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DEVAZIPIDE (MK-329) ADSORPTION ON MICROCRYSTALLINE CELLULOSE

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ABSTRACT

An in-depth evaluation of the adsorption of Devazipide onto microcrystalline cellulose, formulation and/or process optimization procedures to minimize this adsorption are reported. This adsorption was more evident in the 1 mg compared to the 10 mg strength tablet and could be completely eliminated if the microcrystalline cellulose was reduced to <10%. This adsorption followed the Langmuir Isotherm and was dependent on pH with insignificant amount of adsorption occurring at pH 2.0. At this particular pH, adsorbed drug is easily released.

INTRODUCTION

MK-329 is a benzodiazepine derivative (Figure 1). It is a cholecystokinin antagonist proposed for the treatment of inflammatory bowel disease. It is a white essentially amorphous, non-hygroscopic solid. It exhibits satisfactory solid-state stability either neat or in mixtures with common

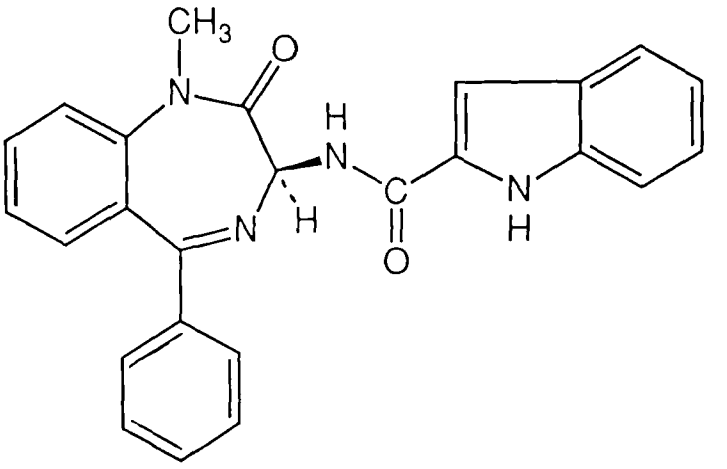

Figure 1: The structure of MK-329

excipients (microcrystalline cellulose, corn starch, pregelatinized starch 1500, magnesium stearate and docusate sodium). It is insoluble in water (equilibrium solubility at room temperature <1 ug/ml). In acetonitrile, acetone and methanol, solubilities at room temperature are 15 mg/ml, 15 mg/ml and 10 mg/ml, respectively. In this study, MK-329 was micronized (particle size $\leq$ 5 micron).

Compounds like benzodiazepines are widely used in low doses (1-6). At these low doses, the potential exists for adsorption onto insoluble tablet excipients which has been reported for benzodiazepines (7) and some antibiotics like ampicillin and amoxycillin (8). These reported studies indicate that excipient interaction, characterized by adsorption of drug onto

microcrystalline cellulose affected the in vitro and in vivo availability of the formulations studied.

Adsorption of a solute by a solid surface can occur during the preparation of suspension dosage forms as well as in a patient's gastrointestinal tract following the co-administration of a soluble drug and an insoluble solid. Adsorption can also occur between a drug and one of the excipients when preparing tablet dosage form (9-16). Most adsorption mechanisms can be classed as either physical adsorption or chemisorption. The attractive forces in physical adsorption are due to van der waals forces, and most likely hydrogen bonding. The attractive forces in chemisorption are much stronger and include coordination complexes and ion exchange (17).

Microcrystalline cellulose has been extensively used in the area of drug formulations as a diluent, disintegrant, and dry binder in tablet formulations prepared by direct compression. Also, microcrystalline cellulose is being used as a tablet excipient in formulations prepared by wet granulation. Usually, this process involves dissolving the drug in granulating fluid. This creates the possibility of adsorption by microcrystalline cellulose or other excipients because of the greater number of dissolved drug molecules that are available to interact.

The availability of medicinal substances adsorbed on solids is important. Reversibility of the adsorption is indispensable to ensure the complete availability of the medicinal substance.

The purpose of this study is to evaluate in-depth the problem of MK-329 adsorption on microcrystalline cellulose and to optimize the formulation and/or process in order to have minimal adsorption.

EXPERIMENTAL

A. Materials

MK-329 (Merck), Microcrystalline cellulose (Avicel pH 101, FMC), Corn Starch (Colorcon), Pregelatinized Starch 1500 (Colorcon), Docusate Sodium (Cyanamid), Acetonitrile (HPLC Grade, Fisher), Potassium Dihydrogen Phosphate (Fisher Certified Primary Standard), Alcohol SD3A (Quantam), Magnesium Stearate (Mallinkrodt).

B. Equipment

Spectrophotometer - Beckman DU-65, Action Wrist Shaker - Borrel (Model 75), Hewlett Packard (Model 1090) HPLC.

C. Preparation of the Tablets

The process of manufacturing is shown in Figure 2. Microcrystalline cellulose, corn starch and MK-329 are mixed in a suitable blender. Then, the mixture is granulated with alcohol SD3A/water (1:10) in which docusate sodium was dissolved. Due to the insolubility of the drug, it was found that it is necessary to add a surfactant, docusate sodium, to the formulation (Table 1). After drying and mixing with magnesium stearate, the tablets were compressed using the single punch press (F-press).

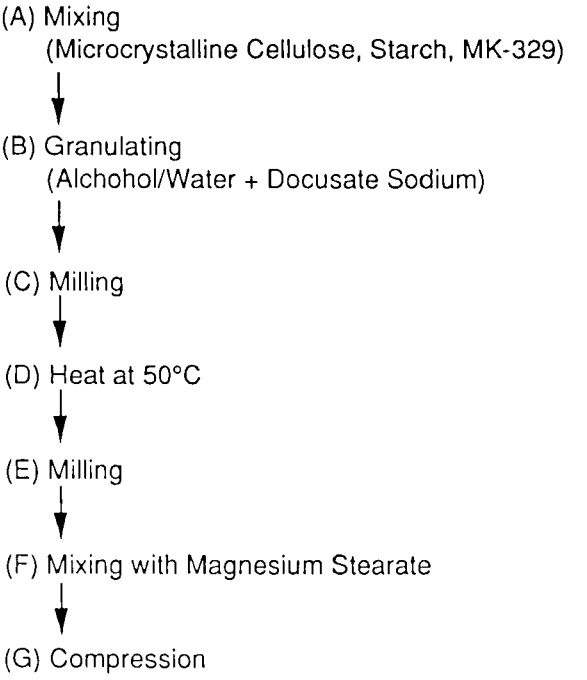

Figure 2: Process I for the manufacture of MK-329 tablet

TABLE 1: MK-329 1.0 mg and 10 mg tablet formulas

Excipients	Tablet Strength	
	1 mg	10 mg
MK-329	1.00	10.0
Microcrystalline Cellulose (Avicel pH 101)	60.0	60.0
Corn Starch 1500	38.0	28.5
Docusate Sodium	0.50	0.50
Magnesium Stearate	0.50	0.50

In this study, 1.0 mg and 10 mg MK-329 were prepared. The weight of the tablets was approximately 100 mg.

D. Analytical Procedure

(1) Assay - two assay procedures were used, an HPLC and a UV method. The HPLC method was used for all the tablet experiments which required high specificity, while the UV method, which is much simpler, was used for all the adsorption-desorption experiments.

(a) HPLC Method

MK-329 tablets were extracted with 50% acetonitrile in water. An amount of water equivalent to 10% of the flask volume (100 ml for 1 mg or 1000 ml for 10 mg tablet) was used to disperse the tablet. The flasks were then put on a mechanical shaker for 10 minutes of shaking to totally disperse the tablet. The flask was brought to approximately 50% volume with acetonitrile and then shaken for an additional 10 minutes. The flask was brought to near volume with water, allowed to come to room temperature and then brought to volume with water. Five to fifty milliliters aliquots of these solutions were diluted with acetonitrile/water, 1/1 and centrifuged. An aliquot from the centrifuged sample was injected onto a Hypersil MOS2 HPLC column (100 mm X 4.6 mm, 5 um particle size) at 40°C using a mobile phase consisting of 0.01M KH_2PO_4 (pH 2.5): acetonitrile, 55:45 and a flow rate of 1.5 ml/min. Detection was by uv absorbance at 290 nm. No interference from the

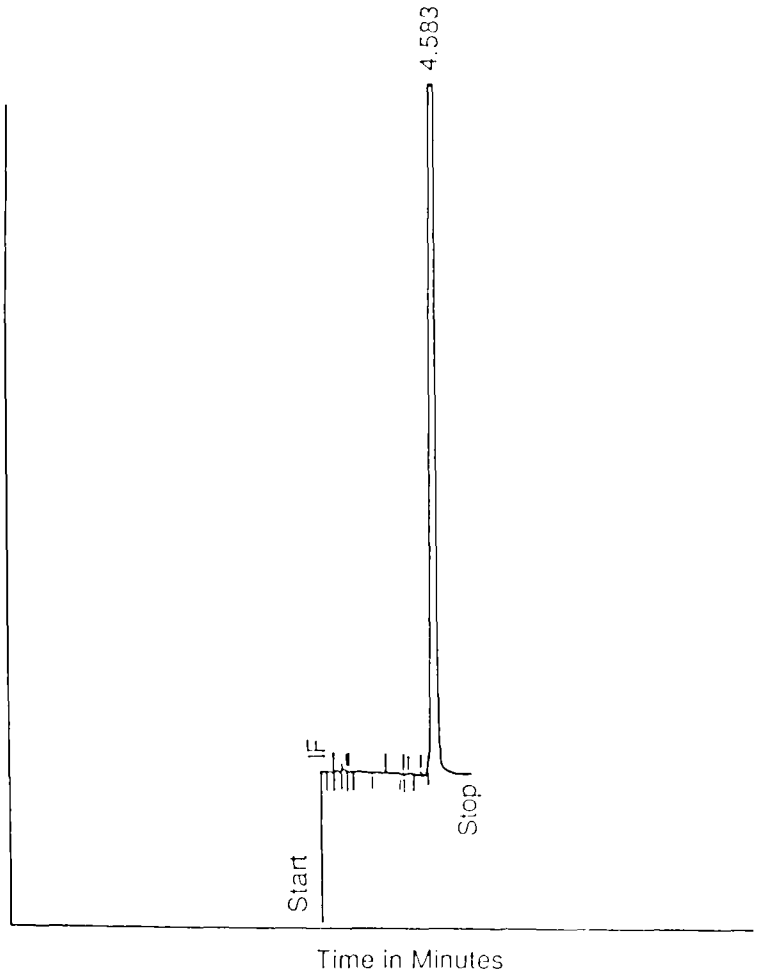

Figure 3: Sample chromatogram of MK-329

excipients in the tablet was present. The method resolved MK-329 from two potential degradates, indole-2-carboxylic acid and the base portion of the molecule (3-amino-benzodiazepam). Figure 3 shows a sample chromatogram of MK-329.

(b) UV Method

Assays were done by uv spectrophotometer at a wavelength of 290 nm.

(2) Dissolution Method

Tablets were dissolved using USP Apparatus II (paddles) at 75 rpm in 900 ml of 2% sodium dodecyl sulfate (SDS). Samples were taken at 30, 45 and 60 minutes. An aliquot was assayed using the HPLC parameters described above.

E. Adsorption Experiments

MK-329 was dissolved in acetonitrile followed by dilution with water (acetonitrile: water - 1:1) to yield initial concentrations of 17-405 mcg/ml. Twenty-five milliliters of these solutions were equilibrated with 1 gm of microcrystalline cellulose by shaking for three hours on the action wrist shaker at 25°C. Samples were taken up to the three hours period. Solutions were then filtered and assayed. The assay results for these solutions are considered the equilibrium concentrations. Controls consisted of drug solutions without microcrystalline cellulose. All the experiments were run in triplicate.

When the effect of pH on adsorption was tested, similar experiments were conducted at a pH range of 2.0-7.0. The pH was adjusted by the addition of either 0.1N NaOH or 0.1N HCl.

F. Desorption Experiments

MK-329 was adsorbed on microcrystalline cellulose in an adsorption experiment similar to the one described above. Solutions were filtered and assayed. The amount of drug adsorbed to microcrystalline cellulose was determined by the

difference between the above assay results and the control (where no microcrystalline cellulose is available). This adsorbed amount was considered to be 100% of the possible drug that could be adsorbed. The microcrystalline cellulose was filtered and dried in the oven. The dried samples were suspended in 25 and 50 ml of acetonitrile/water solution (1:1) at pH 2.0. Then, the suspensions were shaken by a mechanical shaker for 24 hours. Filtered samples were collected at frequent intervals and assayed for MK-329 by UV-spectrophotometry.

RESULTS AND DISCUSSION

A. Tablet Studies

In this study, the prepared tablets (Table 1) were tested. Analytical results obtained for assay, content uniformity and dissolution were in excellent agreement for both potencies. For the 1.0 mg strength, dissolution was 90% after 60 minutes, composite assay was 0.928 mg per tablet and content uniformity was 0.911 mg per tablet (RSD = 0.96%) indicating that adsorption was a significant problem at this potency. For the 10.0 mg strength, the adsorption phenomenon was diminished with a dissolution of 97% after 60 minutes and composite assay was 9.62 mg per tablet and content uniformity was 9.62 mg per tablet (RSD = 0.77%). No degradation products were observed.

Assay results of the 1 mg granulation at various points of the manufacturing process indicate that after mixing the 1 mg

TABLE 2: Assay Results of Granulation and Compressed 1 mg Tablets Prepared with Corn Starch (I) and Pregelatinized Starch 1500 (II)

Sampling	mg/gm I	mg/gm II
1. After mixing	9.00	9.17
2. After milling	9.29	9.37
3. Final granulation	9.38	9.33
4. Whole tablet	9.43	9.45
5. Homogenized tablet (grinding mill, hand held)	9.60	9.79

granulation, only 90.0% of the drug was recovered. However, when the final tablet was homogenized using a grinding mill (hand held) prior to solvent extraction, 96.0% of the drug was recovered. Results show that when pregelatinized starch 1500 was substituted for corn starch, similar recoveries were obtained (Table 2).

Recovery studies as part of the HPLC method validation were done on the crushed placebo tablets formulated with corn starch. These crushed tablets were spiked with MK-329 (solution or solid spike) at different levels (80%, 100% and 120% of MK-329 levels). Recovery results indicate that adsorption is definitely occurring in the manufactured MK-329 tablets since in the spiked placebo tablets, 100.2%-102.2% of the drug was recovered (Table 3).

TABLE 3: Results of Recoveries from Crushed Placebo Tablets

Level	Potency/Assay	% Recovery by Solution Spike	% Recovery by Solid Spike
80%	1 mg/Composite	101.6	102.1
100%	1 mg/Composite	101.3	102.6
120%	1 mg/Composite	100.8	101.9
		X = 101.2	X = 102.2
80%	1 mg/Content Uniformity	100.4	101.8
100%	1 mg/Content Uniformity	100.3	101.2
120%	1 mg/Content Uniformity	100.0	101.1
		X = 100.2	X = 101.4
80%	10 mg/Composite	100.8	-
100%	10 mg/Content Uniformity	100.2	-
120%	10 mg/Content Uniformity	100.8	-
		X = 100.6	

Recovery studies performed by mixing microcrystalline cellulose and MK-329 using the same ratio as in the tablet formula (1.65% MK-329 in microcrystalline cellulose) however, indicated that 100% recovery is not achieved (Table 4). These recovery studies were done using three solvent systems for extraction. In the first system, water was added and then acetonitrile (1:1); in the second system, acetonitrile was added and then water (1:1); and in the third system methanol was added and then water (1:1). Recoveries were almost similar for all these solvent systems. They ranged between 97.1% and 98.4%.

All the above experiments point to the fact that MK-329 was being adsorbed on microcrystalline cellulose.

TABLE 4: Recovery of MK-329 from MK-329/Avicel Mixture (1.647% MK-329 in microcrystalline cellulose), using Different Solvent Systems

Solvent	Recovery, mg/g	% Theory
H_2O/CH_3CN (1:1)	16.00	97.1
CH_3CN/H_2O (1:1)	16.15	98.1
$MeOH/H_2O$ (1:1)	16.20	98.4

Since this type of adsorption occurs mainly between the drug which is dissolved in solution and microcrystalline cellulose, the 1 mg tablets were prepared using another process (Figure 4) by which MK-329 was added to the manufacturing step just prior to the addition of magnesium stearate. Recovery results show that 97.1% (RSD = 0.8%) of the drug was recovered. This was an improvement over the previous data (92-93%). But still about 3% of the drug was not recovered. These results are similar to the recovery data obtained when MK-329 was just mixed with microcrystalline cellulose without the use of any granulating fluid (Table 4).

In another series of experiments, it was found that as the amount of microcrystalline cellulose in the 1.0 mg MK-329 tablets decreases, adsorption decreases. When microcrystalline cellulose was used in small amounts (<10%), almost 100% recovery was achieved (Figure 5).

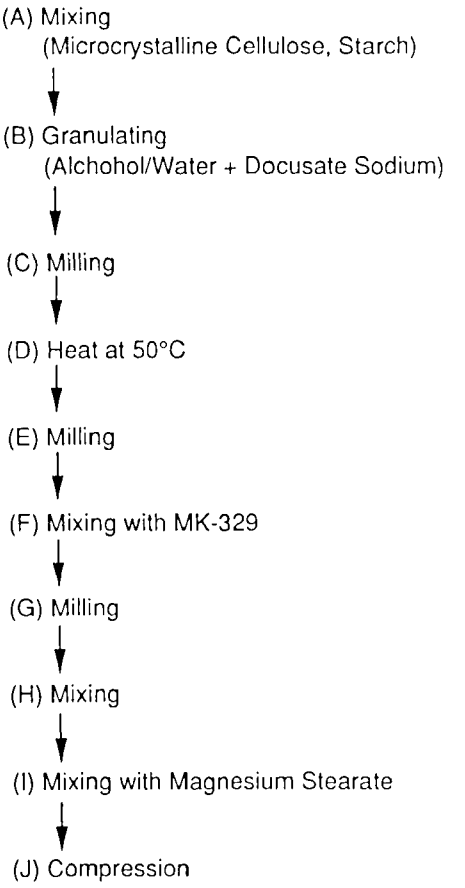

Figure 4: Process II for the manufacture of MK-329 tablet

The above observations indicate that only when microcrystalline cellulose is used at higher levels, adsorption becomes a problem. This data suggests that as the amount of microcrystalline cellulose increases, more adsorption sites will be available for the drug. To study this phenomenon, a thorough adsorption study described in the subsequent paragraphs was carried out.

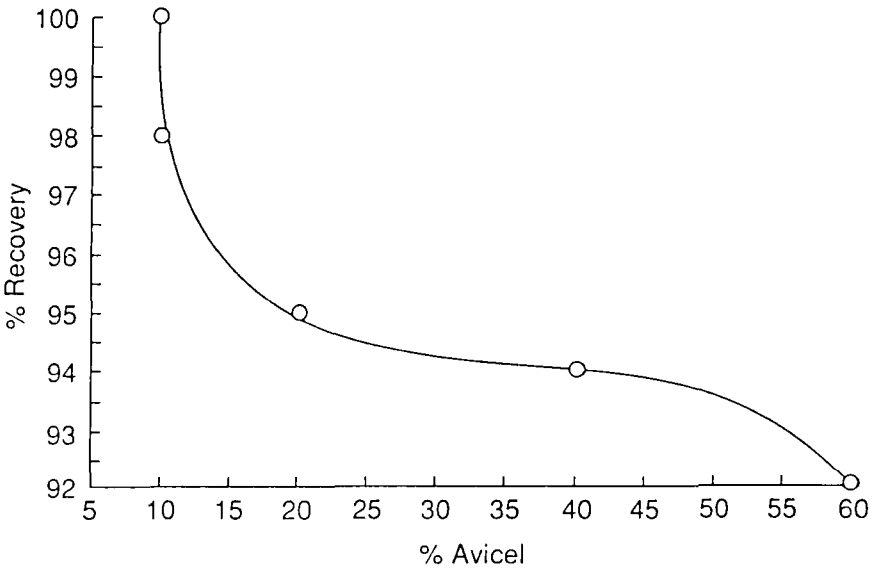

Figure 5 : Recovery of drug from 1 mg MK-329 tablets prepared with different amounts of Microcrystalline Cellulose

B. Adsorption - Desorption Study

In the adsorption - desorption experiments, the percent fraction of drug bound was calculated as:

$$\% \text{ Fraction Bound} = \frac{[\text{Drug}]c - [\text{Drug}]s}{[\text{Drug}]c} \times 100\% \qquad (1)$$

In this equation, [Drug]c is the concentration of MK-329 in the control solution while [Drug]s is the concentration of MK-329 in the sample containing microcrystalline cellulose after being filtered. The adsorption data reported in this study includes the effect of water uptake by microcrystalline cellulose. Although this effect is very minimal, the

TABLE 5: The Amount of MK-329 Adsorbed (xm, mcg/g) as a function of the Equilibrium Concentration (C, mcg/ml)*

Equilibrium Conc **(C) (mcg/ml)	mcg Adsorbed Per Gm of Microcrystalline Cellulose (X/M)***(mcg/g)	C/(X/M)
16.5	12.3	1.33
26.5	18.2	1.45
54.8	34.7	1.58
88.4	54.8	1.61
105	57.8	1.82
220	60.9	3.61
402	68.8	5.85

*TEMP: 25°C

**Drug concentration after equilibration with microcrystalline cellulose.

***Average of three samples (RSD for all results <2%)

adsorption in this study will be considered as the "apparent adsorption" (14).

The amount of MK-329 adsorbed with increasing amounts of equilibrium concentrations is given in Table 5.

The standard adsorption isotherm was plotted in Figure 6. From this figure (Figure 6), it is obvious that the adsorption of MK-329 on microcrystalline cellulose increases rapidly with the increase in equilibrium concentration. Adsorption increases with the increase of equilibrium concentration (c) until a plateau region begins to form after 150 ug/ml.

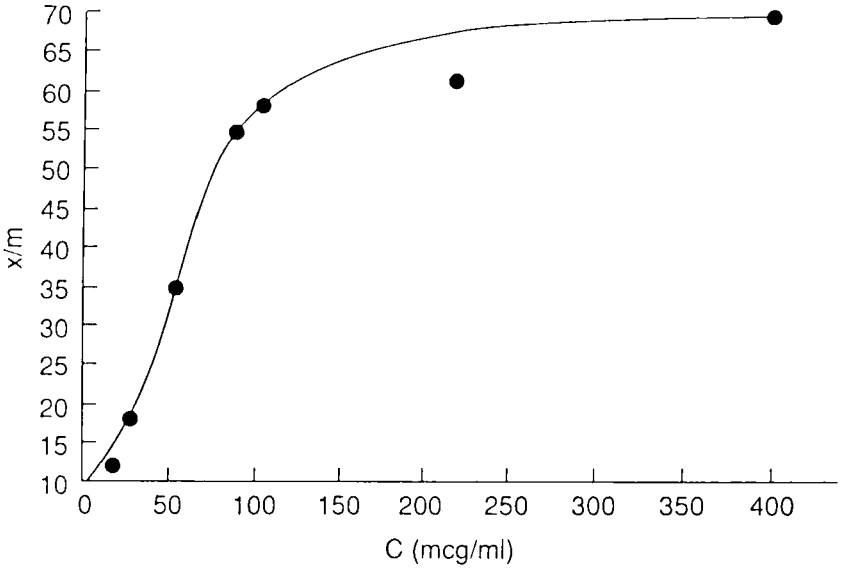

Figure 6: The amount of MK-329 (mcg) adsorbed per gram of Microcrystalline Cellulose (X/M) as a function of the equilibrium concentration (C).

The Langmuir treatment of the adsorption data is given by the following equation:

$$\frac{X}{M} = \frac{K_1 K_2 C}{1+K_1 C} \qquad (2)$$

Linear form of the above equation is:

$$\frac{C}{(X/M)} = \frac{1}{K_1 K_2} + \frac{C}{K_2} \qquad (3)$$

Where C is the concentration of unadsorbed solute at equilibrium, X is the amount of solute adsorbed, M is the mass of adsorbent, K_1 is an affinity constant (adsorbent to adsorbate), and K_2 is the maximum adsorbent capacity.

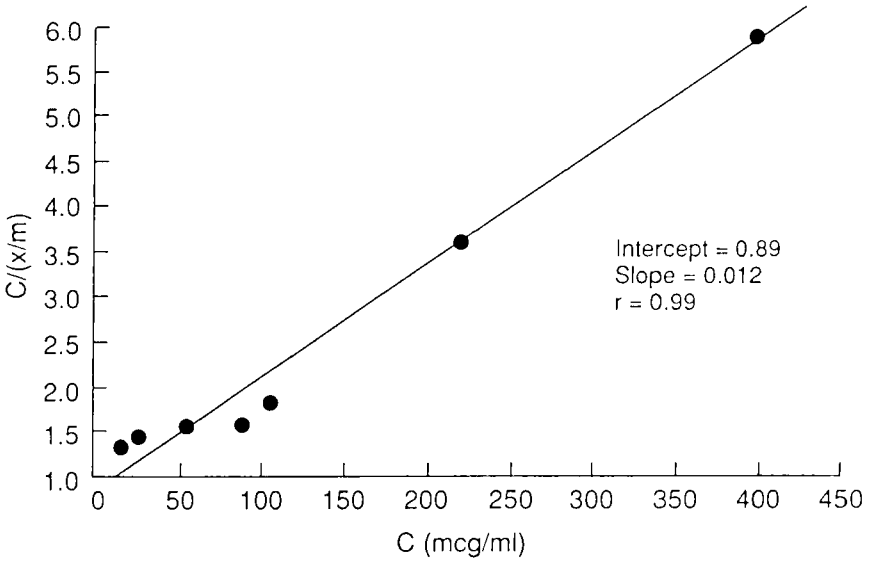

Figure 7: Langmuir plot for the adsorption of MK-329 on Microcrystalline Cellulose

Equation (3) indicates that when plotting C/(X/M) versus C, a slope of $1/K_2$ and an intercept of $1/(K_1K_2)$ should be obtained. The adsorption data were plotted and analyzed by the linear least-squares regression (Figure 7). An excellent fit was obtained (r = 0.99). The maximum adsorptive capacity of microcrystalline cellulose to MK-329 (K_2) was found to be 20.2×10^{-5} mmole/gm of microcrystalline cellulose which is consistent with the adsorptive capacity of other drugs on microcrystalline cellulose (ranges between 0.56×10^{-5} mmole/gm to 30.1×10^{-5} mmole/gm) (15).

The derivation of Equation 2 is dependent upon several assumptions: all the sites of adsorption are equivalent,

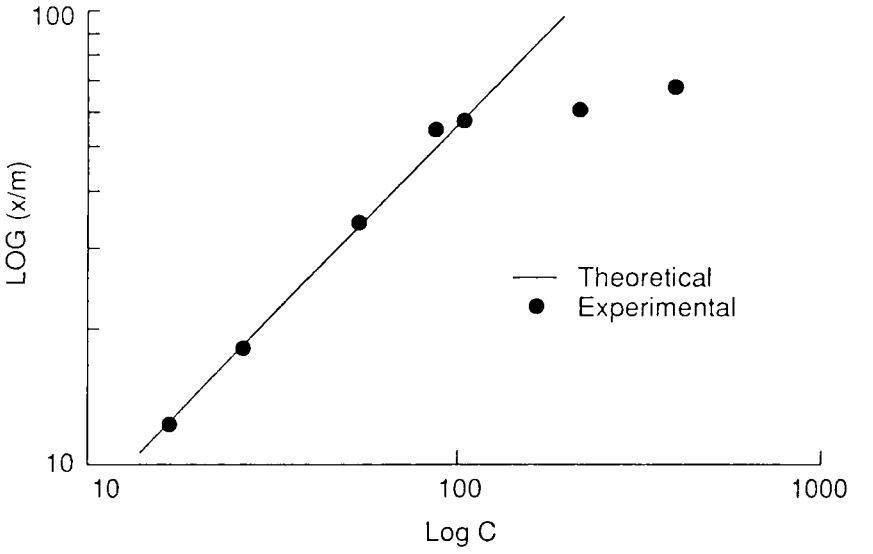

Figure 8: The theoretical Freundlich linear plot for adsorption on Microcrystalline Cellulose with the MK-329 experimental points scattered around it.

adsorbed layer is confined to a monolayer and there are no lateral interactions between the adsorbate molecules.

Some investigators (18, 19) found that the Freundlich equation might be useful in explaining the adsorption phenomenon on tablet excipients. The freundlich equation is:

$$\frac{X}{M} = KC^P \qquad (4)$$

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. The logarithmic transforma-

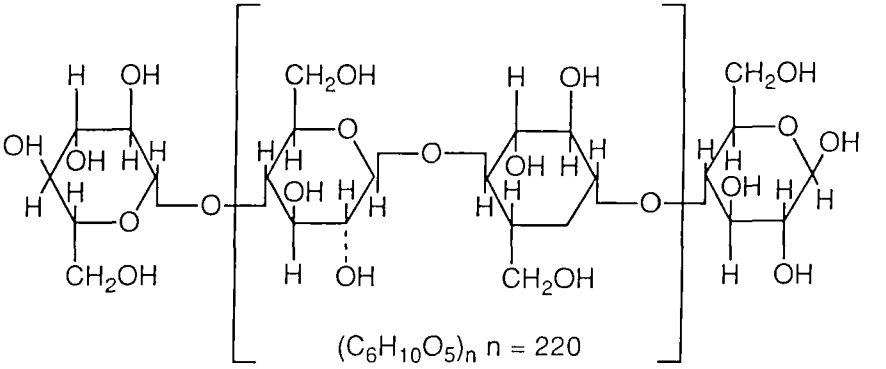

Figure 9: Structure of Microcrystalline Cellulose

tion of the above equation is:

$$\text{Log } \frac{X}{M} = \text{Log } K + P \text{ Log } C \qquad (5)$$

Figure 8 is a theoretical linear plot of the Freundlich adsorption isotherm. The experimental points are scattered around this linear plot which indicates that the isotherm does not follow the Freundlich equation. This was expected since the Freundlich model unfortunately predicts infinite adsorption at infinite concentration.

Several mechanisms have been proposed for the adsorption of drugs on excipients (20-22). One proposed mechanism for this adsorption is that adsorption occurs due to the hydrogen bonds between the hydroxyl groups on the glucose units of microcrystalline cellulose (Figure 9) and the carbonyl groups of MK-329. Since MK-329 has a low pKa (3.9), MK-329 will be ionized at acidic pH. The electronegativity of carbonyl group

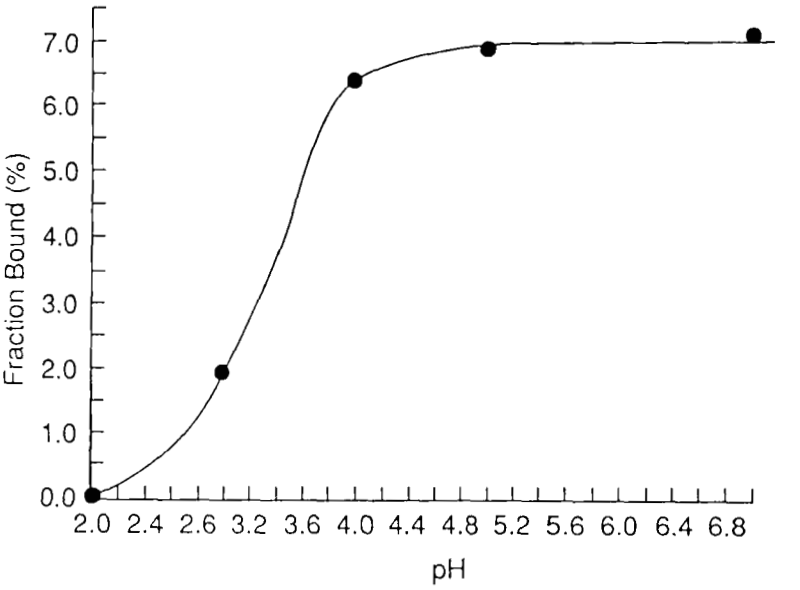

Figure 10: The fraction bound (%) of MK-329 on Microcrystalline Cellulose as a function of pH.

is reduced in acid, thereby, reducing the probability for hydroxyl group to form hydrogen bond with it, resulting in lower adsorption. Figure 10 shows clearly that MK-329 adsorption is pH dependent. It is minimal in the acidic region (below pH 3) and increases gradually with the increase of pH becoming almost constant above pH 4.0. A second mechanism (14) which is less likely, involves the carboxyl groups formed on microcrystalline cellulose as a result of the oxidation of the hydroxy group on individual anhydro glucose units. The pKa of these carboxyl groups is ~4. Thus, as the pH values are increased, the number of negatively charged carboxylate groups

TABLE 6: The Amount of MK-329 Desorbed as a Function of Time*

TIME (Min.)	% Desorbed
5	23.42
20	28.51
35	36.86
45	46.91
55	62.19
65	71.32
75	71.45
85	73.93
105	77.71
135	79.02
150	79.02
160	79.02

*TEMP: 25°C

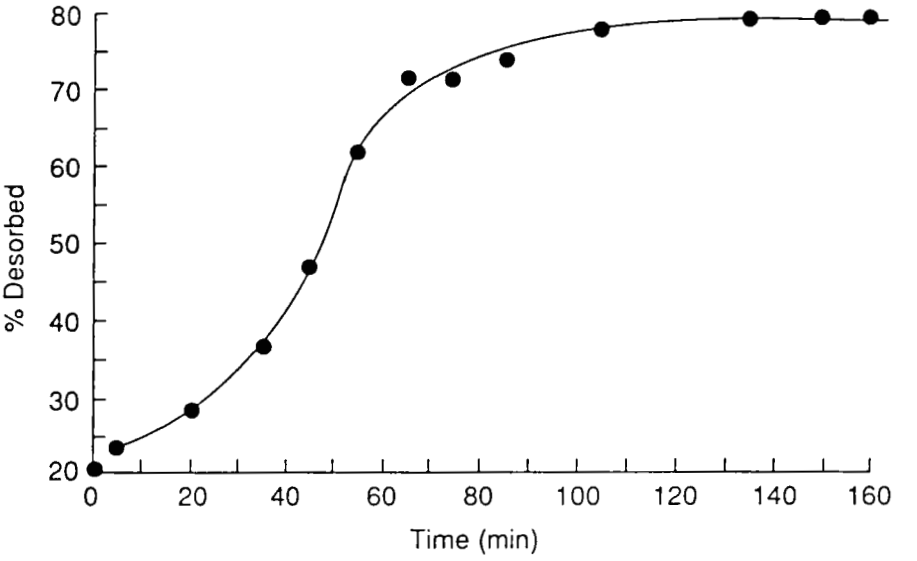

Figure 11: The amount of MK-329 (%) desorbed from Microcrystalline Cellulose as a function of time (pH = 2.00).

on the surface of microcrystalline cellulose particles increases (23). This will lead to an increased adsorption of MK-329 which has a positive electronegativity at the surface.

Because of the above results, desorption studies were carried out at pH 2.0. Table 6 gives the results of these studies. It was found that most of the drug gets desorbed after about two hours (Figure 11). The amounts desorbed were the same when the volume of acetonitrile/water solution was double (50 ml).

CONCLUSION

From the above, it is clear that MK-329 is adsorbed on microcrystalline cellulose. Adsorption is much more significant in the 1.0 mg than the 10 mg strength tablets. Studies were conducted to optimize the formulation and/or process in order to have minimal adsorption. It was found that when the manufacturing process was changed by which the drug was added in the final stages, adsorption decreased, but did not disappear. When microcrystalline cellulose was used in small amounts (<10%), 100% recovery was achieved.

When adsorption-desorption experiments were conducted, it was found that adsorption of MK-329 follows the Langmuir Equation. An insignificant amount of adsorption occurs at a pH of 2.00.

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