Drug Permeation Behavior in Chitosan Film Prepared on the Metal Plate Loaded with Electric Charge

Hiraku IKEDA,* Kozo TAKAYAMA, and Tsuneji NAGAI

Department of Pharmaceutics, Hoshi University, Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan.
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A novel method to prepare chitosan film using a newly developed apparatus was previously reported. An electric charge supplied on the cast plate during preparation of the chitosan film produced a particular fiber arrangement of chitosan macromolecules.

In this study, the drug permeation behavior was investigated in the film prepared on the metal plate loaded with electric charge (VF) in comparison with the film prepared without supplying electric charge (NVF). As model penetrants, caffeine, indomethacin and lidocaine were employed as neutral, acidic and alkaline compounds, respectively. The drug permeation through VF was significantly suppressed compared with NVF. The permeation-suppressing effect of VF was considered to be dependent on the large volume of the crystal-part in VF and fiber orientation of chitosan macromolecules in VF. The permeability coefficient for indomethacin was unusually large compared with those for caffeine and lidocaine, suggesting that the accumulation of indomethacin molecules on the surface of the chitosan films, due to an electric attraction between indomethacin and cationized chitosan molecules.

Key words chitosan film; fiber orientation; drug permeation behavior; electric charge

In previous studies,[1,2] chitosan films were prepared with (VF) or without (NVF) the electric charge and characterized by X-ray diffractometry, differential scanning calorimetry, a microwave molecular orientation analyzer and an optical birefringence analyzer. These analyses indicated that VF had higher crystallinity than NVF and well-crystallized chitosan fibers were formed during the preparation of VF. Furthermore, chitosan fibers in VF had more preferred orientation compared with those in NVF. An electric charge during preparation of chitosan film might help making a particular fiber arrangement of chitosan macromolecules. In this study, we focused on how the crystallinity of chitosan molecules or fiber arrangement affects the drug permeation through chitosan films such as VF and NVF. As model penetrants, caffeine, indomethacin and lidocaine were employed as neutral, acidic and alkaline compounds, respectively.

Experimental

Materials Chitosan, derived from crab shell chitin with about 95% deacetylation, was generously supplied from Dainichi Seika Color & Chemicals Mfg. Co., Ltd., Tokyo, Japan. The relative viscosity of a 0.2% chitosan aqueous solution (at pH 2.0) was 4.11 at 37 °C as determined with an Ubbelohde viscometer. The average molecular weight of chitosan was determined to be 4.4×10^5, using gel permeation chromatography. Caffeine anhydrous [$C_8H_{10}N_4O_2$, M.W. = 194.19, pK_a = 14] was purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Indomethacin [$C_{19}H_{16}C_1NO_4$, M.W. = 357.8, pK_a = 4.5] was purchased from Sigma Chemical Co., U.S.A. Lidocaine [$C_{14}H_{22}N_2O$, M.W. = 234.34, pK_a = 7.86] was purchased from Nacalai Tesque, Inc., Kyoto, Japan. Other chemicals used were of reagent grade. Stainless steel flat plate was purchased from Queen Rose Co., Ltd. Teflon sheet (thickness was 0.3 mm) was purchased from Nichiasu Co., Ltd., Tokyo, Japan. The direct currency power supplier was purchased from Sunhayato Co., Ltd., Tokyo, Japan.

Preparation of Chitosan Film Chitosan films were prepared by using the apparatus as reported previously.[1] Briefly, a 0.8% chitosan solution was prepared by using 1% acetic acid solution as a solvent. This solution (160 ml) was poured on the metal plate in the apparatus. The upper plate was connected with a (−) electrode and the lower one was connected with a (+) electrode of the battery (15 V) which supplies the direct currency. The solvent was naturally dried up in the draft chamber at room temperature. The direct currency was supplied during the drying. Acetic acid and water remaining in the films were removed completely in vacuum for two days. Chitosan films prepared with and without supplying electric charge are hereafter referred to as VF and NVF, respectively.

Drug Permeation Method Two-chamber diffusion cells (Fig. 1; available diffusion area, 5.3 cm^2; volume of each half-cell, 50 ml) held in a water-bath thermostat at 36 °C were used to determine the permeation of model drugs through the films. Firstly the film was soaked in purified water and swollen at equibrium.[1] This swollen film was mounted between two plastic-plane ring-cartridges to fix the film shape. The cartridge wearing the swollen film was then placed and fixed between openings of the lateral two-chamber diffusion cells. The donor cell was filled with the drug-suspended phosphate buffer solution (pH 7.2), and the receiver cell was filled with the buffer solution (pH 7.2). Both cells were stirred by a magnetic stirrer during the experiment. At appropriate intervals, aliquots (40 μl) were withdrawn from the receiver cell.

Solubility Determination A drug-suspended buffer solution was placed in a water bath (36 °C) for 24 h with stirring using a magnetic stirrer. The sample was then placed in and the upper layer was filtered through a 0.45 μm membrane filter (Gelman Science Japan, Ltd., Tokyo, Japan). The concentrations of drugs in the filtrate were determined by

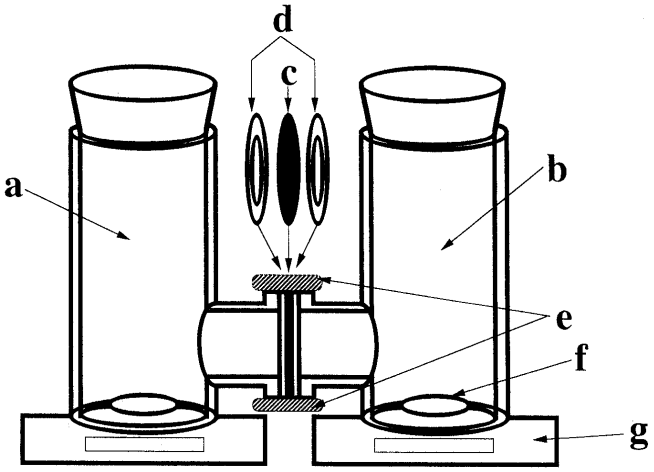

Fig. 1. Schematic Representation of Two-Chamber Diffusion Cells

a, donor chamber; b, receiver chamber; c, chitosan film; d, cartridge for fixation of chitosan film; e, paste to avoid leakage of the buffer solution; f, stirring rod; g, stirrer.

* To whom correspondence should be addressed.

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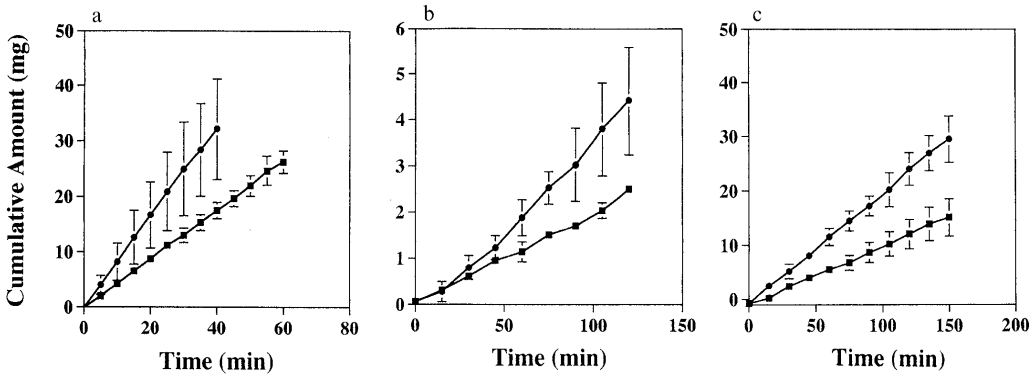

Fig. 2. Permeation Behavior of Caffeine (a), Indomethacin (b) and Lidocaine (c) through VF (■) and NVF (●)
Each point represents the mean ± S.D. for three determinations.

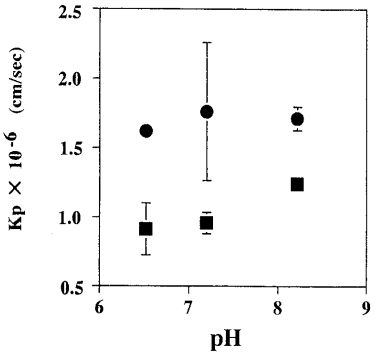

Fig. 3. Effect of pH in the Buffer Solution on the Permeability Coefficient, K_p, of Caffeine through VF (■) and NVF (●)
Each point represents the mean ± S.D. for three determinations.

a UV spectrometer or by an HPLC method.

Analytical Method Caffeine: The sample solution was diluted with the phosphate buffer and the concentration of caffeine was determined using a UV spectrometer (Ubest-30 type, Nihon Bunkou Co., Ltd., Tokyo, Japan) at 273 nm. Indomethacin: The concentration of indomethacin in the sample solution was determined in the same way as caffeine at 319 nm. Lidocaine: The concentration of lidocaine in the sample solution was determined using the HPLC system as follows: Ultraviolet detection (Model SSC UV Detector 3000B, Senshu Kagaku Co., Tokyo, Japan) at 254 nm was employed (sensitivity = 0.08); the column (4.6 mm × 200 mm) was packed with Senshu Pack C8-1201-N (Senshu Kagaku Co., Tokyo, Japan); the column was heated at 37 °C by a Column heater U-620 Type 30 (Sugai Co., Tokyo, Japan); the mobile phase was composed of purified 250 ml of water, 250 ml of methanol and 150 μl of phosphoric acid; the elution was pumped by a LC-10AS liquid chromatograph pump (Shimadzu Corporation, Kyoto, Japan); and the flow rate was 1 ml/min.

Results and Discussion

Figure 2 shows the permeation profiles of caffeine, indomethacin and lidocaine in the buffer solution at pH 7.2. The steady-state flux (J) for these drugs through the membrane were estimated from the slope of the permeation curvatures in Fig. 2, and permeability coefficient (K_p) were calculated from $K_p = J/(C \cdot d)$, where C and d are the drug solubility in the donor solution and the swollen thickness of the film respectively. Since the swelling of these films was achieved at equilibrium within 5 min, the steady-state permeability was thought to be estimated by using the swollen thickness of the films. The results are given in Table 1. For all drugs, both the J and K_p values observed with VF were significantly lower than those with NVF. This suggests that the higher crystallinity and the fiber arrangement of chitosan macromolecules in VF tend

Table 1. Steady-State Flux, J, and Permeability Coefficient, K_p, of Caffeine, Indomethacin and Lidocaine in VF and NVF

	$J \times 10^{-2}$ (μg/s·cm²)		$K_p \times 10^{-3}$ (cm/s)	
	VF	NVF	VF	NVF
Caffeine	139 ± 11	256 ± 72	9.61 ± 0.79	14.9 ± 4.2
Indomethacin	6.13 ± 0.22	11.8 ± 2.9	307 ± 11	499 ± 122
Lidocaine	34.0 ± 8.0	64.1 ± 7.8	9.56 ± 2.26	21.4 ± 2.6

Each datum represents the mean ± S.D. for three determinations.

Table 2. Film Thickness and Film Density of VF and NVF

	VF	NVF
Thickness (μm)	47.5 ± 6.5	56.3 ± 5.6
Swollen thickness (μm)	149 ± 20	174 ± 17
Density[a] × 10^{-3} (g/cm³)	16.8 ± 3.2	23.6 ± 1.1

Each datum represents the mean ± S.D. for three determinations. *a)* The density of film is significantly different between VF and NVF. $p < 0.05$.

to suppress the permeation of drugs through the film. Film thickness was similar between VF and NVF as shown in Table 2. However, the film density of VF was significantly lower than that of NVF. The J and K_p values with VF were nevertheless lower than those with NVF, suggesting that the effect of these differences (Table 2) on the permeation of drugs can be disregarded. No effect of film surface in contact with the donor solution, *i.e.*, the surface side or back side in the films, on the drug permeation was observed in both VF and NVF (data not shown).

The K_p value for indomethacin was unusually large, compared with those for caffeine and lidocaine. This may have been caused by the accumulation or condensation of indomethacin molecules on the surface of the chitosan films, due to an electric attraction between indomethacin and cationized chitosan molecules since indomethacin molecules are negatively ionized in the buffer solution at pH 7.2. Such unusual phenomena were not seen with caffeine and lidocaine because of their neutral and positively ionized molecules in the buffer solution at pH 7.2.

Furthermore, we investigated the effect of pH on the permeation of caffeine through VF and NVF. The electric nature of caffeine as neutral molecules may not be altered by the change of pH in the buffer solution. Thus, the change of pH in the buffer solution only affects on the

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ionized tendency of chitosan molecules in the film. Figure 3 shows the permeability coefficients observed at different pH values in the buffer solution. In both VF and NVF, the K_p values were not greatly changed by the change of pH. Furthermore, the K_p values in VF were significantly smaller than those in NVF irrespective of pH values. These findings suggest that the structure difference between VF and NVF is maintained at the range of pH 6.2—8.2 and the film morphology is not greatly altered by the change of pH in the buffer solution.

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