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Adsorption of Allopurinol and Ketotifen by Chitosan

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Khouloud A. Alkhamis,[1,*], Wasfi M. Obeidat,[1] and Naji M. Najib[1]

[1]Jordan University of Science and Technology, Irbid, 22110, Jordan

ABSTRACT The experimental work of studying the adsorption of ketotifen and allopurinol by chitosan focused on determining the solubilities and the adsorption isotherms of the adsorbates employed in this study. The adsorption of the aforementioned compounds by chitosan was studied using the rotating bottle method. The concentrations, both before and after the attainment of equilibrium, were determined with the aid of a reversed-phase high-performance liquid chromatography column. The results of these studies demonstrated that ketotifen and allopurinol are both adsorbed by chitosan. The nonlinear Langmuir-like and the Freundlich models both were applied to the experimental data. The correlation coefficients obtained from the nonlinear Langmuir-like model were better than those obtained from Freundlich model, suggesting that allopurinol and ketotifen interacted with certain specific binding sites on the chitosan surface. The allopurinol adsorption experiments indicated that the particle size of chitosan and therefore the surface area can significantly affect the Langmuir capacity constant, while the affinity constants are statistically the same. As expected from the solubility studies, the ketotifen adsorption experiments at 2 different pHs (7 and 10) showed that the adsorption affinity at pH 10 was much higher than at pH 7. What was not expected was that the capacity constants were significantly different, suggesting that further studies are needed using common ion buffers and multicomponent adsorption for the proper mechanism to be determined.

KeyWords: Adsorption, Chitosan, Allopurinol, Ketotifen, Particle Size

INTRODUCTION

Recently, natural polymers such as polysaccharides and proteins have received much attention in the pharmaceutical field owing to their good biocompatibility and biodegradability [1]. Among polysaccharides, chitosan, the deacetylated product of chitin, is thought to be one of the most useful natural polymers from the viewpoint of effective utilization of natural resources [2].

Chitosan is insoluble at neutral and alkaline pH values, but forms salts with inorganic and organic acids such as hydrochloric acid. Upon dissolution, the amine groups of chitosan are protonated and the resultant polymer is positively charged. Because chitosan exhibits a positive charge, it has been recently introduced to the market as a weight loss aid and cholesterol-lowering agent. The mechanism behind chitosan may be its effect on lipid transport in the gut, where the positively charged chitosan can bind to the free fatty acids and bile salt components and hence disrupt lipid absorption [3].

The effect of chitosan has been considered mainly because of its positive charge; however, the adsorption process could also be the result of other forces that might exist between molecules, such as hydrogen bonding or van der Waals forces [4]. These interactions might have a strong impact on the absorption and bioavailability of pharmaceutical compounds, especially for drugs that are potent and have low water solubility. Therefore, it was of interest to study the interaction of certain pharmaceutical compounds with chitosan at neutral and alkaline pH values. At neutral and alkaline pH values, the amine groups of chitosan are not expected to be protonated, and the role of other forces that might exist between molecules on the solid surface can be investigated.

Allopurinol and ketotifen were selected as model compounds for the following reasons. Firstly, allopurinol is a weak acid that has an acidity constant (pKa) of 10.2 [5], whereas ketotifen is a weak base that has a pKa of 6.7 [6]. Therefore, allopurinol and ketotifen are expected to be in the unionized form at neutral and alkaline media, respectively. Second, allopurinol and ketotifen exhibit low aqueous

***Corresponding Author:** Khouloud A. Alkhamis, PhD; Jordan University of Science and Technology, Irbid, 22110, Jordan; Telephone: 011-962-2-7095111-23437; Fascimile: 011-962-2-7095019; E-mail: khou@just.edu.jo

solubility at the aforementioned conditions. Last, the presence of a hydroxyl group in allopurinol and a carbonyl group in ketotifen are likely to be important for hydrogen bonding with chitosan.

MATERIALS AND METHODS

Purification of Chitosan Raw Material

Chitosan polymer (lot numbers F971201-200 and F971201-198, JPM, Amman, Jordan) was washed (for 24 hours each time) with deionized distilled water. After each wash, the chitosan was filtered and dried, and then washed again for a total of 6 times for 24 hours each time. After washing, the samples were analyzed using a spectrophotometer and high-performance liquid chromatography (HPLC); the results showed that the washed chitosan was pure enough to be used. The washed chitosan was then dried and used in the adsorption experiments. The characteristics of the chitosan employed in this study are presented in **Table 1**.

Table 1. The Characteristics of Chitosan Employed in this Study

Batch Number	Particle Size	Degree of Deacetylation
F971201-198	8-16 mesh (450-900 μm)	90.2%
F971201-200	80-100 mesh (100-140 μm)	87.3%

General Procedure for the Adsorption Experiments

Chitosan was vacuum dried at 60°C for 24 hours before use using a vacuum oven (Lab-line, Squaroid-Duo-Vac-Oven, Melrose Park, IL). The vacuum oven was connected to an oilless vacuum pump (KNF, model 035 AN.18).

A stock solution was prepared by dissolving ketotifen dihydrogen fumarate (lot number 2790399, Hikma Pharmaceuticals, Amman, Jordan) or allopurinol (lot number 4790498, Hikma Pharmaceuticals, Amman, Jordan) in 500 mL of buffer (phosphate or borate). Aliquots were then removed from the stock solution and diluted to 100 mL using the same buffer. Five milliliters from each dilution were removed and were used as standards for further analysis.

Preliminary studies showed that the extent of adsorption of allopurinol and ketotifen by chitosan increased significantly when the chitosan was hydrated with deionized water. This indicated that chitosan expands in the presence of water molecules; therefore, the dried chitosan was hydrated before use. The time to full hydration was previously determined experimentally in our lab (6 hours), and the chitosan used in the adsorption experiments was fully hydrated.

Chitosan samples (500 mg each) were weighed quickly after removal from the vacuum oven. Each sample was then placed in a screw cap bottle and 25 mL of buffer was added to each sample. The bottles were rotated in a VanKel sustained release apparatus (VanKel Technology Group, VK 7500, model 65-3100, serial no. 6-0411-0399, Cary, NC) for 6 hours (15 rpm and 26°C). Rotation was then stopped and 25 mL of the serial drug dilutions (ketotifen or allopurinol) were added to each bottle. The filled bottles were wrapped with parafilm and the caps were screwed on. The closed bottles were then rotated in the sustained release dissolution apparatus (15 rpm, 26°C) for 1 hour for ketotifen (pH 10) and for 3 hours for allopurinol and ketotifen (pH 7). The time of the adsorption experiment was enough for the equilibrium to be reached. Rotation of the bottles was then stopped with the bottles in an upright position in the water bath; the chitosan was allowed to settle to the bottom of the bottles for 2 hours at 26°C. Aliquots were removed for subsequent dilutions and analysis. The experiments were done at least in triplicate, and statistical analysis was also performed using curve-fitting program (TableCurve 2D V3, Jandel Scientific, San Rafael, CA).

Determination of the Solubilities

An excess amount of each drug (ketotifen or allopurinol) was added to 10 mL of the specified buffer. The samples were rotated in the sustained release dissolution apparatus for 24 hours (30 rpm, 26°C). Rotation was then stopped and the supernatant was taken. The supernatant was filtered using stainless steel filter holders and Teflon membranes, then was diluted and analyzed using HPLC. The time of the experiment allowed equilibrium to be reached, and the experiments were done in triplicate.

HPLC Analysis and Methodology

Samples were analyzed using HPLC and employing a reversed-phase system. The HPLC (10A VP) system consisted of a pump, a UV-VIS detector connected to a personal computer, and a system controller (all from Shimadzu Co, Tokyo, Japan). The HPLC conditions that were used in this study are given in **Table 2**.

Table 2: The HPLC Conditions Used in this Study

Compound	Allopurinol	Ketotifen
Wave length	254 nm	300 nm
Mobile phase	phosphate buffer pH 5	phosphate buffer (0.02, pH 3.6); methanol: THF (48:50:2)
Flow rate	1.0 mL/ min	1.0 mL/min
Column	RP 18, 5µm Lichrocart 125-4	RP 18, 5µm Lichrocart 125-4
Injection volume	100 µL	100 µL

RESULTS AND DISCUSSION

Solubility Studies

The solubility of the adsorbate can significantly affect the extent of adsorption; therefore, it was necessary to determine the solubilities of the adsorbates employed in this study, namely allopurinol and ketotifen. The solubilities, the experimental conditions, and the pKa are presented in **Table 3**.

Table 3: Solubilities, Experimental Conditions, and Acidity Constants of Allopurinol and Ketotifen

Compound	Solubility	Experimental Conditions	pKa
Allopurinol	0.445 mg/mL	phosphate buffer (0.05 M), 26°C, pH =7	10.25 (weak acid)
Ketotifen	10.75 mg/mL	phosphate buffer (0.2 M), 26°C, pH =7	6.76 (conjugate acid of the weak base)
Ketotifen	0.15 mg/mL	boric acid/KCl buffer (0.2 M), 26°C, pH =10	6.76 (conjugate acid of the weak base)

The results in **Table 3** clearly show that the solubility of allopurinol at pH 7 was low. This is expected because allopurinol is a weak acid that is not ionized at pH 7. The results also show that the solubility of ketotifen at pH 7 was much higher than the solubility at pH 10. This was also expected because ketotifen is a weak base (ionization decreases by increasing the pH).

Adsorption of Allopurinol and Ketotifen by Chitosan

Adsorption of allopurinol and ketotifen by chitosan was studied. The concentrations, both before the addition of chitosan and after the attainment of adsorption equilibrium, were determined with the aid of an HPLC system employing a reversed-phase column. The nonlinear Langmuir-like equation was applied to the experimental data. The Langmuir [7] treatment is summarized by the equation

$$\frac{x}{m} = \frac{k_1 k_2 C_{eq}}{1 + k_2 C_{eq}} \qquad (1)$$

Where x is the amount of solute adsorbed, m is the mass of the adsorbent, C_{eq} is the concentration of the unadsorbed solute at equilibrium, k_1 is the capacity constant, and k_2 is the affinity constant. The derivation of the Langmuir-like equation is dependent upon the following assumptions: the heat of adsorption is independent of surface coverage (all of the sites available for adsorption are energetically equivalent), the adsorbed phase is confined to a monolayer, there are no lateral interactions between adsorbate molecules, the adsorbate solution is very dilute, and there is no mixed film formation at maximum solute adsorption.

The parameters of the Langmuir-like equation for the adsorption of allopurinol by chitosan are presented in **Table 4**. A typical nonlinear equilibrium adsorption isotherm for allopurinol is presented in **Figure 1**; the adsorption parameters are presented in **Table 4.**

Table 4: Parameters Obtained from Curve-Fitting of the Nonlinear Langmuir-Like and Freundlich Equations to Allopurinol

	Nonlinear Langmuir-like Equation		Nonlinear Freundlich Equation	
	Lot Number F971201-198	Lot Number F971201-200	Lot Number F971201-198	Lot Number F971201-200
Capacity constant (mg/gm)	1.632	9.739	3.724	15.52
95% Confidence interval	1.175-2.090	5.847-13.63	3.530-3.918	14.20-16.84
Affinity constant (mL/mg)	4.547	3.825	0.932	0.808
95% Confidence interval	2.233-6.982	1.305-6.350	0.738-1.185	0.425-2.127
r (Correlation coefficient)	0.961	0.943	0.944	0.931

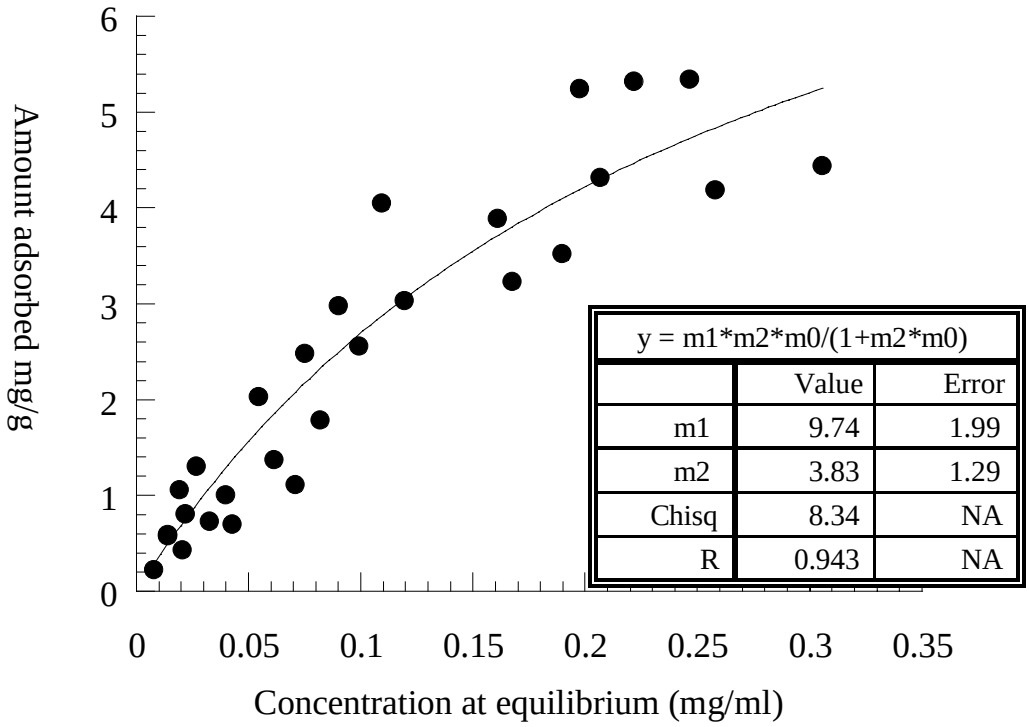

Figure 1. Nonlinear Langmuir-like plot for allopurinol adsorption by chitosan (lot F971201-200, 80-100 mesh, pH 7 and 26°C).

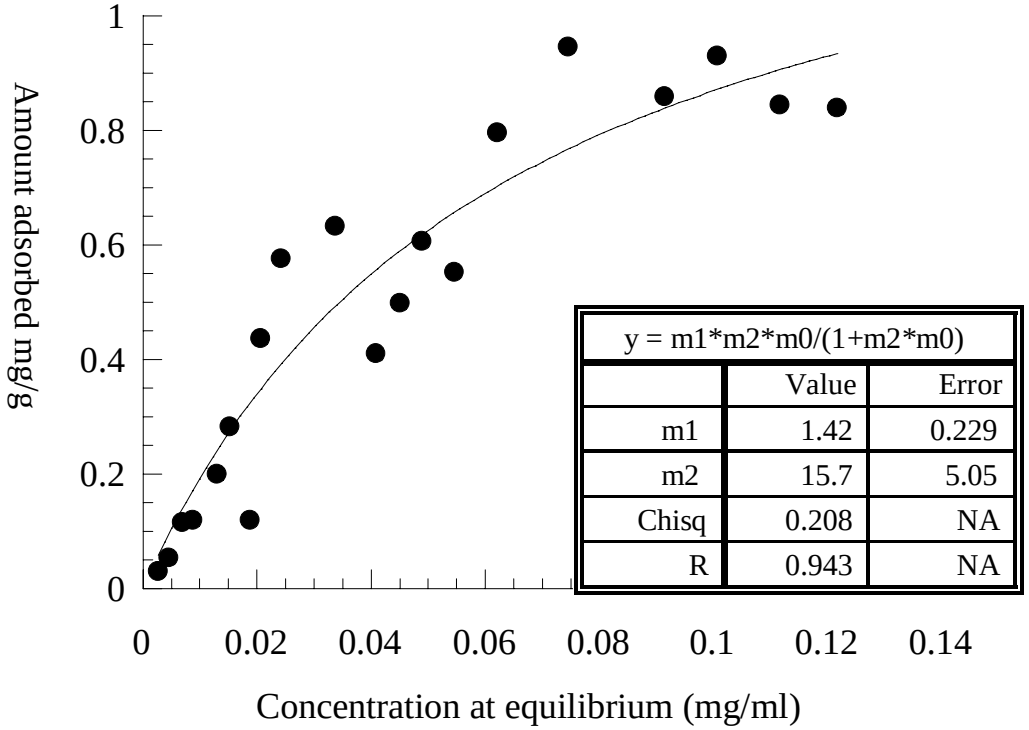

Figure 2. Nonlinear Langmuir-like plot for ketotifen adsorption by chitosan (lot F971201-200, 80-100 mesh, pH 10 and 26°C).

The results in **Table 4** show that the Langmuir capacity constants for the 2 batches are quite different. This is expected because the particle sizes and therefore the surface areas are not the same. The difference in the adsorption capacity resulting from a difference in particle size indicates that the interaction is on the surface (adsorption) and not absorption into the chitosan matrix. The results in **Table 4** also show that the Langmuir affinity constants are quite similar. This is also expected because the strength of the interaction and the equilibrium process should not be affected by the difference in particle sizes.

The good correlation that was obtained using the Langmuir-like equation suggests that allopurinol interacts with certain specific binding sites on the chitosan surface; however, for this result to be confirmed, further studies are needed using solution calorimetry.

The ability of chitosan to act as an adsorbent, in most of the cases, was considered to be mainly the result of ionic interactions; however, the adsorption of allopurinol by chitosan shows that this is not always true. The adsorption of allopurinol by chitosan is likely to be the result of hydrogen bonding between the adsorbate and the adsorbent.

Adsorption of ketotifen by chitosan was also studied at 2 different pHs (7 and 10). The nonlinear Langmuir-like equation was applied to the experimental data. The parameters of the Langmuir-like equation are presented in **Table 5**. A typical nonlinear equilibrium adsorption isotherm is presented in **Figure 2**. The results clearly show that the adsorption affinity at pH 10 is much higher than the adsorption affinity at pH 7. This was expected because the fraction ionized and the solubility of ketotifen at pH 10 are much lower than those of ketotifen at pH 7; the higher the solubility, the lower the extent of adsorption and the lower the affinity constant (assuming that the binding sites are the same). The capacity constants were significantly different, however. This was not expected because the binding sites on the chitosan surface at the beginning were assumed to be constant.

The difference in the capacity constants might be the result of several factors. First, the difference in adsorption capacities could be due to a change in the orientation of the ketotifen molecule on the chitosan surface at different pHs (change in the binding site). Second, the buffer that was used at pH 10 might be competing for the same binding sites (boric acid) as the ketotifen molecules. It is not clear at this moment which mechanism is responsible for the difference in the adsorption capacity at different pHs; however, the second explanation is preferred because previous investigators studied the adsorption of boron by chitosan [8]. Further studies are needed using common ion buffers and multicomponent adsorption in order for the proper mechanism to be determined.

The adsorption of ketotifen (weak base) by chitosan indicates that the interaction cannot be the result of ionic interaction, and that it is likely to be a result of other physical interaction such as hydrogen bonding between the carbonyl group of ketotifen and the hydroxyl group of the chitosan. This conclusion is based on the fact that ketotifen is a weak base, whereas chitosan is either positively charged or neutral at the aforementioned experimental conditions.

Table 5: Parameters Obtained from Curve-Fitting of the Nonlinear Langmuir-Like and Freundlich Equations to Ketotifen (Lot F971201-200)

	Nonlinear Langmuir-like Equation		Nonlinear Freundlich Equation	
	pH = 7	pH = 10	pH = 7	pH = 10
Capacity constant (mg/gm)	4.476	1.424	1.294	7.599
95% Confidence interval	3.730-5.222	0.975-1.874	0.236-2.358	7.364-7.834
Affinity constant (ml/mg)	0.420	15.68	0.507	0.866
95% Confidence interval	0.214-0.620	5.785-25.58	-0.557-1.570	0.631-1.100
r (Correlation coefficient)	0.921	0.943	0.923	0.906

The ability of allopurinol and ketotifen to interact with more than 1 binding site was not excluded; therefore, the Freundlich model was also applied to the experimental data. The Freundlich [7, 9] equation is

$$\frac{x}{m} = KC_{eq}^{P} \qquad (2)$$

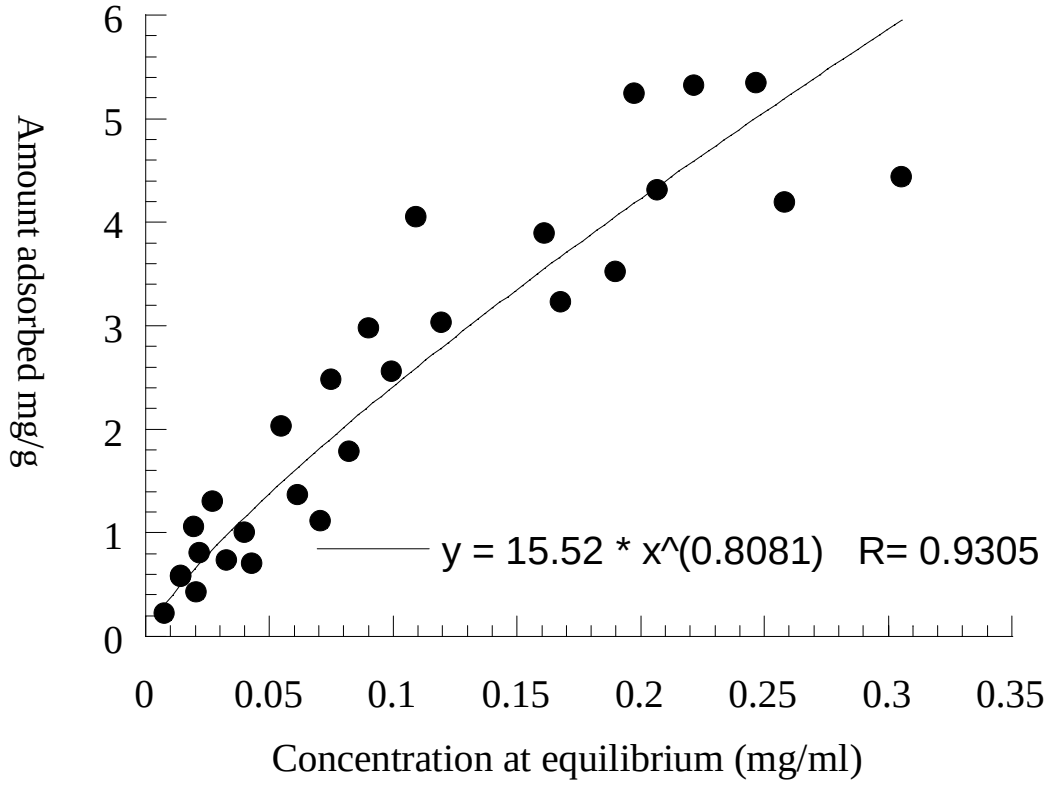

Figure 3. Nonlinear Freundlich plot for allopurinol adsorption by chitosan (lot F971201-200, 80-100 mesh, pH 7 and 26°C).

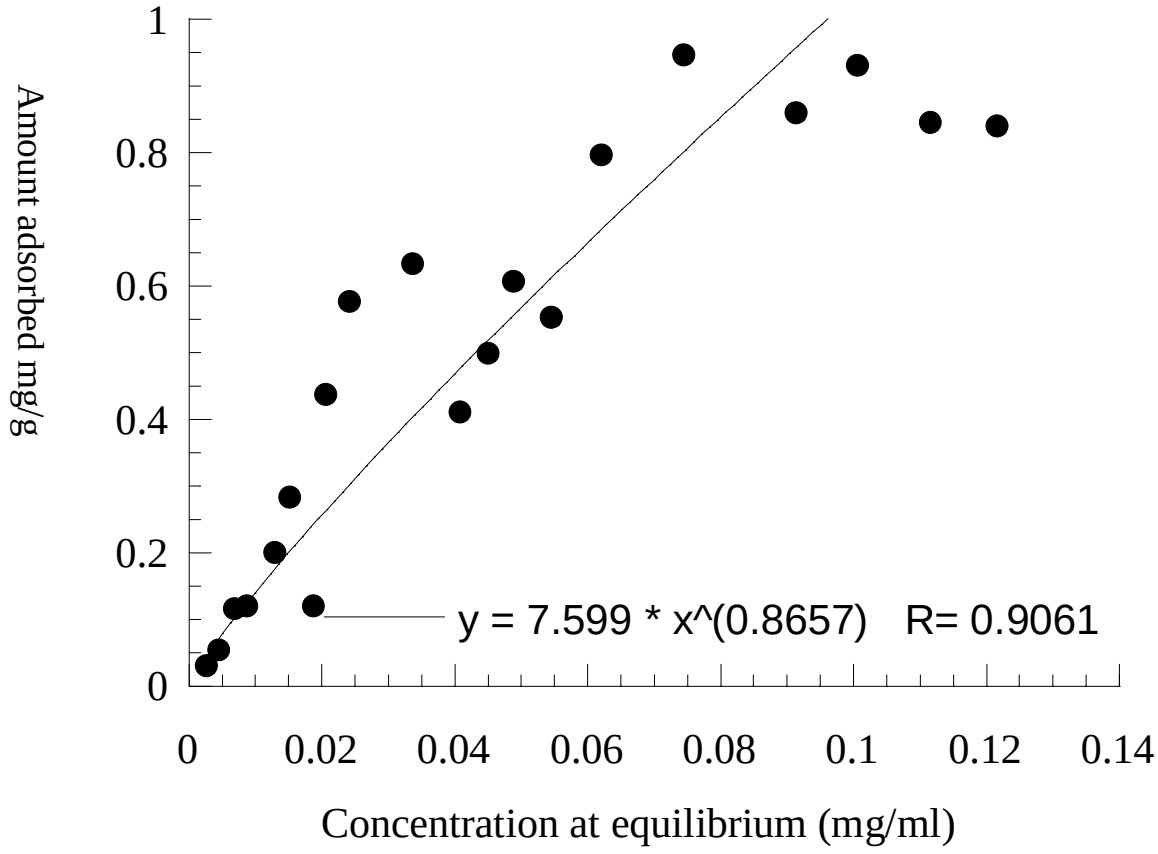

Figure 4. Nonlinear Freundlich plot for ketotifen adsorption by chitosan (lot F971201-200, 80-100 mesh, pH 10 and 26°C).

Where K is a constant related to the capacity of the adsorbent for the adsorbate and P is a constant related to the affinity of the adsorbent for the adsorbate. Although this equation was first employed empirically, it can be derived with the assumption of a continuously varying heat of adsorption. There is no assurance that the derivation of the Freundlich equation is unique; consequently, if data fit the equation, it is only likely, but not proven, that the surface is heterogeneous. The Freundlich model unfortunately predicts both infinite adsorption at infinite concentration and an infinite heat of adsorption at zero coverage.

The parameters of the Freundlich model for the adsorption of allopurinol and ketotifen by chitosan are presented in **Tables 4** and **5**, respectively; the nonlinear adsorption isotherms are presented in **Figures 3** and **4**. The correlation obtained using the Freundlich model was less satisfactory than that obtained from the nonlinear Langmuir-like model. This might indicate that chitosan is interacting with certain specific binding sites. However, the most appropriate model should never be selected solely on the basis of statistical [10] comparisons. Selection must await further studies on heats of adsorption because Langmuir-like and Freundlich treatments differ fundamentally with respect to the requirements placed on the differential heat of adsorption as a function of the extent of adsorbent surface coverage [11].

CONCLUSIONS

First, allopurinol and ketotifen are both adsorbed by chitosan, and the interaction is likely to be the result of hydrogen bonding and not ionic interactions. Second, the particle size and surface area of chitosan can significantly affect the adsorption capacity of chitosan, while the adsorption affinity remains statistically the same. Last, the pH and the ionization of ketotifen can significantly affect the capacity and the affinity constants of the Langmuir-like equation.

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