[Chem. Pharm. Bull. 29(3) 828—833 (1981)]

Interaction of Phenothiazines with Pectin in Aqueous Solution[1,2]

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(Received September 4, 1980)

Interactions between phenothiazines and pectin in aqueous solution were studied by the equilibrium dialysis method, in order to investigate the relation between binding parameters and drug properties.

Scatchard plots were not linear for water-soluble drugs such as promazine hydrochloride, triflupromazine hydrochloride, and so on, and thus the number of binding sites of pectin was considered to be more than two. On the other hand, it was impossible to obtain the r value (mol of drugs bound per mol of pectin) on Scatchard plots for such slightly soluble drugs as levomepromazine maleate, perazine dimaleate and prochlorperazine dimaleate at higher concentrations. Therefore the data were analyzed simply by assuming that they gave a linear plot. The maximum binding number was significantly larger than the values already reported.

A good positive correlation was observed between the binding parameters and adsorption parameters related to hydrophobicity. Among drugs having the same side chain at the 10-position but different substituents at the 2-position, the maximum binding number increased with the bulkiness of the substituents.

The micelle formation of phenothiazines was shown to be related to the interaction and also to the formation of coprecipitate.

Keywords——pectin; phenothiazines; equilibrium dialysis method; Scatchard plot; binding parameters; hydrophobic interaction; micelle formation

It was reported that pectin interacts with cationic drugs mainly with its carboxy residues, probably by hydrogen bonding or van der Waals forces.[4–6] It was also reported that pectin forms water-insoluble complexes with several nonsteroidal antiinflammatory drugs, suggesting that it may be useful as an additive for sustained-release preparations.[7]

In the present work, we attempted to study the interaction of phenothiazines with pectin in aqueous solution, in order to investigate the relation between the binding parameters and various properties of phenothiazines already reported. Phenothiazines were chosen because they are cationic drugs and have many derivatives. We also hoped to obtain information that would be useful for the development of sustained-release preparations based on coprecipitates of drugs with pectin.

Experimental

Materials——Low methoxyl pectin from Sunkist Growers Inc., the molecular weight of which was determined to be 120000 by gel chromatography on Sephadex G-100, was used.[7] Thirteen derivatives of phenothiazines used are listed in Table I. The drugs are very soluble in water except for levomepromazine (LPZ), prochlorperazine (CPeZ) and perazine (PeZ). The solubilities of LPZ, CPeZ and PeZ in purified water at 37° were determined to be 6.14×10^{-3}, 1.56×10^{-3} and 5.56×10^{-3} M, respectively.

Equilibrium Dialysis Method for Determination of the Amount of Drugs Bound to Pectin——Visking cellulose tubing containing 10 ml of 0.5% pectin in purified water was immersed in 30 ml of purified water solution of a drug in a light-resistant Nessler tube, at 37°. The initial concentration of drugs was usually 2.8×10^{-4}—2.8×10^{-2} M. The drug concentration in the Nessler tube was determined at appropriate intervals and the amount of drug bound to pectin was calculated. The drugs were assayed by the ultraviolet (UV) absorption method using a Hitachi 124 spectrophotometer.

Determination of Critical Micelle Concentration (cmc) of Drugs——The cmc of drugs were determined

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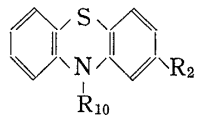
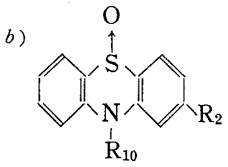
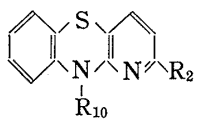
by a well known pH method.[8,9] The pH of drug solutions in purified water in the concentration range of 1.0×10^{-3}—1.0×10^{-1} M was measured with a Hitachi-Horiba pH meter at 37°.

Table I. Phenothiazines used in This Study

Compound	R_{10}	R_2
Anergen[a,d,i]	$-(CH_2)_2-N(CH_3)(CH_3)$	H
Diethazine[a,d,j]	$-(CH_2)_2-N(CH_2-CH_3)(CH_2-CH_3)$	H
Promazine[a,d,h]	$-(CH_2)_3-N(CH_3)(CH_3)$	H
Chlorpromazine[a,d,h]	$-(CH_2)_3-N(CH_3)(CH_3)$	Cl
Trifupromazine[a,d,h]	$-(CH_2)_3-N(CH_3)(CH_3)$	CF_3
Promethazine[a,d,i]	$-CH_2-CH(CH_3)-N(CH_3)(CH_3)$	H
Methdilazine[a,d,i]	$-CH_2-CH(CH_2-N-CH_3)(CH_2-CH_2)$	H
Levomepromazine[a,f,h]	$-CH_2-CH(CH_3)-CH_2-N(CH_3)(CH_3)$	OCH_3
Perazine[a,g,h]	$-(CH_2)_3-N\,N-CH_3$	H
Prochlorperazine[a,g,h]	$-(CH_2)_3-N\,N-CH_3$	Cl
Trifluoperazine[a,e,h]	$-(CH_2)_3-N\,N-CH_3$	CF_3
Isothipendyl[c,d,i]	$-CH_2-CH(CH_3)-N(CH_3)(CH_3)$	H
Chlorpromazine-sulfoxide[b,d]	$-(CH_2)_3-N(CH_3)(CH_3)$	Cl

a) 

b) 

c) 

d) Hydrochloride. *e*) Dihydrochloride. *f*) Maleate.
g) Dimaleate. *h*) Tranquilizer. *i*) Anti-histaminic.
j) Anti-Parkinsonic.

Results and Discussion

Binding Properties of Phenothiazines to Pectin as Determined by the Equilibrium Dialysis Method

The binding of drugs to pectin was found to reach equilibrium after 8 hr, and the sampling was done after 24 hr. The data were analyzed according to a usual Scatchard plot. The Scatchard plots of water-soluble drugs were not linear, indicating that the number of binding sites of pectin to drugs was more than two. However, the binding parameters of water-soluble drugs were calculated on the assumption that the number of binding sites of pectin was two. On the other hand, it was impossible to obtain the r values (moles of drugs bound per mole of pectin) in Scatchard plots for such slightly soluble drugs as LPZ, CPeZ and PeZ at higher concentrations. Therefore the data were analyzed simply as if they corresponded to a linear plot. The Scatchard plots of trifluoperazine (TPeZ) and CPeZ are illustrated in Fig. 1, as an example. The solubility of CPeZ was small, but if the solubility of CPeZ were large as

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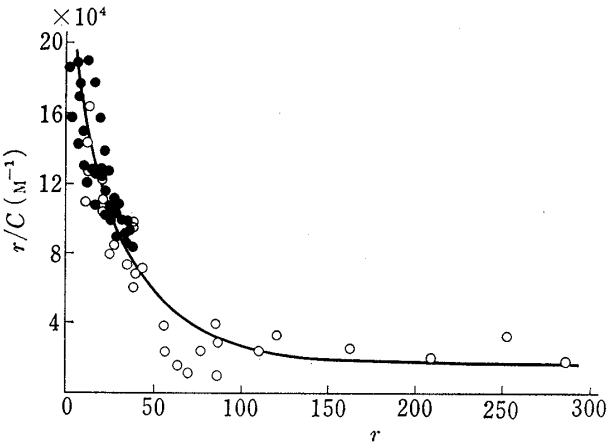

Fig. 1. Scatchard Plot for the Binding of Trifluoperazine and of Prochlorperazine to Pectin at 37° as Measured by the Equilibrium Dialysis Method in Purified Water

○: trifluoperazine, ●: prochlorperazine. r: mol of drug bound per mol of pectin. C: equilibrium concentration (M).

that of TPeZ, a similar type of binding curve might be obtained.

Table II summarizes the binding parameters calculated by means of a Tosbac 7/10 computer.[10,11] In general, the binding constants K_1, K_2 and K were small and the maximum binding numbers n_1, n_2 and n were large compared with the binding parameters already reported for the interaction of phenothiazines with protein[12] and of various drugs with protein.[13,14] However, a case of large n value has been reported for the interaction of antihistamines with hydrocolloids, including pectin, by Graham *et al.*[5]

Relation between Binding Parameters and Some Properties of Phenothiazines

Nambu *et al.* reported the adsorption of a series of phenothiazines on carbon black and graphite,[15] and also their binding to bovine serum albumin.[12] They reported that the hydrophobic interaction plays a predominant role in the adsorption, and Langmuir-type adsorption isotherms were observed. The relations between the binding parameters obtained in the present study and the adsorption parameters were investigated. There was a coefficient of correlation of 0.997 between the a-value on graphite[15] and the maximum binding number n to pectin, excluding LPZ and chlorpromazine-sulfoxide (CPZ-O), as shown in Fig. 2. A coefficient of correlation of 0.860 was obtained between the a-value on carbon black[15] and n-value to pectin. The reason for the large deviation of LPZ and CPZ-O is not clear, and further investigations are required. A coefficient of correlation of 0.938 was obtained between the binding constant K obtained in this study and the b-value to graphite,[15] as shown in Fig. 3.

Table II. Binding Constants and Maximum Binding Numbers for Phenothiazines to Pectin as Determined by the Equilibrium Dialysis Method at 37° in Purified Water

Drug	K_1(M^{-1})	n_1	K_2(M^{-1})	n_2	K_1n_1(M^{-1})	K_2n_2(M^{-1})	K(M^{-1})	n	Kn(M^{-1})
Anergen	1430	66.7	9.88	505	95400	4990	1280	79.2	101000
Diethazine	740	108	1.58	3090	79900	4880	1800	72.5	131000
Promazine	5280	33.3	50.6	385	176000	19500	1580	78.5	124000
Chlorpromazine	597	164	9.50	598	97900	5680	1450	92.5	134000
Triflupromazine	1042	31.2	291	202	32500	58800	1080	112	121000
Promethazine	1764	54.6	42.1	168	96300	7070	1460	70.4	103000
Methdilazine	211	168	8.14	646	35400	5260	2240	62.0	139000
Levomepromazine							1030	91.4	94100
Perazine							6840	24.4	167000
Prochlorperazine							2690	68.4	184000
Trifluoperazine	6250	34.5	18.6	1005	216000	18700	2330	69.2	161000
Isothipendyl	917	86.4	61.7	75.6	79200	4660	1170	82.0	95900
Chlorpromazine-sulfoxide	1280	79.5	4.53	536	102000	2430	1230	83.6	103000

K_1: primary binding constant.
n_1: primary maximum binding number.
K_2: secondary binding constant.
n_2: secondary maximum binding number.
K and n: These parameters were calculated on the assumption that there is one binding site in the initial concentration range of drug between 2.8×10^{-4}—2.6×10^{-3} M.

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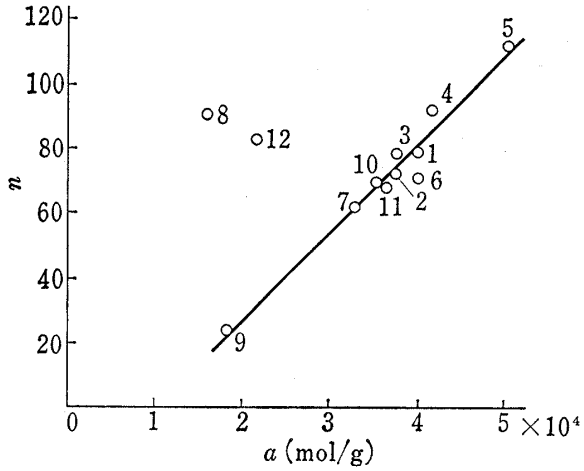

Fig. 2. Relation between Adsorbability of Phenothiazines on Graphite (a)[15] and Maximum Binding Number to Pectin (n) obtained in This Study

a: amount of drugs adsorbed on graphite according to the Langmuir equation.
1: anergen, 2: diethazine, 3: promazine, 4: chlorpromazine, 5: triflupromazine, 6: promethazine, 7: methdilazine, 8: levomepromazine, 9: perazine, 10: prochlorperazine, 11: trifluoperazine, 12: chlorpromazine-sulfoxide.

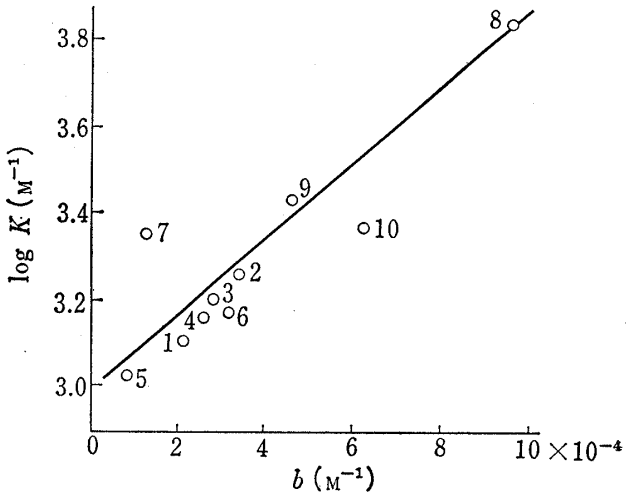

Fig. 3. Relationship between Equilibrium Constant for Adsorption of Phenothiazines on Graphite (b)[15] and Binding Constant to Pectin (K) obtained in This Study

b: value of equilibrium constant for adsorption of drugs on graphite according to the Langmuir equation.
1: anergen, 2: diethazine, 3: promazine, 4: chlorpromazine, 5: triflupromazine, 6: promethazine, 7: methdilazine, 8: perazine, 9: prochlorperazine, 10: trifluoperazine.

Hydrophobic bonding was considered to be involved in the adsorption of phenothiazines on carbon black and graphite. Therefore, in view of the present results, hydrophobic bonding might also be considered to play a predominant role in the interaction of phenothiazines with pectin.

Promazine-group [promazine(PZ), CPZ, triflupromazine(TPZ)] and perazine-group [PeZ, CPeZ, TPeZ] drugs have the same side chain at R_{10}, but different substituents at R_2. As shown in Table III, among these two groups, the maximum binding number n increased with the bulkiness of the substituents as follows: PZ<CPZ<TPZ; PeZ<CPeZ<TPeZ. This tendency was similar to that of the parameters indicating hydrophobicity.[16] Therefore, the results demonstrated that substituents at the 2-position might influence the interaction between phenothiazines and pectin. On the other hand, a reciprocal relation was observed between the parameter π and the primary binding parameter n_1K_1, suggesting that there are two binding sites of pectin, as shown in Table III.

Table III. Influence of the Side Chain R_2 on Hydrophobicity and Binding Parameters

Compound	R_2	$\pi^{a)}$	Binding parameters			
			K_1n_1(M^{-1})	n	K(M^{-1})	Kn(M^{-1})
Promazine	H	0	176000	78.5	1580	124000
Chlorpromazine	Cl	1.04	97900	92.5	1450	134000
Triflupromazine	CF_3	1.49	32500	112	1080	121000
Perazine	H	0		24.4	6840	167000
Prochlorperazine	Cl	1.04		68.4	2690	184000
Trifluoperazine	CF_3	1.49		69.2	2330	161000

a) Parameter indicating hydrophobicity.[16]

Keipert *et al.* investigated the interaction of phenothiazines with sodium carboxymethylcellulose, an anionic polysaccharide like pectin, in aqueous solution.[17] They reported that hydrophobic interaction was observed over the whole concentration range of drugs and that

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ionic interaction also was observed at higher concentrations. In this study, n was calculated for lower concentrations of drugs on Scatchard plots. n_1K_1, however, was calculated over the whole concentration range of drugs. The order of the maximum binding number n was same as that of the values of a and b (Figs. 2 and 3), and that of the parameter π (Table III), both of which are measures of hydrophobicity. On the other hand, the order of the primary binding parameter n_1K_1 was opposite to that of the parameter π indidation hydrophobicity, as shown in Table III. Judging from the relation between the binding parameters and hydrophobicity of drugs shown in Table III, we considered that ionic bonding was also involved in the interaction at higher concentrations of drugs.

Relation between the Binding Parameters and cmc

Many phenothiazine hydrochlorides are known to self-associate and thus to form micelles in aqueous solution.[8,18] It was also reported that the micelle formation influences the interaction of drugs with polyvinylpyrrolidone.[19,20]

The plots of pH against the concentrations of TPZ and of isothipendyl (ITP) are shown in Fig. 4, as an example. The drugs, except for ITP and CPZ–O, showed curves with an

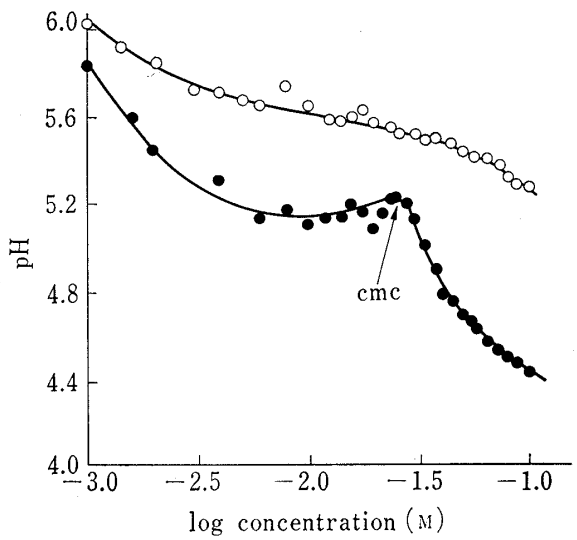

Fig. 4. Plots of pH *versus* Logarithmic Concentration of Drugs at 37° in Purified Water

●: triflupromazine. ○: isothipendyl.

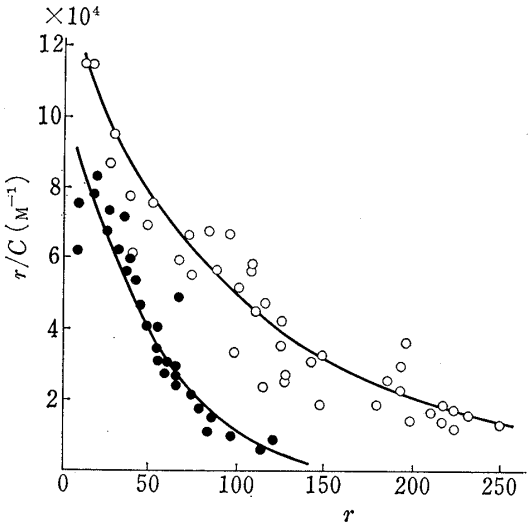

Fig. 5. Scatchard Plot for the Binding of Chlorpromazine (○) and Chlorpromazine-sulfoxide (●) to Pectin at 37° in Purified Water

r: mol of drug bound per mol of pectin.
C: equilibrium concentration (M).

Table IV. Values of cmc Determined by pH Measurement in Purified Water at 37°

Compound	cmc × 10^z (M)
Promazine	3.05[a]
Chlorpromazine	2.95
Trifluprazine	2.90
Anergen	5.40
Diethazine	3.72
Promethazine	4.40[a]
Methdilazine	2.95
Trifluoperazine	3.40
Isothipendyl	np
Chlorpromazine-sulfoxide	np

a) In distilled water at 34° by pH measurement[8] np: no inflexion point was observed.

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inflexion point (*e.g.*, TPZ), which corresponds to the cmc of drug.[8,9] The cmc of each drug thus obtained is shown in Table IV. These values are higher than the highest initial concentration of the drugs, 2.8×10^{-2} M, used, in the equilibrium dialysis method. This suggests that micelle formation of the drugs does not have any relation to the binding data obtained in this study.

On the other hand, CPZ–O, like ITP (Fig. 4), did not show an inflexion point within the experimental concentration range. A similar pattern of CPZ–O was reported by Nakagaki *et al.*[9] During equilibrium dialysis, white precipitate were observed in the cellulose tubing when very high concentration of drugs were used, except with ITP and CPZ–O. It was reported that self-association of a conjugated acid into micelles may be accelerated.[21] Therefore, it appears that pectin strongly interacts with cationic drugs such as TPZ, and this gives rise to coprecipitate formation. This is being investigated in detail.

Furthermore, the values of K_2n_2 of ITP and CPZ–O are smaller than those the other drugs (see Table II). As shown in Fig. 5, the r values (n_1 plus n_2) of CPZ–O are apparently smaller than those of CPZ. The absence of micelle formation and the small values of binding parameters, K_2n_2, of secondary binding sites for ITP and CPZ–O suggest that the secondary binding sites may relate to ionic bonding and not to hydrophobic bonding.

Acknowledgement The authors are very grateful to Dr. Kozo Takayama of Hoshi Institute of Pharmaceutical Sciences for Scatchard plot analysis by computer, and to Messers. Toshio Kuroda and Yoshiaki Saito of Wakamoto Pharmaceutical Company for their support in this work. Thanks are also due to Misses Tomoko Ando and Michiyo Honda for their assistance in the experimental work.

References and Notes

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2) A part of this work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1979, and at the 2nd International Conference on Pharmaceutical Technology, Paris, June 1980.
3) Location: *a) Kanade-378, Oi-machi, Ashigarakami-gun, Kanagawa 258, Japan*; *b) Fbara-2-4-41, Shinagawa-ku, Tokyo 142, Japan.*
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