Transport of pharmaceuticals through silk fibroin membrane

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The transport of pharmaceuticals through silk fibroin membranes has been investigated. The membrane was prepared from Chinese cocoon. The permeability coefficients of five kinds of pharmaceutical, i.e. 5-fluorouracil (5FU), L-(+)-ascorbic acid (Vc), resorcinol (Res), sodium phenolsulfonate (SPS) and benzyltrimethylammonium chloride (BTAC), were measured in the pH range from 3.0 to 9.0. The silk fibroin membrane was an amphoteric ion-exchange membrane composed of both weak acidic and weak basic groups. Membrane-potential measurements revealed that the isoelectric point of the membrane was about pH 4.5. The positively charged pharmaceutical, BTAC, was excluded from the membrane below the isoelectric point. On the other hand, the negatively charged one, SPS, had a permeability that decreased as the pH increased. The neutral molecule, Res, however, penetrated through the membrane independently of pH value over the measured pH range. In the case of 5FU and Vc, the permeability coefficients decreased steeply above pH 7 and pH 4, respectively, because 5FU and Vc were negatively charged above pH 7 ($pK_a = 8.0$) and pH 4 ($pK_a = 4.25$), respectively.

(Keywords: drug transport; silk fibroin membrane; permeability)

INTRODUCTION

Silk fibroin membranes are produced from silk *Bombyx* cocoon. One of us has already suggested that they are widely applicable to medicine, for instance in contact lenses and as artificial skin[1,2]. The permeabilities of saccharides[3] and amino acids[4] through silk fibroin membranes have already been reported by Sugiura and Yoshimura *et al.*, respectively. We have studied transport phenomena of inorganic electrolytes through silk fibroin membranes by measuring membrane potentials and permeability coefficients above the isoelectric point of this membrane[5,6]. In addition, the fixed charge density and ion mobility in the membrane were estimated by the curve fitting method of the Teorell–Meyer–Sievers (TMS) theory[7,8] to experimental data.

In this study, transport of pharmaceuticals through the membranes was studied as a function of pH. Silk fibroin membrane is an amphoteric ion-exchange membrane composed of both weak acidic and weak basic groups. It is of great interest that the membrane and the penetrant can vary charge density and degree of dissociation, respectively, by pH change in external solution.

EXPERIMENTAL

Materials

Silk fibroin membrane was prepared from Chinese cocoon. The silk thread was degummed with aqueous boiling 0.5% (w/v) Na_2CO_3 solution, and subsequently dissolved in calcium chloride/ethanol/water solution ($CaCl_2/C_2H_5OH/H_2O = 1/2/8$ mole ratio) at 70°C for 2 h. After the solution was filtered, dialysis was continued for 3 days against running ion-exchanged water to remove $CaCl_2$ using a cellulose semipermeable membrane. Thin membranes were cast onto polystyrene Petri dishes and dried at 30°C in air for 48 h. After that the membranes were treated with a 75 vol% aqueous methanol solution for 15 min[9]. The membrane thickness was 0.027 mm.

In order to measure the water content of the membranes at different pH values, the membranes were immersed for 3 days in the corresponding pH (NaOH or HCl) solution. After removal from the solution, the wet membranes were dried at 75°C for 24 h in a vacuum oven. The weights of wet and dry membranes were measured to decide the water content W from the following equation:

$$W(\%) = \frac{W_o - W_d}{W_o} \times 100 \quad (1)$$

where W_o is the weight of wet membrane and W_d is the weight of dry membrane.

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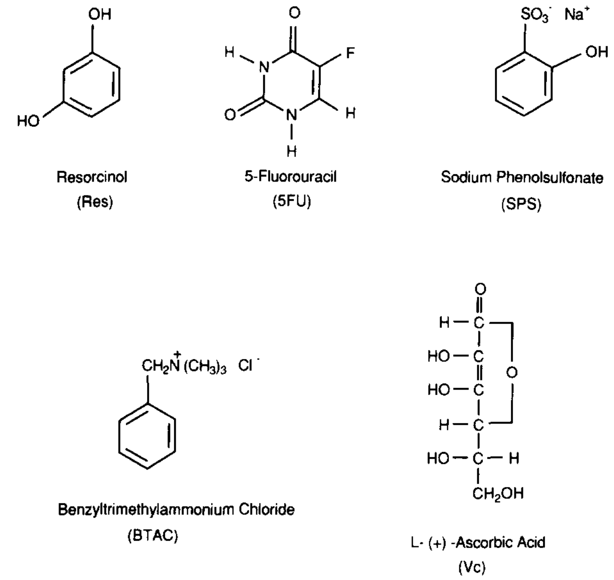

Figure 1 Chemical structures of penetrants

Penetrants

The five kinds of pharmaceutical shown in *Figure 1* were used without further purification. They are: 5-fluorouracil (5FU), an anti-cancer material; vitamin C, L-(+)-ascorbic acid (Vc); resorcinol (Res), which is used as a disinfectant and in dermatology; sodium phenolsulfonate (SPS), an antiseptic; and benzyltrimethylammonium chloride (BTAC), which is used as a disinfectant.

Permeation experiments

Permeation measurements were carried out at 25°C in a glass cell composed of two compartments of equal volume (200 ml). A silk fibroin membrane, whose diameter was 30 mm, was clamped between these compartments. Then 10^{-3} mol l^{-1} of pharmaceutical was added to one compartment and ion-exchanged water to the other. The solutions of both compartments were adjusted to have the same pH value by use of HCl or NaOH, and the pH range was limited from 3 to 9. The solutions of both compartments were vigorously stirred during the permeation experiments.

In order to analyse the concentration C_d of the pharmaceutical transported to the water compartment, a 3.5 ml sample was taken every hour from the low-concentration side of the cell, and replaced with the same quantity of ion-exchanged water. The u.v. absorption of the sampled solution was measured with a Hitachi UV 330 Spectrophotometer. The wavelengths used are listed in *Table 1*. The concentrations C_d were calculated using calibration curves. The concentration C_o of the higher-concentration side of the membrane was regarded as constant (10^{-3} mol l^{-1}) during the permeation experiments. The experiment was continued for 8 h. The permeability coefficient P (cm^2 s^{-1}) was calculated by using the following equation:

$$P = \frac{Vd\Delta C_d}{S(C_o - C_d)\Delta t} \quad (2)$$

where S is the effective area of the membrane, V is the volume of the low-concentration side of the membrane, d is the thickness of the wet membrane, and $\Delta C_d/\Delta t$ is the concentration change of the solution in the low-concentration side of the cell per unit time.

The u.v. spectra of 10^{-4} mol l^{-1} solutions of 5FU were recorded over a pH range of 3–10.

Titration experiments

The dissociation of 5FU was measured using an Automatic Titrator (Mitsubishi Kasei Co., type G-T-05). The titrant was 1 mol l^{-1} NaOH and the titrated solution was 0.01 mol l^{-1} 5FU plus 0.1 mol l^{-1} HCl solution at 25°C.

Membrane-potential experiments

The membrane potential was measured as a function of pH using an aqueous KCl solution. The measurements were carried out at 25°C in a poly(methyl methacrylate) cell composed of two compartments of equal volume (330 ml). A silk fibroin membrane, whose diameter was 20 mm, was clamped between these compartments. The potential was measured using a pair of Ag–AgCl reference electrodes (TOA HS205). The KCl concentration ratio of the low- to high-concentration compartment was adjusted to be 1/100, and the pH was adjusted by adding the same amount of HCl to both sides.

RESULTS AND DISCUSSION

The water content of the membrane is shown in *Figure 2*. This demonstrates a minimum value near pH 6 (about 3.5% lower than at pH 3).

The membrane potential generated between two aqueous KCl solutions, which are separated by a

Table 1 Experimental conditions

		MW	U.v. wavelength (nm)	pH (10^{-3} M)
5FU	$C_4H_3FN_2O_2$	130.08	266	6.0
Vc	$C_6H_8O_6$	176.13	265 250 (pH 3)	3.7
Res	$C_6H_4(OH)_2$	110.11	273	6.4
SPS	$HOC_6H_4SO_3Na$	196.19	230	5.8
BTAC	$C_6H_5CH_2N(CH_3)_3Cl$	185.70	208	6.0

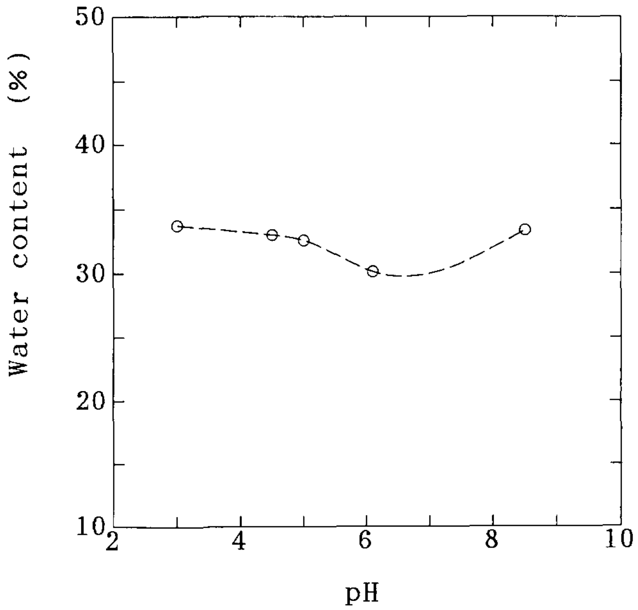

Figure 2 Water content of silk fibroin membrane

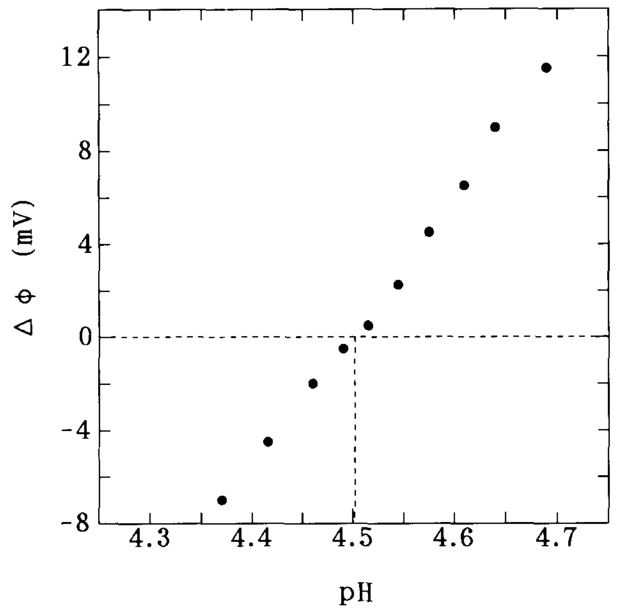

Figure 3 Membrane potential vs. pH in silk fibroin membrane—0.01 M KCl + HCl aqueous solution system

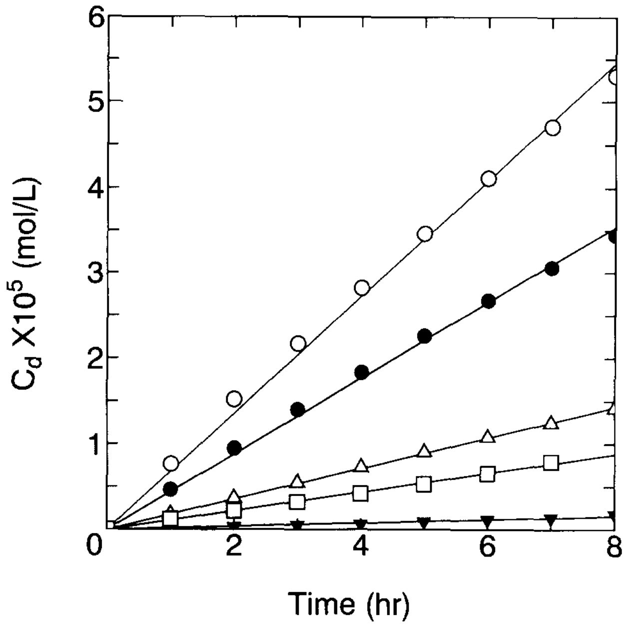

Figure 4 Permeation curves for silk fibroin membrane: (●) 5FU, (○) Res, (△) Vc, (□) BTAC, (▼) SPS

membrane, is affected by the fixed charge group in the membrane. The isoelectric point was determined from the membrane potential as a function of pH of the aqueous KCl solution as shown in *Figure 3*. Since the isoelectric point of this membrane was about pH 4.5, it is considered to operate as an anion exchanger below pH 4.5 and as a cation exchanger above pH 4.5. In our previous paper, the fixed charge density QC_x of the membrane was calculated as $0.015 \, mol \, l^{-1}$ by curve fitting of the TMS theory to the experimental results of the membrane potential, and the ion mobility of Cl^- in the membrane was found to be about 1/100 of that in water[5,6].

Concentration changes for the five permeating pharmaceuticals are shown as a function of time in *Figure 4*. The pH values in these permeation experiments of each pharmaceutical are listed in *Table 1*. Each penetrant has a different permeation rate. Every permeation curve seems to be a straight line. From the slope of the permeation curves, permeability coefficients were calculated using equation (2). *Figure 5* shows the pH dependence of the permeability coefficients.

Around the isoelectric point (about pH 4.5) of the membrane, the permeability coefficient of SPS decreased strikingly with increasing pH. This is due to Donnan exclusion of the SPS anion by negatively charged groups fixed in the membrane. Since BTAC is cationic in aqueous solution, the permeability coefficient of BTAC increased with increasing pH. This increase may be attributable to electrostatic interaction between the cationic penetrants and negatively charged groups fixed in the membrane.

In addition, at the isoelectric point (about pH 4.5) the membrane becomes neutral, and the fixed charge on the membrane has no influence on permeability. Since the molecular size of the SPS anion is nearly the same as that of the BTAC cation, the actual permeability coefficients of SPS and BTAC at the isoelectric point are almost the same.

As would be expected, the neutral molecule (Res) in the measured pH range (dissociation constant of Res is $pK_{a1} = 9.15$ and $pK_{a2} = 11.32$ at 30°C in H_2O)[10] can penetrate through the membrane without being influenced by isoelectric point and pH change.

The permeability coefficient of 5FU did not depend on pH below pH 7, while above pH 7 the permeability coefficient decreased. The isoelectric point did not affect the permeability coefficient of 5FU, but the permeability at pH 9 was reduced by a factor 1/25 of that at pH 6.

The pH titration experiments of 5FU showed that the dissociation constant pK_a was about 8.0 as shown in *Figure 6*. This means that 5FU exists as an anion above pH 7 as shown in *Figure 7*. The u.v. absorption spectra of 5FU, which are shown in *Figure 8*, support this conclusion. The reason for the steep decrease of the permeability coefficient is then explained by the Donnan exclusion of the 5FU anion by negatively charged groups fixed in the membrane.

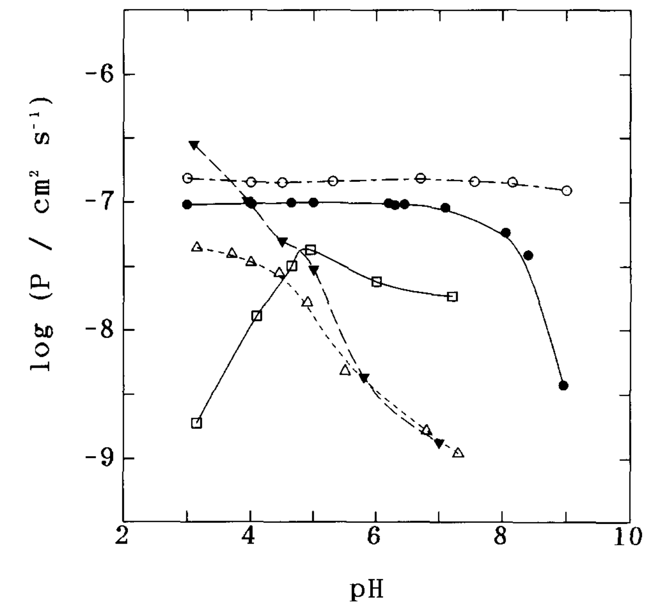

Figure 5 Permeability coefficients *P* of pharmaceuticals vs. pH; the solutions of both compartments were adjusted to have the same pH value by use of HCl or NaOH: (●) 5FU, (○) Res, (△) Vc, (□) BTAC, (▼) SPS

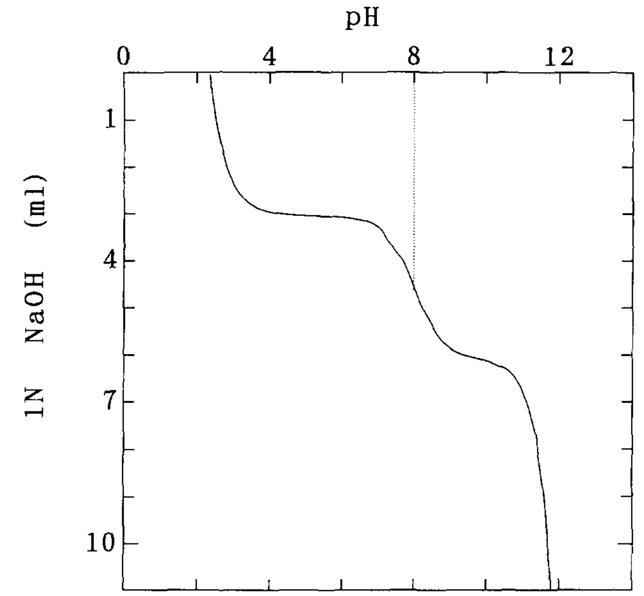

Figure 6 Titration curve of 5FU

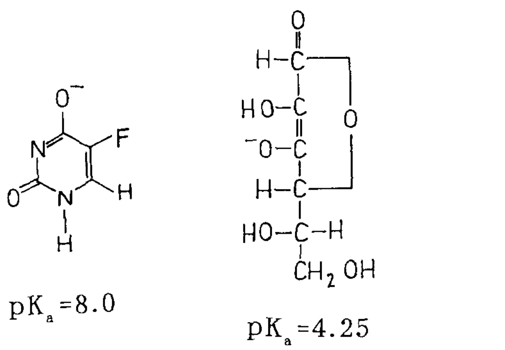

Figure 7 Chemical structures of dissociated forms of 5FU and Vc

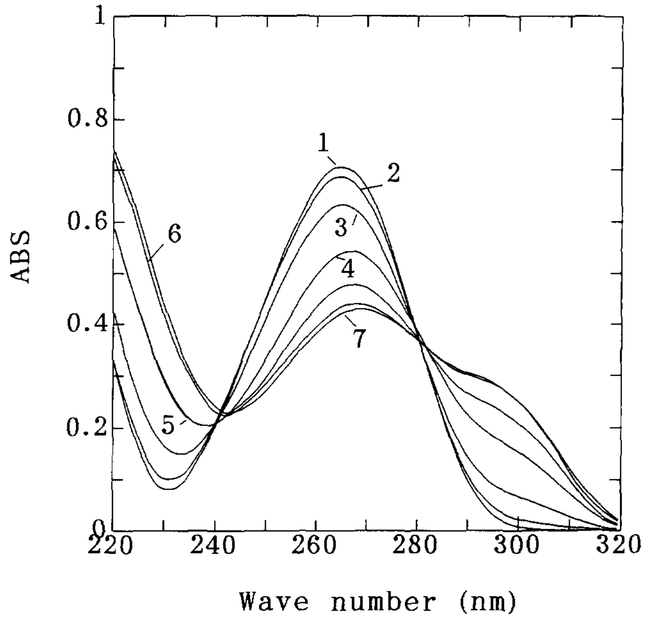

Figure 8 U.v. absorption spectra of 10^{-4} M 5FU solution for different pH values: (1) pH 6.0, (2) pH 7.0, (3) pH 7.5, (4) pH 8.0, (5) pH 8.7, (6) pH 9.5, (7) pH 10.0

The permeability coefficient of vitamin C suddenly decreased above pH 4. Since vitamin C is also known to become an anion ($pK_{a1}=4.25$)[11], as shown in *Figure 7*, the cause of this decrease is similar to that of 5FU.

It is concluded that, owing to the amphoteric ion-exchange property of the silk fibroin membrane, the permeability of silk fibroin membrane to pharmaceuticals can be regulated by changing the pH value of the external solution. It is expected that silk fibroin membrane could be used as the matrix of a drug delivery system with pH-responsive functions.

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