quantities (Xm) adsorbed for each temperature at certain equilibrium concentration (say Ce=2mg/L). The natural logarithms of these Xm values were plotted versus the reciprocal of temperature to obtain the graphic representation of vant Hoff's equation (Figure (5)). From these two figures the thermodynamic parameters values were:

(ΔH^o=-19.86 KJ.mol^{-1}, ΔG^o=-17.994 KJ.mol^{-1}, and ΔS^o=6.25 J.mol^{-1}$^oK^{-1}$).

These values indicate that the adsorption process is spontaneous with very small heat of adsorption process indicating a physical adsorption as a main mechanism for the adsorption of metronidazole on attapulgite. At very low concentration of drug the quantities adsorbed were decreased as the temperature increases. This fact may be due to desorption process which occurred as temperature increased. The binding between drug molecules and the attapulgite surface active sites was very weak and easy to dissociate as temperature increased.

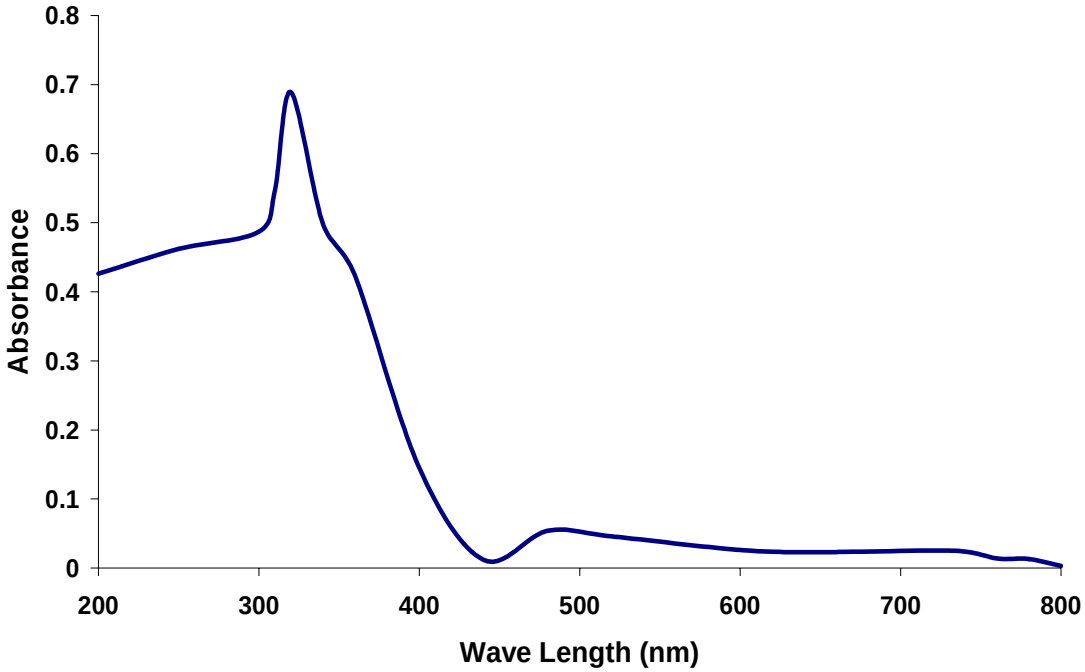


Figure (1): Maximum wave length estimation of the metronidazole solution (10mg/L) at 25°C.

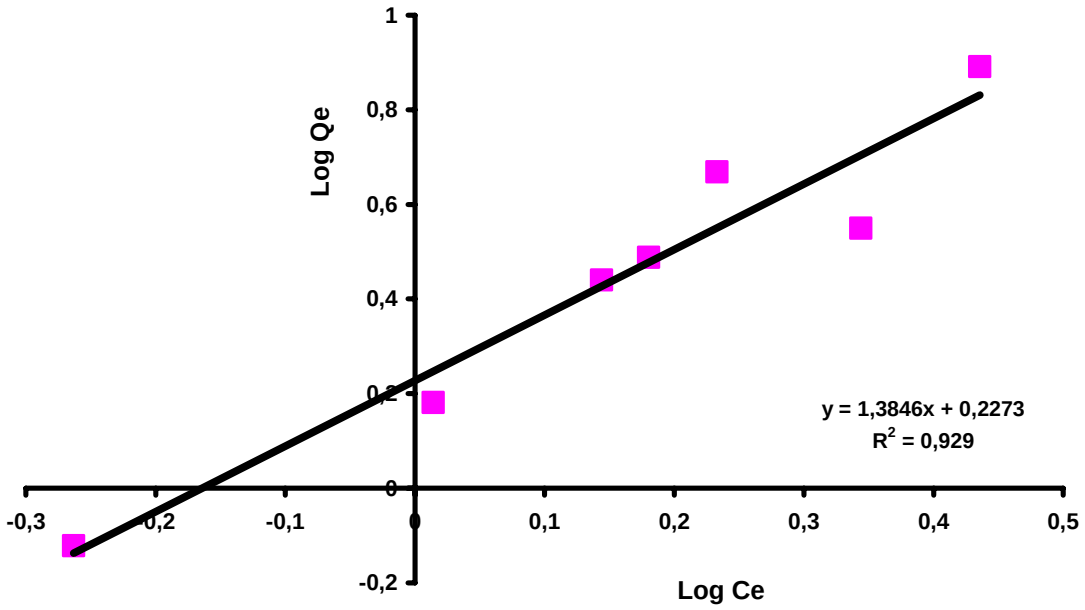


Figure (2): Linear form of Freundlich equation of adsorption of metronidazole on attapulgite clay surface.

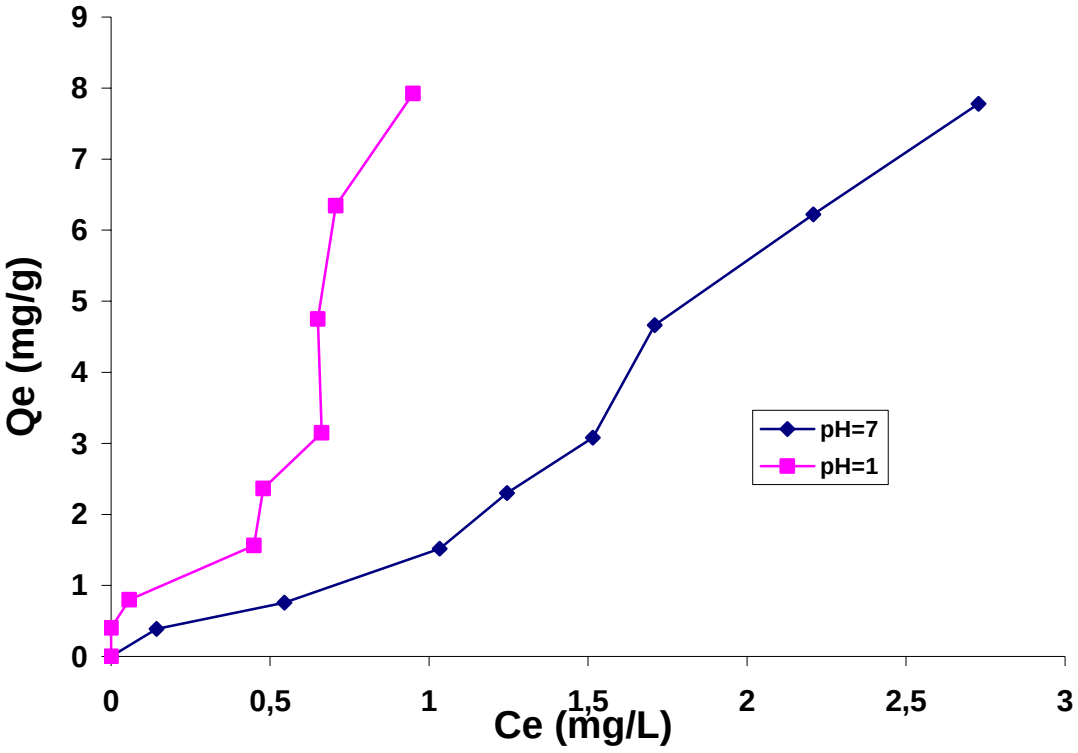


Figure (3): Adsorption isotherm of metronidazole on attapulgite at pH=1 and 7 at 25 ºC.

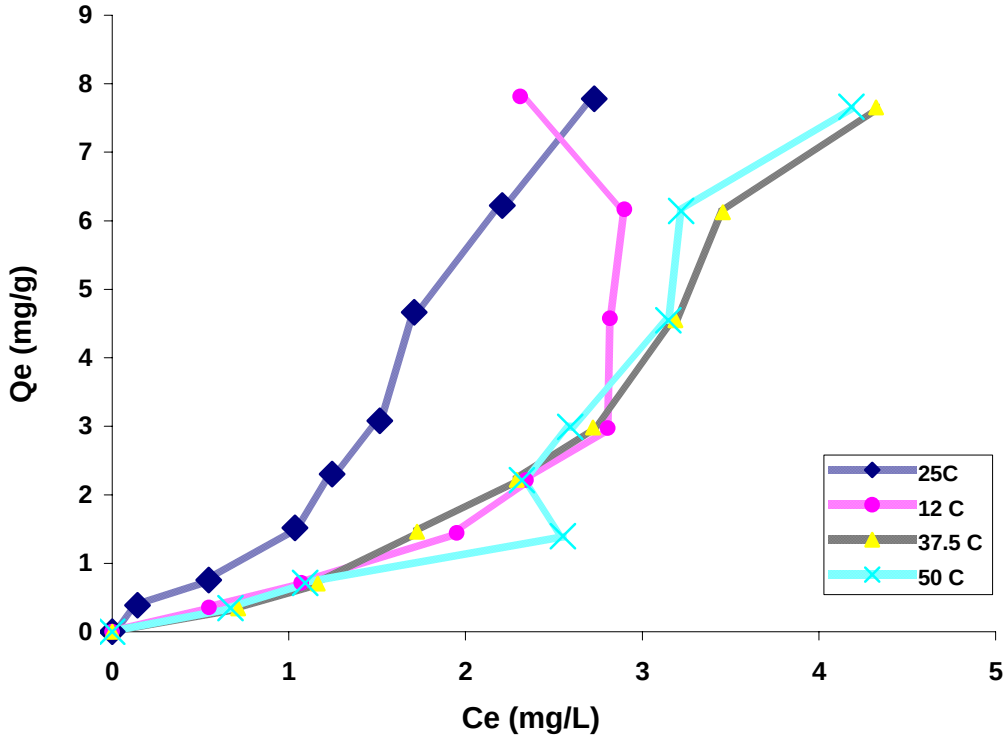


Figure (4): Adsorption isotherms of metronidazole on attapulgite at different temperatures (12, 25, 37.5, and 50ºC).

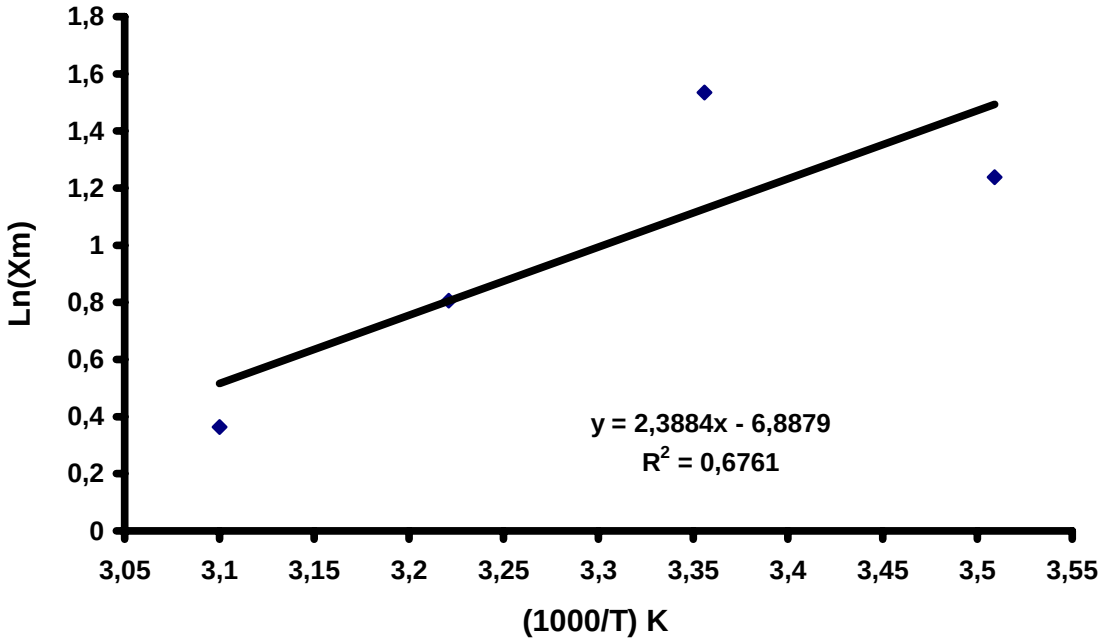


Figure (5): Correlation of maximum quantities of metronidazole drug adsorbed on attapulgite at equilibrium concentration (Ce=2mg/L) and different temperatures (12, 25, 37.5, and 50ºC) according to the modified vant Hoff's equation.

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