Effect of Carboxylic Acids on Permeation of Chlorpromazine Through Dimethyl Polysiloxane Membrane

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Abstract □ The effect of carboxylic acids on the permeation of chlorpromazine was investigated through a dimethyl polysiloxane nonpolar membrane. The permeability of the diffusate, at pH 5.8, increases considerably in the presence of carboxylic acids or phosphate, probably due to an ion-pair formation between the relative anions and chlorpromazine.

Keyphrases □ Chlorpromazine—permeation through a dimethyl polysiloxane membrane, effect of carboxylic acids □ Diffusion—chlorpromazine, through a dimethyl polysiloxane membrane, effect of carboxylic acids □ Carboxylic acids—effect on chlorpromazine diffusion through a dimethyl polysiloxane membrane

Previously reported studies (1–3) on the diffusion and permeation of drugs through polymeric membranes have contributed much to the knowledge of drug diffusion; a dimethyl polysiloxane polymer would be useful to investigate drug permeation through a membrane.

The permeation of molecules through a nonpolar membrane in the presence of other molecular species, has been studied previously (2, 4, 5). Many factors, including complex and ion-pair formation (6, 7), can influence the physiological availability of drugs. Nakano (8) studied the influence of a variety of substances, such as adsorbents and excipients, on the permeation of chlorpromazine through a model membrane. All the examined compounds, dissolved in citrate or dimethylglutarate buffers, decrease the permeation of the drug.

The present study examined the variation of chlorpromazine permeation through a nonpolar membrane in the presence of many acids to understand the effect of different organic anions on drug permeability. The selected carboxylic acids, the majority of them being physiological, had different structures.

EXPERIMENTAL

Materials—Nonreinforced dimethyl polysiloxane[1] sheeting in a labeled thickness of 5 ml (12.5×10^{-3} cm), thoroughly rinsed and treated as described previously (2), was used.

Chlorpromazine hydrochloride[2] as well as, citric[3], tartaric[3], acetic[3], glutaric[3], adipic[3], isocitric[4], oxaloacetic[4], malic[3], α-ketoglutaric[3], succinic[3], fumaric[3], and aconitic[4] acids were obtained commercially.

Instruments—A pH meter[5], a spectrophotometer[6], and a plate tensiometer[7] were used.

Determination of Critical Micelle Concentration (CMC)—The CMC of chlorpromazine in the presence of bicarboxylic acids was determined by measurement of surface tension with a plate tensiometer at 37 ± 0.5° in 50×10^{-3} *M* solution of acid. The ionic strength was maintained at 0.146 *M* by adding sodium chloride.

Diffusion Studies—The diffusion cell was constructed according to one used previously (9). The glass cell consisted of donor and receptor compartments (the volume of each compartment was 22 ml) and a membrane (available area was 3.14 cm^2) placed between them. Twenty-two milliliters of 0.01 *N* HCl was added to one arm and an equal volume of the test solution was placed in the other arm. The concentration of diffusible chlorpromazine in the desorbing solution was kept to a zero value to maintain diffused chlorpromazine in a dissociated form. The diffusing solutions were always corrected with sodium hydroxide or with hydrochloric acid, according to the desired pH, since preliminary experiments showed that phosphate and carboxylate anions can change drug permeation considerably. All solutions were warmed in a jacketed container maintained at 37 ± 0.1°. The content of each compartment was rotated by a magnet attached to an electric motor; the rotating speed was ~300 rpm.

Analytical Methods—At scheduled times an aliquot (0.5 ml) of the receptor solution was pipetted out for UV determination, and the same volume of 0.01 *N* HCl was added to the receptor compartment to replace the reduced volume. The 0.5-ml aliquot of the desorbing solution was transferred into a volumetric flask, and 4.5 ml of water was added. The drug concentrations were determined spectrophotometrically at 254 nm (log ε 4.49) for more diluted solutions and at 305 nm (log ε 3.63) for more concentrated solutions, or when additives interfered with the measurement at 254 nm.

Determination of the Apparent Diffusion Constant—Equation 1, derived by Garrett and Chemburkar (1) for a steady-state diffusion, was used to obtain the apparent diffusion constant *D*:

$$D = \frac{C_1 X V}{t C_2 S} \qquad \text{(Eq. 1)}$$

where C_1 is the concentration of diffusate in the desorbing solution, X is the thickness of the membrane, S is the available area of membrane, V is the volume of the desorbing solution, and C_2 is the concentration of the diffusate.

Diffusion Studies with Increasing Phosphate Concentration—Solutions at pH 5.8 with 2×10^{-3} *M* chlorpromazine and increasing phosphate concentrations ($1–70 \times 10^{-3}$ *M*) were prepared. The steady-state diffusion of chlorpromazine through the membrane was studied at 37° (Fig. 1).

Diffusion Studies of Chlorpromazine at Various pH—The solutions of chlorpromazine were kept at a concentration of 2×10^{-3} *M* when the pH range was within 3.5–6.0 and at a concentration of 5×10^{-4} *M* when the pH was >6.0. The required pH was obtained with hydrochloric acid or sodium hydroxide. The steady-state diffusion of chlorpromazine from these solutions through a 5-ml thick dimethyl polysiloxane membrane into 22 ml of 0.01 *N* HCl was studied at 37°. The pH values of diffusing solutions were noted before and after the experiments and were unchanged. The samples were removed at intervals of 20 min over a 2-hr period (Fig. 2, curve a).

In another series of tests, the effect of phosphate on the steady-state diffusion of chlorpromazine was studied at different pH. Diffusing 2 ×

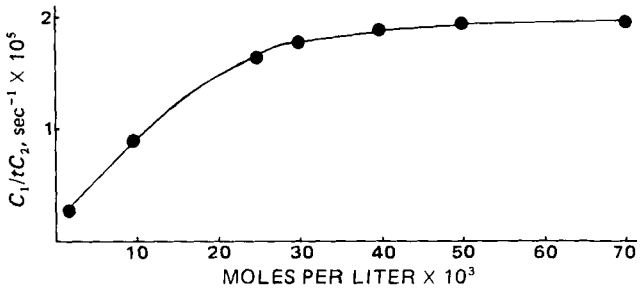

Figure 1—*Specific concentration rate increase of chlorpromazine (in the presence of phosphate) through a membrane into 0.01* M *HCl solution* versus *phosphate concentration.*

[1] Silastic, Dow Corning.
[2] Rhone-Poulenc.
[3] Merck.
[4] Fluka.
[5] Orion model 701/A.
[6] Perkin-Elmer EPS-3T.
[7] Dognon Abribat.

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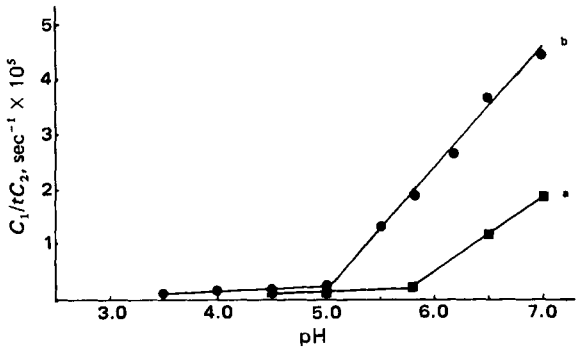

Figure 2—*Specific concentration rate of chlorpromazine through a membrane into 0.01 M HCl desorbing solution at 37° versus the pH of the diffusing drug solution. Key: a, without phosphate; and b, with a phosphate concentration 25 times the molarity of chlorpromazine.*

10^{-3} *M* chlorpromazine solutions in the pH range 3.5–6.0 and 5×10^{-4} *M* at pH >6.0, each containing phosphate concentration 25 times the molarity of the drug, were prepared (Fig. 2, curve b) and examined.

Diffusion Studies of Chlorpromazine in the Presence of Carboxylates—Experiments relative to the permeation of chlorpromazine in the presence of carboxylates were carried out at pH 5.8 and 37°. The diffusing solutions contained $1–3 \times 10^{-3}$ *M* of chlorpromazine and a carboxylate concentration 25 times the molarity of the drug.

RESULTS AND DISCUSSION

Effect of Phosphate on the Diffusion Rate of Chlorpromazine—The rate of steady-state diffusion through dimethyl polysiloxane membranes of an almost constant concentration (C_2) of chlorpromazine increases with the increasing phosphate concentration (Fig. 1). The specific diffusion rate of chlorpromazine is invariant at an acid concentration 21 times higher than the molarity of the drug. The permeation increase could be ascribed to ion-pair formation between chlorpromazine and the phosphate anions. An ion-pair interaction between dialkylaminoalkylphenothiazines and pteridines was also verified (10). The same interaction was proved between phosphate and antidepressive tricyclic drugs (11).

These results show that it is impossible to study the influence of a carboxylic anion on the permeability of chlorpromazine in the presence of other anions (such as phosphate). Therefore, all diffusion experiments were carried out without buffering the solutions, but using only hydrochloric acid or sodium hydroxide to adjust the pH.

Effect of pH on the Diffusion Rates—The diffusion of chlorpromazine varies according to pH. As shown by Fig. 2, curve a, chlorpromazine diffusion is very low at low pH values, because with a pKa of 9.3 (12), it is almost completely ionized. As pH increases, the chlorpromazine diffusion rate also increases, owing to the formation of undissociated hydrochloride. Figure 2, curve b, shows that the specific diffusion rates of chlorpromazine, in the presence of a constant excess of phosphate in the pH 5.5–7.0 range, are higher than those obtained using only the drug as a consequence of the formation of an ion-pair.

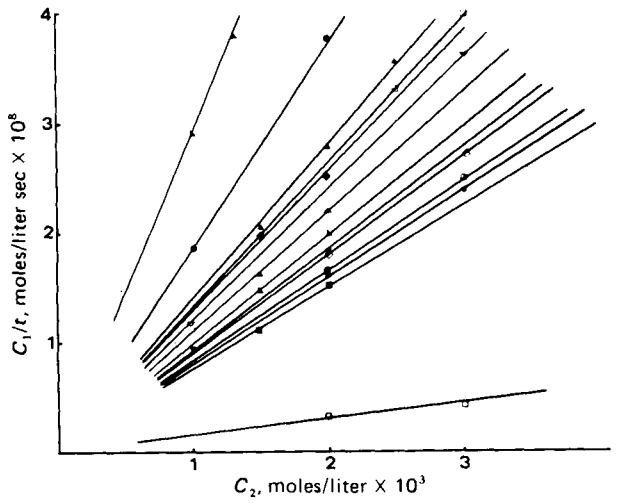

Figure 3—*Rates (C_1/t) of concentration increase in 22 ml of 0.01 N HCl desorbing solution versus chlorpromazine solution concentrations in the presence of carboxylic acid at a concentration 25 times the molarity of the drug. Key: □, none; ■, aconitic; ★, adipic; △, malic; ○, isocitric; ◇, malonic; ▼, fumaric; ◺, tartaric; ▲, glutaric; ▽, citric; ◆, α-ketoglutaric; □, acetic; ◢, succinic; ●, phosphoric; and ◭, oxaloacetic acids.*

Table I—Apparent Diffusion Constants of Chlorpromazine through a Dimethyl Polysiloxane Membrane in the Presence of Various Acids at a Concentration 25 Times Higher than That of the Drug at 37°

Acid	C_1/tC_2[a] ×10^5, sec^{-1}	Apparent Diffusion Constant $D \times 10^{10}$, liter cm/sec
None	0.15	1.31
Aconitic	0.74	6.48
Adipic	0.80	7.01
Malic	0.83	7.25
Isocitric	0.84	7.35
Malonic	0.92	8.05
Fumaric	0.93	8.14
Tartaric	1.00	8.75
Glutaric	1.10	9.62
Citric	1.13	9.89
α-Ketoglutaric	1.28	11.21
Acetic	1.34	11.73
Succinic	1.38	12.08
Phosphoric	1.91	16.72
Oxaloacetic	2.91	25.42

[a] Specific rate of concentration increase of desorbing solution.

Effect of Carboxylates on Rate Diffusion of Chlorpromazine—Since the rate of steady-state diffusion of an almost constant concentration (C_2) of chlorpromazine increased in the presence of oxaloacetic acid, the permeation rate of chlorpromazine was determined in the presence of other carboxylic acids. The linear dependence of the diffusion rates for the same membrane area, thickness, and volume of the diffusing solution, showed that rate diffusion through the membrane was directly proportional to the concentration of the diffusing drug. The lag times are negligible.

Rates of chlorpromazine concentration increase in 22 ml of 0.01 *N* HCl desorbing solution, C_1/t, are shown in Fig. 3 as a function of the respective concentration C_2 of the drug in the diffusing solution.

Apparent diffusion constants (D) were computed from the specific rates and are summarized in Table I. From D values it can be observed that the effect of permeation varies among the carboxylic acids. The apparent diffusion constant (D) depended on the acid concentrations up to a maximum of 21 times that of the drug and afterward remained invariant for all examined solutions where the acid concentration was 25 times higher than that of the drug.

The difference in the drug permeability in the presence of acids could also be explained by an ion-pair formation, probably dependent on D for its stability. To understand the behavior of the acids, the apparent diffusion constants of chlorpromazine in the presence of bicarboxylic acids were plotted against the chlorpromazine critical micelle concentration (CMC), determined in the presence of a fixed concentration of the same

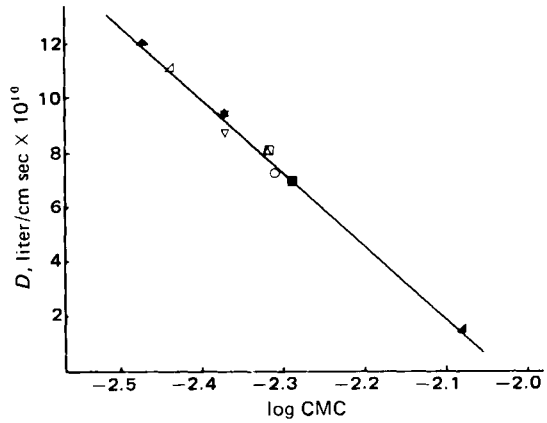

Figure 4—*Apparent diffusion constant D of chlorpromazine into 0.01 N HCl desorbing solution in the presence of bicarboxylic acids versus log CMC of chlorpromazine determined in 50 × 10^{-3} M solution of the same acid (μ = 0.146 M). Key: ◢, sodium chloride; ■, adipic; ○, malic; □, malonic; △, fumaric; ▽, tartaric; ★, glutaric; ◺, oxoglutaric; and ▲, succinic acids.*

acid. The CMC describes the lipophilicity of amphiphilic substances (13); the CMC value of chlorpromazine in the presence of acid could be an indirect measure of the lipophilicity of the ion-pair. Figure 4 shows an almost linear correlation between D and the CMC for the different bicarboxylic acids.

Chlorpromazine was selected for this study since it is representative of many tricyclic drugs and its behavior toward carboxylic acids may be important in GI absorption. Ion-pair formation between chlorpromazine and dietary carboxylic acids (such as citric, tartaric, and acetic acid) would give a favorable absorption in GI lumen. Furthermore, these findings could be useful for the development of dosage forms. It was shown (14) that certain drugs can be absorbed in their undissociated state, either directly or by ion-pair or complex formation. The behavior of chlorpromazine in humans might also be ascribed to the interactions of the drug with some of the physiological acids studied.

REFERENCES

(1) E. R. Garrett and P. B. Chemburkar, *J. Pharm. Sci.*, **57**, 949 (1968).

(2) M. Nakano and N. K. Patel, *ibid.*, **59**, 77 (1970).

(3) G. L. Flynn and J. Roseman, *ibid.*, **60**, 1788 (1971).

(4) R. H. Reuning and G. J. Levy, *ibid.*, **58**, 79 (1969).

(5) T. R. Bates, J. Galownia, and W. H. Johns, *Chem. Pharm. Bull.*, **18**, 656 (1970).

(6) G. Levy and E. J. Mroszczak, *J. Pharm. Sci.*, **57**, 235 (1968).

(7) T. Higuchi, A. Michaelis, and A. Hurwitz, *Anal. Chem.*, **39**, 974 (1967).

(8) M. Nakano, *J. Pharm. Sci.*, **60**, 571 (1971).

(9) K. Juni, M. Nakano, and T. Arita, *Chem. Pharm. Bull.*, **25**, 2807 (1977).

(10) M. R. Gasco, M. E. Carlotti, and M. Trotta, *Pharm. Acta Helv.*, **56**, 55 (1981).

(11) A. Tilly, *Acta Pharm. Suec.*, **12**, 89 (1975).

(12) A. L. Green, *J. Pharm. Pharmacol.*, **19**, 89 (1966).

(13) E. Tomlinson, S. S. Davis, and G. I. Mukhayer, in "Solution Chemistry of Surfactants," vol. 2, Mittal, Ed. 1980, pp. 889.

(14) M. J. Cho, and R. Schnabel, *J. Pharm. Sci.*, **64**, 1894 (1975).

Effect of Albumin Conformation on the Binding of Phenylbutazone and Oxyphenbutazone to Human Serum Albumin

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Abstract □ The binding of phenylbutazone (I) and oxyphenbutazone (II) to human serum albumin over pH 6.9–9.3 was studied by difference spectrophotometry and equilibrium dialysis. At each pH tested, there was higher binding affinity of I to human serum albumin than II. Equilibrium dialysis showed that over the pH 7–8.2 range both agents had a single high-affinity site and several sites of lower affinity, with the highest binding constant and number of binding sites at pH 7.4 for both I and II. Both techniques showed that the affinity of both drugs to albumin was higher for the neutral form than for the basic form and this transition occurred in both cases around the neutral region (7–7.4). Both the ionized and unionized forms of I and II participated in the binding. In the neutral region, magnesium ion increased the affinity of both drugs to albumin while chloride ion decreased it slightly.

Keyphrases □ Phenylbutazone—effect of albumin conformation on binding to human serum albumin □ Oxyphenbutazone—effect of albumin conformation on binding to human serum albumin □ Albumin, human serum—effect of conformation on binding of phenylbutazone and oxyphenbutazone □ Binding—of phenylbutazone and oxyphenbutazone, effect of albumin conformation, human serum albumin

Drug–protein binding studies are important for prediction of drug dynamics in the body (1). The affinity of such interaction can possibly be used to correlate therapeutic and toxicological effects, as well as drug distribution and excretion. Most *in vitro* drug–protein interaction studies are conducted in isotonic pH 7.4 buffer. Data reduction in terms of binding constants assumes that for a partially ionized drug each species is bound with equivalent affinity (2).

It was reported (3) that conformational changes occur in serum albumin over pH 6–9. Zurawski and Foster (4) established that two conformational states exist in bovine serum albumin over this pH region. They called the form at neutral pH (pH 6–7) the "N" form and the form at higher pH (around pH 9) the "B" (base) form. Thus, the conformational change that occurs is the N to B or B to N transition. It was recently shown (5–7) that the same conformational change occurs in human serum albumin over pH 6–9. The N to B transition is seemingly dependent on pH but may also occur to some extent with ionic strength of the buffer and buffer ion composition. Calcium ion and chloride ion affect this transition (5–10) as well as the binding of drug to the protein.

BACKGROUND

While the macromolecule conformation changes with varying pH, ionization of the drug may also occur over such a pH range. A method was presented recently (2) to distinguish which species of a partially ionized acidic or basic drug bind to the protein and to enable determination of the binding site constant (3) for the interaction. In addition, this method aids in the detection of significant changes in the protein binding site that may result from pH perturbation. Since the blood pH in patients may vary between 6.8 and 7.8 (11), significant changes in drug binding to albumin or other plasma proteins could take place with a change in blood or local change in organ (*e.g.*, liver) pH. This pH range could not be predicted in any one individual; at best these pH differences usually would be only a few tenths of a pH unit (*i.e.*, respiratory acidosis or alkalosis).

The present study used equilibrium dialysis and UV difference spectroscopy to investigate the effect of albumin conformational state on the binding of phenylbutazone and oxyphenbutazone to human serum albumin at various pH values.

EXPERIMENTAL

Materials—The human serum albumin used was previously investigated for purity[1] (12). Phenylbutazone (I) and its metabolite oxyphen-

[1] Armour Pharmaceutical Co., Kankakee, Ill.

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