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In Vitro Adsorption–Desorption of Fluphenazine Dihydrochloride and Promethazine Hydrochloride by Microcrystalline Cellulose

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Abstract □ Fluphenazine dihydrochloride and promethazine hydrochloride were adsorbed *in vitro* from suspensions of the tableting excipient, microcrystalline cellulose. Studies were undertaken to determine how this adsorption phenomenon was affected by the type of phenothiazine derivative, the type of microcrystalline cellulose, and pH and ionic strength adjustment. The smaller the microcrystalline cellulose particle size, the more drug was adsorbed. Changes in the pH, the ionic strength, and the valency of the cation used to adjust the ionic strength all had a major effect on the extent of adsorption. The adsorption process was rapidly and completely reversed *in vitro* at gastric pH and ionic strength values.

Keyphrases □ Fluphenazine dihydrochloride—*in vitro* adsorption-desorption by microcrystalline cellulose, promethazine hydrochloride □ Promethazine hydrochloride—*in vitro* adsorption-desorption by microcrystalline cellulose, fluphenazine dihydrochloride □ Adsorption—*in vitro* desorption of fluphenazine dihydrochloride and promethazine hydrochloride by microcrystalline cellulose

Microcrystalline cellulose has been extensively used as a tablet diluent, disintegrant, and dry binder in tablet formulations prepared by direct compression. More recently it has been used as a tablet excipient in formulations prepared by wet granulation (1). This process often involves dissolving the active ingredient or ingredients in the granulating liquid. When the active ingredient is dissolved, the possibility of its being adsorbed by microcrystalline cellulose or other tablet excipients is increased due to the greater number of drug molecules available for interaction with the sorbent surface.

Cellulose derivatives and their adsorption properties have been studied extensively in the textile and paper industries. These studies have often involved dye and surfactant adsorption (2–7). Cellulose, including microcrystalline cellulose, has also been used to coat TLC plates for use in the separation of various chemical and biological agents (8, 9). The adsorptive properties of cellulose cannot be disputed. Even so, very few studies have been undertaken to determine if drug substances (*i.e.*, phenothiazines) adsorb to the insoluble cellulose derivatives that are used as excipients in the manufacture of tablets (10).

This study was undertaken to characterize the interaction, if any, between the phenothiazine derivatives, fluphenazine dihydrochloride (I) and promethazine hydrochloride (II), and two different grades of microcrystalline cellulose. It was hoped that by understanding the basic principles of drug–microcrystalline cellulose interactions a greater insight into the formulation of phenothiazine tablets could be gained.

EXPERIMENTAL

Materials—Fluphenazine dihydrochloride[1], promethazine hydrochloride[2], and all other chemicals used in this study were either USP, NF, or reagent grade. All water was doubled distilled, deionized, and degassed. Microcrystalline cellulose, in both a medium grade (III)[3] and a fine grade (IV)[4] was obtained directly from the supplier and was used without further modification. Actinic glassware was used to avoid photodecomposition of the phenothiazines.

Methods—The analytical procedure used for determining drug concentrations in solutions or suspension supernatants was a 3-point UV spectrophotometric technique (11) subsequently adopted for phenothiazine analysis (12, 13). This method was used in order to remove linear background absorbance due to UV absorbing trace substances released into the suspension media by the microcrystalline cellulose. Wavelengths used for the adsorption measurements are shown in Table I.

[1] Fluphenazine dihydrochloride, Schering Corp., Kenilworth, N.J.
[2] Promethazine hydrochloride, Wyeth Laboratories, Philadelphia, Pa.
[3] Avicel pH 101, FMC Corp., Philadelphia, Pa.
[4] Avicel pH 105, FMC Corp., Philadelphia, Pa.

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Table I—Wavelengths Used for UV Absorption Measurements

Compound	Wavelengths, nm		
	WL^{a}_{short}	WL^{b}_{max}	WL^{c}_{long}
I	234	256	268
II	225	250	260

[a] Wavelength shorter than WL_{max}. [b] Wavelength of maximum absorption. [c] Wavelength longer than WL_{max}.

The accuracy of the assay was determined at different drug concentrations, as well as at the different ionic strengths and pH values used in this study. The range of measured drug concentrations was 97.60–105.17% of the theoretical drug concentrations. The appropriate pH and ionic strength adjusted solution was used in the reference cell.

Adsorption *versus* pH—The preparation of a series of drug–cellulose suspensions involved the addition of 100 ml of distilled water to a 250-ml actinic glass flask containing 3 g of the appropriate grade of microcrystalline cellulose. The suspensions were then shaken or placed in a bath-type sonifier[5] for 30 sec to ensure dispersion. To each suspension was added 1.3 ml of a 1 mg/ml stock solution of the appropriate drug. The pH of these suspensions was adjusted to 2.1, 3.0, 4.0, 5.0, or 6.1, using 0.1 *N* HCl or 0.009 *N* KOH, while the added[6] ionic strengths were adjusted to 0.0107 or 0.107 using 2 *N* KCl. The pH and ionic strengths of the suspensions were adjusted by the addition of ~95% of the total 2 *N* KCl necessary for ionic strength adjustment (determined by a trial run), followed by pH adjustment using 0.1 *N* HCl or 0.009 *N* KOH, and finally the addition of enough 2 *N* KCl to bring the added ionic strengths to the desired level. This was necessary since the initial addition of 2 *N* KCl caused a significant drop in the pH of the suspensions. The final small addition of 2 *N* KCl to bring the added ionic strength to the desired level caused no significant pH change. A final small dilution with distilled water brought the total volume of the suspensions to 120 ml. The pH was monitored using a combination hydrogen ion electrode[7] and pH meter[8].

Preliminary experiments indicated no significant drug degradation took place at the pH values used in this study, nor in the presence of

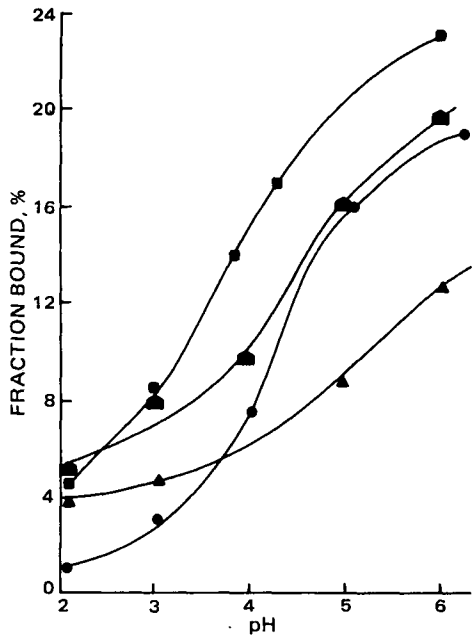

Figure 1—*Effect of pH on the percent fraction of fluphenazine dihydrochloride bound to 3 g of microcrystalline cellulose (1.3 mg of drug/120 ml of external phase). Key: (●) III, added ionic strength (μ′) = 0.0107; (■) IV, μ′ = 0.0107; (▲) III, μ′ = 0.107; (▲) IV, μ′ = 0.107.*

[5] Coulter Ultrasonic Bath, Branson Instruments, Stamford, Conn.

[6] The added ionic strength refers to the total contribution from the hydrochloric acid or the potassium hydroxide and the potassium chloride used in pH and ionic strength adjustment, respectively. It does not include contributions from ions released from the microcrystalline cellulose itself, nor does it include contributions from the drug molecules themselves.

[7] Combination pH Electrode, Sargent-Welch Scientific Co., Skokie, Ill.

[7] Expandomatic SS-2 pH meter, Beckman Instruments, Inc., Scientific Instruments Div., Fullerton, Calif.

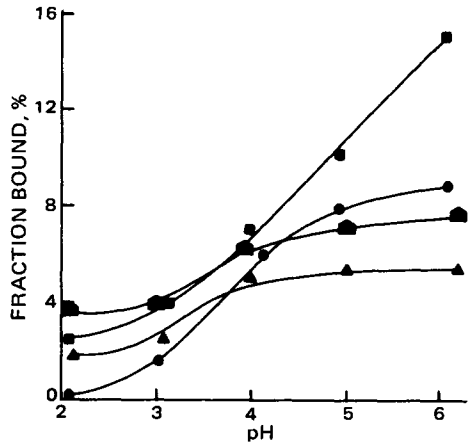

Figure 2—*Effect of pH on the percent fraction of promethazine hydrochloride bound to 3 g of microcrystalline cellulose (1.3 mg of drug/120 ml of external phase). Key: (●) III, μ′ = 0.0107; (■) IV, μ′ = 0.0107; (▲) III, μ′ = 0.107; (▲) IV, μ′ = 0.107.*

microcrystalline cellulose. Blanks were prepared at the same drug concentrations, pH, and added ionic strengths as the samples, but they contained no microcrystalline cellulose. The suspensions and blanks were equilibrated for 1 hr at 23 ± 0.5° on a mechanical shaker water bath[9]. Preliminary experiments performed over a 24-hr period indicated that equilibrium between the adsorbates and adsorbents was reached in <30 min. The suspensions were then centrifuged for 20 min at 23 ± 0.5° on a refrigerated centrifuge[10] at 15,100 rpm. The supernatants were decanted and recentrifuged for an additional 20 min at the same temperature and rpm. The supernatants and blanks were then assayed by the previously described UV spectrophotometric technique. Two separate readings were performed on each sample, and the average was used in the final calculations. The pH values of the final supernatants were monitored and they did not differ by > ±2% of the original adjusted value. The percent fraction of drug bound was calculated as:

$$\% \text{ Fraction Bound} = \frac{[\text{Drug}]_B - [\text{Drug}]_S}{[\text{Drug}]_B} \times 100\% \qquad \text{(Eq. 1)}$$

where $[\text{Drug}]_B$ was the concentration of drug found in the blank and $[\text{Drug}]_S$ was the concentration of drug found in the suspension supernatant. Throughout this study it was assumed that only a minimal amount of water was adsorbed by the microcrystalline cellulose. Even so, the uptake of water by the adsorbent will alter slightly the actual drug concentrations in the suspension supernatants. For this reason, the adsorption discussed throughout this work is actually the apparent adsorption and includes the effect of water uptake by microcrystalline cellulose.

Adsorption Isotherms—Adsorption isotherms were determined for suspensions where the external medium was distilled water (*i.e.*, no pH or ionic strength adjustment), as well as for pH and ionic strength adjusted suspensions. The suspensions were prepared as previously described, with only the drug concentrations being different. For the un-

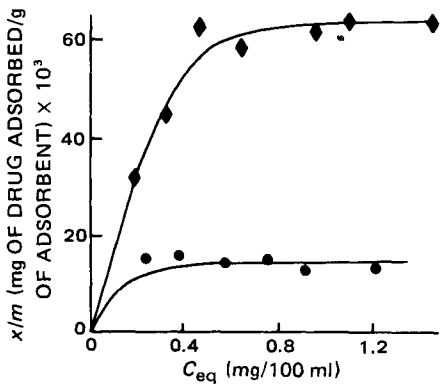

Figure 3—*Adsorption isotherms of fluphenazine dihydrochloride on III at an added ionic strength of 0.107. Key: (●) pH 2.1; (♦) pH 6.1.*

[9] Thermo Shake Incubator Shaker, Forma Scientific, Inc., Marietta, Ohio.

[10] Sorvall Superspeed RC 2-B, Ivan Sorvall, Inc., Newtown, Conn.

Table II—Summary of Constants Obtained from Linear Plots of Langmuir and Freundlich Equations for Fluphenazine Dihydrochloride

Suspension pH	2.1				6.1				Unadjusted (Distilled Water)	
Added ionic strength	0.0107		0.1070		0.0107		0.1070		Unadjusted	
Type of microcrystalline cellulose	III	IV	III	IV	III	IV	III	IV	III	IV
Langmuir constants:										
Adsorptive capacity $k_2 \times 10^3$ (mg of drug adsorbed/g of III or IV) $\times 10^3$	9.6	16.1	11.3	17.6	77.6	106.2	74.9	96.4		
Affinity constant k_1k_2	0.137	0.284	−0.121	9.804	1.218	1.068	0.353	0.767		
Freundlich constants:										
Relative adsorptive capacity $K \times 10^3$									15.2	36.1
Affinity constant N									0.572	0.414
R^2 for linear plots	0.96	0.99	0.98	0.98	0.98	0.99	0.98	0.96	0.98	0.99

adjusted suspensions, the initial drug concentration ranged from 2.5×10^{-3} to 3.33×10^{-2} mg/ml, while for the pH and ionic strength adjusted suspensions the initial concentration of drug was between 2.5×10^{-3} and 1.67×10^{-2} mg/ml. The pH values of the adjusted suspensions were held at 2.1 and 6.1, while the added ionic strengths were held at 0.0107 and 0.1070. The pH values were considered to approximate gastric and intestinal conditions. Blanks were prepared at the same drug concentration, pH, and added ionic strength as the appropriate suspension, only containing no microcrystalline cellulose. The suspensions and blanks were adjusted to the above-mentioned pH and added ionic strength values, equilibrated, and assayed as described previously. The amount of drug adsorbed to the microcrystalline cellulose was determined by the difference in drug concentrations between the suspension supernatant and the appropriate blank. The amount of drug adsorbed in milligrams per gram of microcrystalline cellulose was calculated.

Adsorption *versus* Electrolyte Addition—Suspensions and blanks were prepared at constant pH values of 2.1 and 6.1 exactly as described in the Adsorption *versus* pH section except the added ionic strengths were adjusted. Only III was used. Suspensions prepared at pH 6.1 were adjusted to added ionic strengths of 0, 0.000107, 0.00107, 0.0107, 0.107, and 1.070. Appropriate dilutions were made in a 2 *N* KCl solution so that measurable volumes could be added to the suspensions for ionic strength adjustment. At pH 2.1 the suspensions were adjusted to added ionic strength values of 0.0107, 0.107, and 1.07, using 2 *N* KCl. The suspensions and blanks were equilibrated and assayed as previously described. The percent fraction of drug adsorbed was calculated usng Eq. 1.

Adsorption *versus* Ionic Species—Individual suspensions were prepared by adding 100 ml of distilled water to 250-ml actinic glass flasks containing 3 g of III. Compound III was dispersed, and 1 ml of the appropriate 1-mg/ml drug solution was added. Blanks were prepared containing no adsorbent. Enough sodium chloride, potassium chloride, magnesium chloride, or calcium chloride was added to make the suspensions and blanks 9.804×10^{-4} *M* in added electrolyte. All salt solutions used were 0.1 *N*. These electrolytes were selected due to their frequency of occurrence in the GI tract. Another group of suspensions and blanks were adjusted to a constant added ionic strength of 9.804×10^{-4} using the same salts. Total volume was kept constant at 102 ml, and no pH adjustments were made. The suspensions and blanks were equilibrated, centrifuged, and assayed as previously described. The percent fraction of drug adsorbed was calculated using Eq. 1, and the supernatant pH was monitored.

Desorption *versus* Elution—Individual adsorption complexes were prepared by the addition of 39 ml of distilled water to 50-ml plastic centrifuge tubes containing 1 g of III or IV. The adsorbent was dispersed by shaking. To these suspensions was added 1 ml of a 1-mg/ml solution of the appropriate drug. Blanks were prepared without III or IV. The suspensions and blanks were then equilibrated, centrifuged, and assayed as previously described. Preliminary experiments performed over a 24-hr period indicated that desorption equilibrium was attained in <30 min.

The amount of drug adsorbed to III or IV in the suspension sediment was calculated as the difference between the total drug concentration in the blank and the total drug concentration in the supernatant. This adsorbed amount was considered to be 100% of the possible drug that could be desorbed during an elution. After carefully decanting the supernatant, the remaining drug–microcrystalline cellulose sediment was eluted with either distilled water, or medium adjusted to pH values of 2.1 or 6.2 and added ionic strengths of 0.0107 or 0.1070. The pH and added ionic strength adjustments were performed as previously described after the addition of 35 ml of distilled water to resuspend the sediment. The total volume of elution medium was kept constant at 40 ml. Sediment and elution media were equilibrated, centrifuged, and assayed as described previously, and the elution procedure was repeated a maximum of five times on each drug–microcrystalline cellulose sediment. The pH values of the supernatants were determined and they did not differ significantly from the original adjusted pH values. The cumulative percent desorbed was calculated after each elution using the following equation:

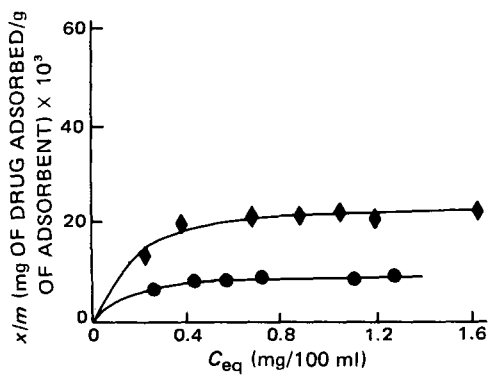

Figure 4—*Adsorption isotherms of promethazine hydrochloride on III at an added ionic strength of 0.107. Key: (●) pH 2.1; (♦) pH 6.1.*

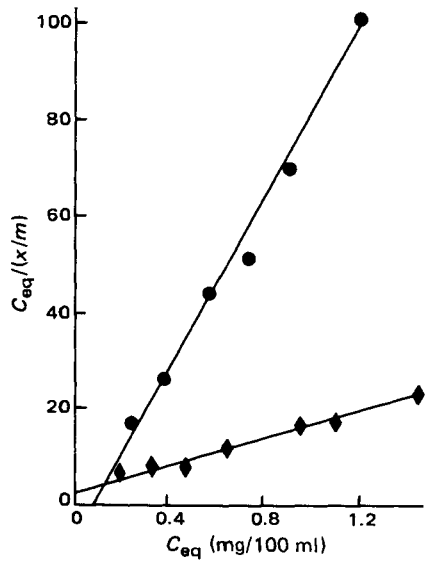

Figure 5—*Langmuir plots for the adsorption of fluphenazine dihydrochloride on III at an added ionic strength of 0.107. Key: (●) pH 2.1; (♦) pH 6.1.*

Table III—Summary of Constants Obtained from Linear Plots of the Langmuir and Freundlich Equations for Promethazine Hydrochloride

Suspenion pH	2.1				6.1				Unadjusted (Distilled Water)	
Added ionic strength	0.0107		0.1070		0.0107		0.1070		Unadjusted	
Type of microcrystalline cellulose	III	IV	III	IV	III	IV	III	IV	III	IV
Langmuir constants:										
Adsorptive capacity $k_2 \times 10^3$ (mg of drug adsorbed/g of III or IV) $\times 10^3$	0	10.1	9.9	19.1	70.3	93.6	25.0	40.5		
Affinity constant k_1k_2	0	0.465	0.073	0.093	0.080	0.185	0.168	0.164		
Freundlich constants:										
Relative adsorptive capacity $K \times 10^3$									4.6	5.8
Affinity constant N									0.703	0.628
R^2 for linear plots	—	0.99	0.99	0.96	0.96	0.94	0.97	0.98	0.99	0.99

$$\text{Cumulative \% Desorbed} = \frac{[\text{Drug}]_C - \sum_{i=1}^{m} [\text{Drug}]_i}{[\text{Drug}]_C} \times 100\% \qquad \text{(Eq. 2)}$$

where $i = 1, 2, 3, \ldots m$ elutions, $[\text{Drug}]_C$ is the total amount of drug initially adsorbed in the drug–microcrystalline cellulose adsorption complex, and $[\text{Drug}]_i$ is the total amount of drug found in the supernatant after the ith elution. Each elution was performed on two separate samples and the values were averaged.

RESULTS AND DISCUSSION

Effect of pH on Adsorption—Figures 1 and 2 illustrate the effect of pH on the adsorption of fluphenazine dihydrochloride and promethazine hydrochloride from suspensions of microcrystalline cellulose adjusted to two different added ionic strengths. Both figures show that at a given added ionic strength and pH, IV always adsorbs a greater amount of drug than does III. This is probably due to the greater total surface area of IV when compared with an equal weight of III. The average particle size of III is 50 μm, while the average particle size of IV is 20 μm (1).

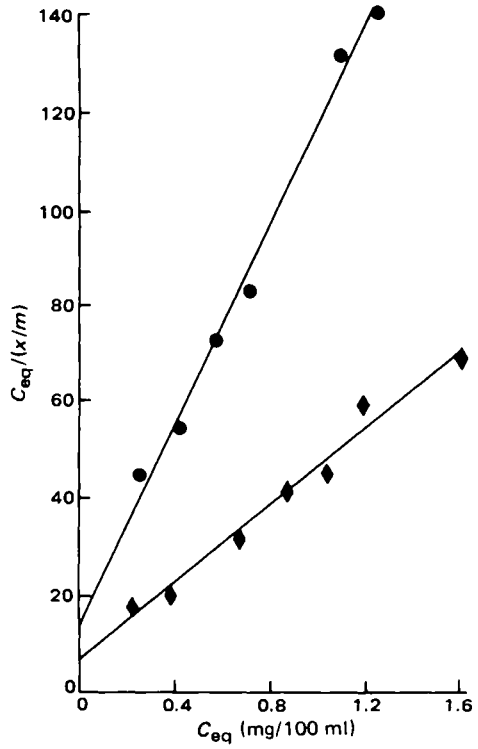

Figure 6—*Langmuir plots for the adsorption of promethazine hydrochloride on III at an added ionic strength of 0.107. Key: (●) pH 2.1; (♦) pH 6.1.*

Table IV—Percent Fraction of Drug Adsorbed by III (3 g) from Suspensions Containing 1.3 mg of Drug/120 ml of Adjusted Medium [a]

Total Added Ionic Strength	Fraction of Drug Adsorbed, %	
	I	II
0.0	82.48	68.98
0.000107	65.17	54.33
0.00107	42.04	27.05
0.0107	16.83	8.77
0.107	13.50	5.28
1.07	11.99	5.84

[a] At a fixed pH of 6.1.

As the pH increases from 2.1 to 6.1, the amount of drug adsorbed by the microcrystalline cellulose also increases (Figs. 1 and 2). This is most likely due to the ionization of carboxyl groups on the cellulose surface. These carboxyl groups are formed by oxidation of the hydroxy groups on individual anhydro-glucose units (14–16). The pKa of these carboxyl groups is ~4.0 (14). As the pH values of the suspensions are increased from 2.1 to 6.1, the number of negatively charged carboxylate groups on the surface of the microcrystalline cellulose particles increases. The increased number of anionic surface sites leads to increased adsorption of the predominantly positively charged drugs at the surface of the particles. The pKa values of these weakly basic drugs are 3.90 and 8.05 for fluphenazine dihydrochloride and 9.08 for promethazine hydrochloride (13).

The possibility also exists that these hydrophobic drugs are adsorbed from solution as the nonprotonated free bases. As the pH values of the suspensions approach the highest pKa values of the phenothiazines, more free base will be found in solution. The free base could then be removed from solution by adsorption to the microcrystalline cellulose surface. As free base is removed from solution, new free base will replace it from the

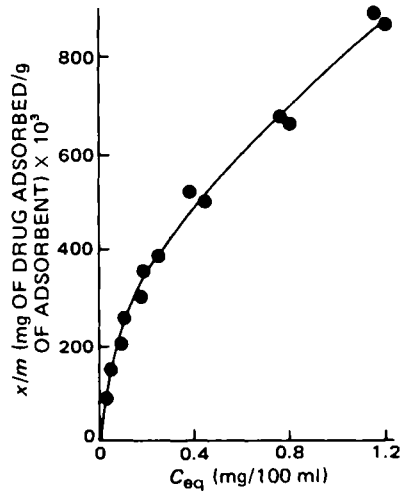

Figure 7—*Adsorption isotherm of fluphenazine dihydrochloride on III in distilled water.*

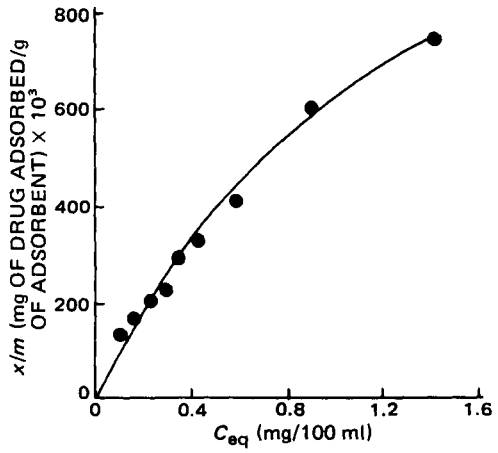

Figure 8—*Adsorption isotherm of promethazine hydrochloride on III in distilled water.*

protonated excess. This will continue until an equilibrium is established between the free base in solution and the base adsorbed to the cellulose surface. This explanation does not seem as likely as the one previously described since pH levels are kept two or more units below the highest pKa of the drugs, and there is an abundance of literature describing the negative surface of microcrystalline cellulose (14, 17–19) as well as other cellulosic materials (2, 15, 20).

Increasing the added ionic strength has different effects on the extent of adsorption depending on the pH of the suspension (Figs. 1 and 2). At high pH values (*i.e.*, pH 6.1), an increase in added ionic strength caused a decrease in the amount of drug adsorbed. This is most likely caused by increased competition between positively charged drug molecules and potassium and hydrogen ions for the negatively charged microcrystalline cellulose surface.

At low pH values (*i.e.*, pH 2.1), where the cellulose carboxy groups are predominantly in their nonionized form, an increase in the added ionic strength causes a slight increase in the amount of drug adsorbed by a particular type of microcrystalline cellulose (Figs. 1 and 2). This could be due to the increased surface activity of these phenothiazines at higher ionic strengths (21). The increased surface activity of these drugs at higher ionic strengths could be caused by the suppression of adsorbed drug–drug electrical repulsions due to the screening effect of the added ions. This would allow more drug to be adsorbed to the cellulosic surface. At higher pH values, where the surface of the microcrystalline cellulose is predominantly negative, this suppression effect may be absent or hidden by the attraction between oppositely charged particles. There is also a possibility that these drugs adsorb by different mechanisms depending on the pH of the suspension and the interrelated surface charge of the microcrystalline cellulose.

The experimental results of this adsorption study give only indirect evidence for the mechanism of adsorption. Further experiments using IR spectroscopy and X-ray diffraction would be needed to determine the exact adsorption mechanism (22, 23).

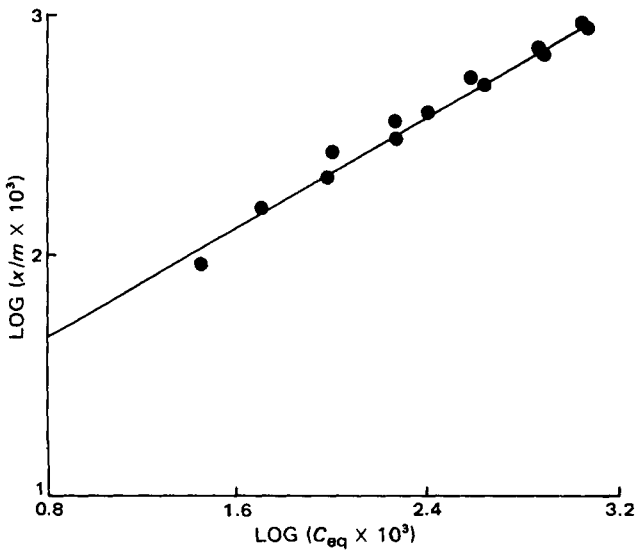

Figure 9—*Freundlich plot for the adsorption of fluphenazine dihydrochloride on III in distilled water.*

Table V—Percent Fraction of Drug Adsorbed by III (3 g) from Suspensions Containing 1.3 mg of Drug/120 ml of Adjusted Medium [a]

Total Added Ionic Strength	Fraction of Drug Adsorbed, % I	II
0.0107	1.01	0.00
0.107	4.66	1.85
1.07	6.27	4.23

[a] At a fixed pH of 2.1.

Table VI—Effect of Electrolytic Species on the Fraction of Promethazine Hydrochloride (0.98 mg/100 ml) Bound to III (3 g)

Electrolyte	Constant Molarity of Added Electrolyte (9.804×10^{-4} *M*) Fraction Bound, %	pH of Supernatant	Constant Ionic Strength of Added Electrolyte (9.804×10^{-4} *M*) Fraction Bound, %	pH of Supernatant
Potassium chloride	28.6	5.55	28.6	5.55
Sodium chloride	31.8	5.52	31.8	5.52
Magnesium chloride	9.9	5.45	14.2	5.60
Calcium chloride	9.5	4.90	15.4	5.30

Adsorption Isotherms—Adsorption isotherms prepared from suspension data where the pH and ionic strength were adjusted to fixed values adhered to the theoretical Langmuir equation, while those prepared from distilled water conformed to the empirical Freundlich equation (24–26). Fit to one or the other type of isotherm was based solely on the comparison of R^2 values obtained from the linear plots (using least squares) of the respective isotherms.

Drug aggregates often exist below the apparent critical micelle concentration for surface active agents such as the phenothiazines (27). The apparent critical micelle concentration will change with varying pH and ionic strength levels (28). Adsorption of aggregates of drug molecules (*i.e.*, ion-pair adsorption) has been reported in the literature (2). This type of phenomenon is often accompanied by a drastic increase in adsorption as the drug concentration approaches its apparent critical micelle concentration (2). No such changes in the adsorption isotherms were observed during this study. It was felt that this fact, coupled with the low concentrations of drugs used, indicated that adsorption was predominantly occurring at a monomolecular level.

The linear form of the Langmuir adsorption isotherm is given by:

$$\frac{C_{eq}}{x/m} = \frac{1}{k_1 k_2} + \frac{C_{eq}}{k_2} \qquad \text{(Eq. 3)}$$

where C_{eq} is the concentration of drug remaining in the suspension supernatant in mg/100 ml after equilibrium adsorption is obtained, x/m is the amount of drug adsorbed in milligrams per gram of microcrystalline cellulose, and k_1 and k_2 are constants. The constant k_2 is the limiting adsorptive capacity. It is the maximum amount of adsorbate, in milligrams, that can be adsorbed by 1 g of adsorbent. Due to the lack of data points at extremely low drug concentrations (*i.e.*, infinite dilution), k_1k_2, instead of k_1, is often used as a measure of the relative affinity of the adsorbate for the adsorbent (13, 29, 30). Typically, calculated k_1k_2 values have been shown to be subject to some error (31). Figures 3 and 4 are typical examples of the isotherms obtained from pH and ionic strength adjusted suspensions. Figures 5 and 6 are the linear plots of these same Langmuir adsorption isotherms.

Adsorption isotherms determined for the phenothiazine–microcrystalline cellulose suspensions where the external phase was distilled water conformed to the Freundlich equation given in the linear form as:

$$\log \frac{x}{m} = \log K + N \log C_{eq} \qquad \text{(Eq. 4)}$$

where x/m and C_{eq} are defined as before and K and N are constants. According to Adamson, the constant K gives a rough measure of the relative adsorbent capacity for a given drug, while N gives a general idea of the affinity of the adsorbate for the adsorbent (32). Typically, the

Table VII—Summary of Desorption *versus* Elution Data for Fluphenazine Dihydrochloride

Elution pH	2.1				6.2				Unadjusted (Distilled Water)	
Added ionic strength	0.0107		0.1070		0.0107		0.1070		Unadjusted	
Type of microcrystalline cellulose	III	IV	III	IV	III	IV	III	IV	III	IV
Cumulative percent desorbed										
Elution 1	97.2	94.4	93.7	92.0	88.5	81.9	86.5	84.9	6.6	5.7
Elution 2	100	100	100	100	98.6	94.2	96.1	97.3	9.9	9.5
Elution 3					100	97.8	97.1	100	12.9	13.1
Elution 4									16.3	16.3
Elution 5									20.5	18.1

Table VIII—Summary of Desorption *versus* Elution Data for Promethazine Hydrochloride

Elution pH	2.1				6.2				Unadjusted (Distilled Water)	
Added ionic strength	0.0107		0.1070		0.0107		0.1070		Unadjusted	
Type of microcrystalline cellulose	III	IV	III	IV	III	IV	III	IV	III	IV
Cumulative percent desorbed										
Elution 1	96.0	96.3	95.1	96.8	86.6	90.5	89.6	92.7	11.8	14.0
Elution 2	100	100	100	100	96.6	100	97.8	100	21.6	24.3
Elution 3									30.6	32.7
Elution 4									37.7	39.0
Elution 5									44.3	45.4

constants k_1 and k_2 (Langmuir constants) are not compared to the constants K or N (Freundlich constants) since they are determined from different types of isotherms (*i.e.*, theoretical *versus* empirical). Figures 7 and 8 are typical examples of the isotherms obtained from suspensions where the external medium was distilled water. Figures 9 and 10 are the linear plots of these same Freundlich adsorption isotherms.

Tables II and III show the constants obtained from the different isotherms, as well as the squared correlation coefficients of the linear plots for fluphenazine dihydrochloride and promethazine hydrochloride, respectively. Comparison of isotherms determined from suspensions in which the external medium was distilled water (Figs. 7 and 8) to isotherms where the external medium was pH and ionic strength adjusted (Figs. 3 and 4) indicates that the electrolyte concentration of the suspension has a major effect on the extent of adsorption.

Effect of Added Electrolyte—Tables IV and V show how changes in the added ionic strength of the suspensions affects the percent fraction of drug adsorbed at constant pH values of 6.1 and 2.1, respectively.

Effect of Ionic Species—The results shown in Table VI indicate that divalent cations have a major effect on the extent of promethazine hydrochloride adsorption by III at both constant electrolyte molarity and added ionic strength. There seems to be little difference in the fraction of drug bound when comparing cations with equal valences. Divalent cations seem to cause a major decrease in the amount of drug adsorbed to III, as compared with monovalent cations. This could be due to divalent cations neutralizing twice as much negative charge on the surface of the microcrystalline cellulose as monovalent cations, which would decrease the number of anionic surface sites available for the protonated drug molecules to interact with, and therefore less drug would be adsorbed. The divalent cations may also have a greater affinity for the surface of III than the monovalent cations and, therefore, less drug would be adsorbed due to increased competition for the negative surface of the microcrystalline cellulose.

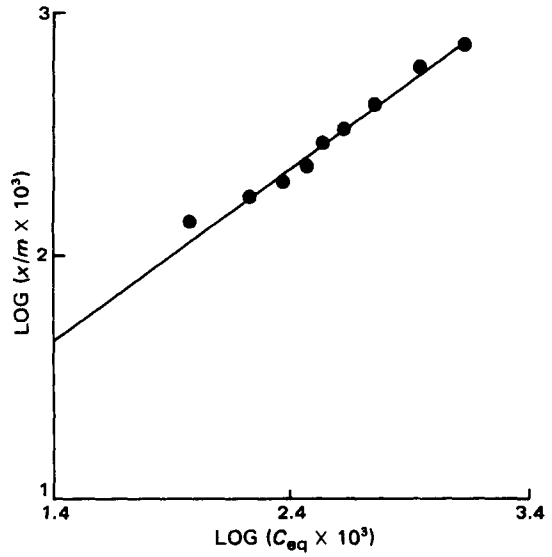

Figure 10—*Freundlich plot for the adsorption of promethazine hydrochloride on III in distilled water.*

Even though the above are hypothesized mechanisms, they seem likely due to the fact that oxidized celluloses, such as microcrystalline cellulose (14, 18), have been shown to adsorb inorganic cations (16, 20, 33). The pH values of the final supernatants were approximately the same after the addition of potassium chloride, sodium chloride, magnesium chloride, and only slightly lower for calcium chloride, indicating that pH was not the reason for the differences seen in the fraction of drug adsorbed.

Desorption—Preliminary experiments indicated that desorption equilibrium was attained in <30 min. Tables VII and VIII show the results of the desorption experiments for fluphenazine dihydrochloride and promethazine hydrochloride, respectively. These results demonstrate the importance that electrolytes play in the desorption phenomenon. It would appear that the inorganic cations in the elution medium have an affinity for the negative surface of the microcrystalline cellulose and, therefore, displace the adsorbed drugs into the external media. This is evidenced by small amounts of the drugs being desorbed from microcrystalline cellulose after five elutions with distilled water, as compared with almost 100% being desorbed with pH and ionic strength adjusted media after three or less washes (Tables VII and VIII). The pH values of the elution supernatants of samples washed with distilled water were close to the adjusted elution pH of 6.2. This indicates that the added electrolytes in the adjusted elution samples must cause the increased desorption, not differences in pH.

The results of these *in vitro* desorption studies indicate that the adsorption of these drugs by microcrystalline cellulose should be rapidly and completely reversed at gastric and intestinal pH values and ionic strengths.

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Isolation, Identification, and Synthesis of the Major Sulpiride Metabolite in Primates

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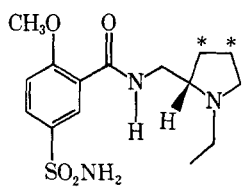
Abstract □ The major metabolite of sulpiride, *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-sulfamoyl-2-anisamide (I), in the monkey is *N*-[(1-ethyl-5-oxo-2-pyrrolidinyl)methyl]-5-sulfamoyl-2-anisamide (II). It is also a metabolite in other laboratory animal species and possibly at very low levels in humans. Treatment of the urine from a monkey dosed orally with ^{14}C-I by dry column chromatography and high-pressure liquid chromatography (HPLC) produced the major metabolite in pure form. Characterization of the purified ^{14}C-radiolabeled metabolite by proton NMR, TLC, HPLC, and chemical ionization mass spectroscopy, along with subsequent comparison of a synthetically prepared sample, gave unequivocal structural confirmation.

Keyphrases □ Sulpiride—isolation, identification, and synthesis of major metabolites, monkeys □ High-pressure liquid chromatography—analysis, major sulpiride metabolites, monkeys □ Metabolites—sulpiride, isolation, identification, and synthesis, monkeys

Sulpiride (I) is a structurally unique antipsychotic drug. Studies utilizing ^{14}C-labeled I indicated that, while this drug is metabolized to a very small extent in humans, the monkey produces a major metabolite which accounts for 10–30% of a single dose (1). Column chromatography on a strong cation exchange resin, with dilute acid, rapidly eluted this major metabolite, whereas I was retained. This behavior suggested that the metabolite was rendered less basic than the parent drug by a metabolic change on the pyrrolidine ring.

There are several model chemical compounds that possess a pyrrolidinyl moiety either as a fused five-membered ring or as the saturated heterocyclic structure analogous to I. Among these are mazindol (III), prolintane (IV), and tremorine (V).

One of the major biotransformations of these chemical models (Structures II, III–V) in analogous animal species is the oxidation of the alpha position of the five-membered ring to the lactam structures (VI, VII, and VIII). These metabolic changes suggest that I would be similarly biotransformed. It has been reported (2) that the metabolite oxytremorine (VI) is physiologically active and provided



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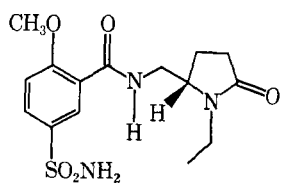

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