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Permeation of Drugs through Chitosan Membranes[1,2]

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The permeation of several drugs such as promethazine hydrochloride, chlorpromazine hydrochloride, diethazine hydrochloride, trifluprom azine hydrochloride, flufenamic acid, ketoprofen, indomethacin, and tolbutamide through a chitosan membrane was investigated as part of a series of studies on pharmaceutical applications of chitin and chitosan.

The membrane constant of the chitosan membrane was found to be 0.0443, which is close to the reported value for cellulose membrane. The permeability decreased with increase in the molecular volume of the drugs. The activation energy of the permeation was found to be close to that of diffusion of usual organic drugs in water. Greater permeabilities were observed for acidic drugs than basic drugs. The observed effect of pH on the permeation of drugs through the chitosan membranes was considered to be attributable to the cationic state of the chitosan membrane. These results suggested that the permeation of drugs through the chitosan membrane is controlled mainly by diffusion through pores, especially in the case of drugs of smaller molecular volume, and depends upon the cationic state of the chitosan membrane.

Keywords——chitosan membrane; permeation membrane constant; permeability constant; diffusion constant; charge state of membrane; diffusion through pore

Chitosan is a polyaminosaccharide and can be obtained by alkaline deacetylation of chitin, which is a wide-spread naturally-occurring, structural material. It occurs as a principal constituent of the protective cuticles of crustacea and insects, and also in the cell walls of some fungi and microorganisms. Although chitin is insoluble to water and usual organic solvents, chitosan is soluble in dilute acidic solution and a chitosan membrane can easily be prepared.[3]

Recently, chitosan has been used in biomedical fields because of its favorable characteristics, such as good biocompatibility, and was reported to be useful for pharmaceutical preparations.[3,4] Few studies have been done on the permeation of drugs through chitosan membranes except for some work with urea, uric acid[5] and vitamin B_{12}[6] to evaluate chitosan for use as a dialysis membrane. It is worthwhile to evaluate chitosan membranes with a view to finding a possible utility for the control of drug release.[4a,c] Therefore, the permeation of several drugs through chitosan membranes was investigated in the present work.

Experimental

Materials——Chitosan for fine chemical use (degree of deacetylation, 92.7% as calculated from amino group content) was purchased from Kyowa Oil and Fat Co., Ltd. Visking tubing No. 101 was also obtained commercially. Promethazine hydrochloride, chlorpromazine hydrochloride and diethazine hydrochloride were supplied by Yoshitomi Pharmaceutical Co., Ltd. Trifluprom azine hydrochloride was supplied by Nippon Squibb Co., Ltd. Flufenamic acid was supplied by Sankyo Co., Ltd. Ketoprofen was supplied by Sumitomo Chemical Co., Ltd. Tolbutamide was supplied by Hoechst Japan Co., Ltd.

Preparation of Chitosan Membranes——Colorless, transparent chitosan membranes were prepared according to the modified method reported by Yaku *et al.*[7] Procedures for the preparation of chitosan membranes are shown in Fig. 1.

Quantitative Determination of Sodium Chloride——The concentration of sodium chloride was determined with a Corning ion meter model 130. Experiments were carried out in triplicate.

Quantitative Determination of Drugs——The concentration of drugs were determined by the ultraviolet absorption method, using a Hitachi 124 spectrophotometer: promethazine at 253 nm; chlorpromazine at 254 nm; diethazine at 249 nm; trifluprom azine at 257 nm; flufenamic acid at 288 nm; ketoprofen at 260 nm; indomethacin at 265 nm; tolbutamide at 226 nm. All experiments were carried out in triplicate.

chitosan (≃325 mesh) 10 g+1.5% acetic acid solution 300 ml

↓ kept for 1 d

acetic acid solution of chitosan

↓ i) 10 ml of acetic acid solution of chitosan was poured into a frame on flat glass plate
ii) solidified on drying at room temperature for 3 d

chitosan acetate membrane

↓ i) soaked in 10% NaOH solution for 3 h
ii) washed with a large amount of water
iii) kept at 80°C for 1 h

chitosan membrane

Fig. 1. Preparation of Chitosan Membrane

Procedures and Apparatus for Permeation through Membranes——Permeation through membranes was carried out according to the usual equilibrium dialysis method in a red-brown glass cell as described in the previous papers.[8] One compartment (A) contained 10^{-3} M of a drug in 1/30 M phosphate buffer solution (unless otherwise stated), while the other (B) contained the same buffer solution only. The assembled cell was shaken mechanically at 30°C (unless otherwise stated) in a Taiyo M-1 incubator. Three ml of sample solution was withdrawn at appropriate intervals and immediately replaced with an equal volume of the buffer solution. It was confirmed that the drugs were satisfactorily stable under these conditions.

Measurement of the Membrane Thickness——Immediately after the final sampling, the thickness of the membrane at 12 different points[9] was measured with a Mitsutoyo 101—103 micrometer, and the mean was obtained as 0.010 and 0.0086 cm for the chitosan and the cellulose membranes, respectively, with a very small deviation.

Results and Discussion

Membrane Constant of Chitosan Membranes

The permeation of a drug through a membrane can be expressed as follows:[8a]

$$\log\{(C_0-2C)/C_0\} = -(2P\cdot S/2.303\cdot L\cdot V)\cdot t \quad (1)$$

$$P = f\cdot D \quad (2)$$

where C is the concentration in compartment B at time t, C_0 the initial concentration in compartment A, P the permeability constant, L the thickness of the membrane, f the membrane constant, D the diffusion constant, V the volume of solution in the compartments, and S the effective surface area of the membrane.

The permeability constant, P, is thought not to vary with the initial concentration, C_0, in equation (1), but practically, P is known to be influenced by the concentration of solute molecules.[10] In this study, the values of P were obtained with $C_0=10^{-2}$ M for sodium chloride, $C_0=10^{-3}$ M for phenothiazines and tolbutamide, and $C_0=0.5\times10^{-3}$ M for non-steroidal anti-inflammatory drugs. The membrane constant of the cellulose membrane used was 0.0562.[8a] It was confirmed that a linear relation according to equation (1) was obtained up to 24 h for chlorpromazine hydrochloride permeating through membranes.

Assuming that the permeation of a drug through a chitosan membrane proceeds on the basis of diffusion through pores, and also that sodium chloride has no special interaction with the chitosan membrane, the membrane constant of the chitosan membrane was calculated from the permeability constant, P, obtained for sodium chloride.[11] It was confirmed preliminarily that sodium chloride was not adsorbed by chitosan powder.

The values of permeability of sodium chloride through the chitosan membrane at 30°C in Table I were calculated from the data up to 6 h. The D value of Table I was obtained by

proportional calculation as described elsewhere.[12] The membrane constant of the chitosan membrane was found to be 0.0443, which is close to the value for cellulose membrane reported by Nakagaki *et al.*[11,13]

TABLE I. Membrane Constant of Chitosan Membrane Evaluated from the Diffusion Constant of NaCl

Temperature (°C)	30
$C_0 \times 10^2$ (M)	1.00
L (cm)	0.0101
$P \times 10^7$ (cm²/s)	8.38
$D \times 10^6$ (cm²/s)	18.9
f	0.0443

Permeability of Drugs

The values of permeability of drugs through the chitosan membrane at 30°C shown in Table II were calculated from the data up to 6 h. The values of permeability of drugs through the cellulose membrane are also shown in Table II. The values for phenothiazines and tolbutamide through the cellulose membrane were taken from previous papers.[8a,10] Acidic drugs such as non-steroidal antiinflammatory drugs and tolbutamide showed larger permeabilities through the chitosan membrane than basic drugs such as phenothiazines. It was also found that the D values of acidic drugs with the chitosan membrane were 2 or 3 times larger than those through the cellulose membrane, while the D values of basic drugs through the chitosan membrane were a little smaller than those through the cellulose membrane. These results might be attributed to the charge state of the chitosan membranes and will be discussed later.

TABLE II. Permeability Constants and Diffusion Constants of Drugs through Chitosan and Cellulose Membranes at 30°C[a]

Compound	Mv (ml/mol)	pH	$C_0 \times 10^3$ (M)	$P \times 10^{7}$ [b] (cm²/s) Chitosan membrane[c]	$D \times 10^{6}$ [b] (cm²/s) Chitosan membrane[c]	$P \times 10^7$ (cm²/s) Cellulose membrane[d]	$D \times 10^6$ (cm²/s) Cellulose membrane[d]
Promethazine	335.8	6.0	1.00	1.51±0.02	3.42±0.05	2.36	4.20
Chlorpromazine	353.7	6.0	1.00	1.37±0.09	3.10±0.20	2.19	3.90
Diethazine	356.6	6.0	1.00	1.38±0.04	3.11±0.10	1.98	3.52
Triflupromazine	373.0	6.0	1.00	1.28±0.06	2.88±0.14	2.03	3.61
Flufenamic acid	271.7	7.0	0.50	4.63±0.11	10.45±0.24	1.82	3.24
Ketoprofen	285.4	7.0	0.50	4.04±0.24	9.12±0.52	1.69	3.01
Indomethacin	370.6	7.0	0.50	3.10±0.27	6.99±0.50	1.45	2.60
Tolbutamide	293.6	7.0	1.00	3.48±0.19	7.84±0.41	2.09	3.71

a) $V=200$ ml, $A=\pi \times 2.15^2$ (cm²).
b) Mean of 3 determinations ± S.D.
c) $f=0.0443$, $L=0.010\pm0.001$ cm.
d) $f=0.0562$, $L=0.0086$ cm.

The concept of molecular volume, Mv, was presented by Kopp,[14] and Arnold reported that Mv is closely related to the diffusion of the solute in a solvent.[15] Thus, the molecular volume Mv was calculated according to the method described,[16] and the relation between the diffusion constant D, and molecular volume at boiling point, Mv, is shown in Fig. 2. The deviation for indomethacin shown in Fig. 2 cannot be explained clearly, but it could be said that the diffusion constant decreased with increase in molecular volume. This tendency was especially clear for drugs of small molecular volume.

The temperature dependence of permeation of chlorpromazine, ketoprofen and tolbutamide through the chitosan membrane is shown in Table III and Fig. 3. The values of activ-

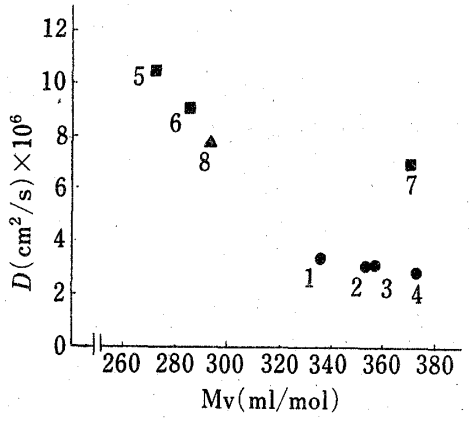

Fig. 2. Relation between Diffusion Constant D at 30°C and Molecular Volume Mv

1: promethazine; 2: chlorpromazine; 3: diethazine; 4: triflupromazine; 5: flufenamic acid; 6: ketoprofen; 7: indomethacin; 8: tolbutamide; ●: phenothizines; ■: non-steroidal antiinflammatory drugs; ▲: sulfonylurea.

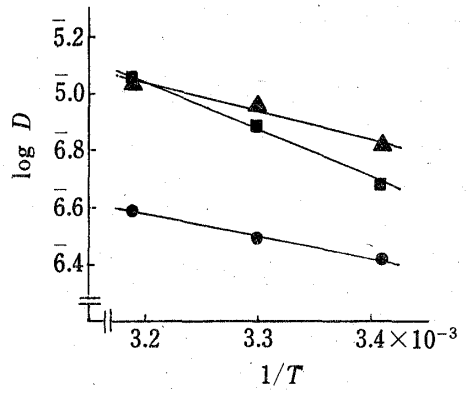

Fig. 3. Temperature Dependence of Diffusion of Drugs through the Chitosan Membrane

●: chlorpromazine; ▲: ketoprofen; ■: tolbutamide

ation energy, E, obtained from Fig. 3, are also shown in Table III, and these values are close to those reported for diffusion-controlled dissolution.[17] In addition, the values of activation energy of chlorpromazine and tolbutamide through the chitosan membrane and those through the cellulose membrane[8a,10] were of the same order.

Table III. Temperature Dependence of Permeability Constant and Diffusion Constant, and Activation Energy of Permeation of Drugs through Chitosan Membrane[a]

Compound	pH	Temperature (°C)	$C_0\times10^3$ (M)	$P\times10^{7}$ [b] (cm^2/s)	$D\times10^{6}$ [b] (cm^2/s)	E (kcal/mol)
Chlorpromazine	6.0	20	1.00	1.16±0.08	2.62±0.18	3.26
		30	1.00	1.37±0.09	3.10±0.20	
		40	1.00	1.72±0.14	3.88±0.30	
Ketoprofen	7.0	20	0.50	2.92±0.16	6.59±0.35	4.37
		30	0.50	4.04±0.24	9.12±0.52	
		40	0.50	4.83±0.25	10.90±0.54	
Tolbutamide	7.0	20	1.00	2.10±0.18	4.74±0.38	7.90
		30	1.00	3.48±0.19	7.84±0.41	
		40	1.00	5.01±0.22	11.31±0.50	

a) $f=0.0443$, $V=200$ ml, $A=\pi\times2.15^2$ (cm^2), $L=0.010\pm0.001$ cm.
b) Mean of 3 determinations±S.D.

The effect of the charge state of the chitosan membrane on the permeation of chlorpromazine, ketoprofen and tolubtamide through the chitosan membrane is summarized in Table IV with the values of % ionized drug and chitosan at each pH calculated from the pK_a values. The pK_a value of chitosan is reported to be 6.3.[18] Concerning the effect of pH on the permeation of drug through an ionized membrane, Nakano *et al.*[19] reported for collagen membrane that drugs in molecular form permeated faster than those in ionic form. In the pH range of this experiment, chlorpromazine exists in a cationic state, and ketoprofen and tolbutamide exist almost wholly in an anionic state, as shown in Table IV. Data for drugs in molecular form were not obtained. Therefore a significant change of the charge state was observed only in the chitosan membrane. Comparing the D values with % ionized chitosan values, the less ionized the chitosan was, the smaller were the D values of the anionic drugs ketoprofen and tolbutamide, and the larger were the D values of the cationic drug chlorpromazine.

TABLE IV. pH Dependence of Permeation of Drugs through Chitosan Membrane at 30°C[a]

Compound	pH	$C_0 \times 10^3$ (M)	$P \times 10^{7}$ [b] (cm²/s)	$D \times 10^{6}$ [b] (cm²/s)	% ionized	
					Drug	Chitosan[c]
Chlorpromazine	6.0	1.00	1.37±0.09	3.10±0.20	100.0	66.6
(pK_a=9.3)	7.0	1.00	1.79±0.10	4.04±0.21	99.5	16.6
Ketoprofen	6.0	0.50	4.96±0.20	11.20±0.45	99.2	66.6
(pK_a=3.9)	7.0	0.50	4.04±0.24	9.12±0.52	100.0	16.6
	8.0	0.50	3.30±0.20	7.45±0.45	100.0	2.0
Tolbutamide	6.0	1.00	4.09±0.13	9.23±0.27	79.9	66.6
(pK_a=5.4)	7.0	1.00	3.48±0.19	7.84±0.41	97.5	16.6
	8.4	1.00	2.72±0.10	6.14±0.21	99.8	0.8

a) f=0.0443, V=200 ml, $A=\pi \times 2.15^2$ (cm²), L=0.010±0.001 cm.
b) Mean of 3 determinations±S.D.
c) pK_a=6.3.

In conclusion, the permeation of drugs through the chitosan membrane followed equation (1) and was thought to proceed mainly by diffusion through pores,[8a,11,13] especially for drugs of smaller molecular volume; it also depended upon the charge state of the chitosan membrane, that is, its cationic state. Therefore, greater permeabilities through the chitosan membrane were observed for acidic drugs than basic drugs. Further studies are necessary to clarify the mechanism of permeation, especially the effects of charge state, hydrophobicity and concentration of drugs.

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