

Modification of the Physicochemical Properties of Minocycline Hydrochloride Ointment with Cyclodextrins for Optimum Treatment of Bed sore

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Modification to find the best physicochemical properties of minocycline hydrochloride ointment for optimum treatment of bed sore was investigated by coformulating various types of cyclodextrins (CyD) in the ointment base. It was found that the drug release rate from the ointment base was modified according to the preparation method of ointment base and the type of CyD admixed. The physicochemical properties, such as viscosity, elution volume, water absorption of ointment base were also modified by those factors. The mechanism of physicochemical modification with CyD was explained by the structural change of ointment base and the change of surface tension of emulsifying agent solution with the CyD. The stability of ointment was investigated by confirming the reproducibility of drug release rate after storage at ambient and cooled temperature conditions. In conclusion, a fused mixed ointment with β -CyD was found to be preferable for treatment of bed sore, because of the improved drug release rate, lowered viscosity and increased elution volume of the resultant ointment.

Key words minocycline hydrochloride; release rate constant; viscosity; elution volume; cyclodextrin; surface tension

We have been developing ointment bases of minocycline hydrochloride (MH) used in the medical treatment of bed sores and refractory skin ulcers to improve therapeutic effects. In our previous report,²⁾ the water absorption, the elution, the viscosity and the release of drug of various types of ointment base were investigated. We found that by adding purified lanolin (PL) into the hydrophilic ointment (HO), the amount of penetration of the drug into the epidermis was increased with the improvement of drug release rate and water absorption capacity of the base. In the present study, further modification of physicochemical properties of this ointment base for better treatment of bed sore was sought.

In our hospital, bed sore treatment has been conducted using only a lipophilic ointment base admixed with antibiotics, irrespective of disease stage including the infectious period, necrosis and agglutination period, proliferation period of granulation and the formative period of epidermis. The treatment, therefore, has not always been ideal. We wanted to develop a desirable ointment base having a high water absorbing capacity and improved drug releasing property for the incipient stages of bed sores: the infectious period and necrosis and agglutination periods. In the infectious period, an antibiotic substance is applied to lower the degree of infection, where it is necessary to enhance the drug release rate in order to shorten the period of treatment and avoid creating tolerant bacteria.³⁾ Identification of bed sore bacteria made during our treatment showed that MH had the highest efficacy, so MH was chosen for the present study.

It is known that the physicochemical properties of the ointment base can be modified by formulating various kinds of additives.^{4–9)} As an additive candidate cyclodextrins (CyDs) were chosen because of their characteristic to form inclusion complexes with the lipophilic constituents in the ointment base. This interaction was assumed to occur at the interface of the emulsion droplet, modifying the physicochemical property of the ointment base. This event might change the

fluidity, *i.e.* viscosity, of the ointment base for better dispensing. Furthermore, the drug release behavior might be changed according to the modification of the ointment structure. In this study, the effects of the type of CyD derivative used as the additive on the physicochemical properties of the resultant ointment base and their modification mechanism were elucidated to find the best formulation for optimum treatment of bed sore.

Experimental

Materials Powdered MH, (Japan Lederle) for injection, passed through a JIS sifter of 180 μ m opening was used as the model drug. Ointment bases used were HO, (Maruishi Pharm. Co.), and PL (Yoshida Pharm. Co.). Lactated Ringer's injection (Lactec[®]) as a medium for the release tests (Otsuka Pharm. Co.) was used as received. We used α -cyclodextrin (α -CyD, Nakalai Tesque Co.), β -cyclodextrin (β -CyD, Wako Pure Chemicals Ind. Co.), γ -cyclodextrin (γ -CyD, Sanraku Ocean Co.), heptakis (2,6-di-*O*-methyl)- β -cyclodextrin (DM- β -CyD), heptakis (2,3,6-tri-*O*-methyl)- β -cyclodextrin (TM- β -CyD, Toshin Chemical Co.), and dextrin made at Mitsui Chemical Drug Co., as additives.

Preparation of Ointment Containing MH The ointment bases and MH were kneaded and homogenized with an ointment spatula on a ceramic slab. The kneaded mixture was homogeneously mixed with CyD when required and dispersed in a warmed water bath at 80 °C and the system was then cooled to room temperature. The concentrations of MH and CyD (or dextrin) in ointment were 1% and 5%, respectively (Fusing method).

Release Test of MH from Ointment A Franz diffusion cell with membrane installed horizontally was used to evaluate the drug release from the ointment.¹⁰⁾ Seamless cellulose tubing (Visking Co., size 30/32) was used as the membrane after washing for 2 h in distilled water at 80 °C. Five grams of ointment were mounted on the cellulose membrane placed on the receiver cell (the area of the membrane in contact with the ointment, 8.03 cm²; volume of the cell, 45 ml). Then, 50 ml of distilled water or lactated Ringer's injection solution was introduced into the receiver cell and stirred with a magnetic stirrer. The assembled cell was placed in the water bath thermally controlled at 37 °C. Every 30 min for 3 h, 1 ml of the solution was withdrawn and was replaced by 1 ml of the dissolution medium. The MH released in the medium was measured spectrophotometrically at 349 nm (105–40 type, ultraviolet spectrophotometer, Hitachi, Japan). The data of the drug release test are represented by the mean value of triplicate runs.

Observation of Ointment Structure We observed the structure of emulsion fabricated in ointment base with an optical microscope (Olympus,

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BH-2). About 200 mg of ointment base containing various types of CyDs, was spreaded smoothly on a slide glass with 0.3 mm in thickness, and covered with the cover glass for the microscopic observation.

Measurement of Apparent Viscosity The apparent viscosity of ointment was measured by a rheometer (NRM 100-0 model type, Japan Rheology Co.). The radius and edge angle of corn were 6.4 cm and 20', respectively. The maximum shear rate applied during the measurement was 1800 s^{-1} , and the acceleration and deceleration of corn speed was programmed to be constant within 60 s. The shear rate vs. stress curve of all tested ointment bases exhibited almost a straight line as found by Ōishi *et al.*¹¹⁾ The apparent viscosity of the ointment base at room temperature was determined by calculating the ratio of its slope of straight line to that of standard calibration liquid.

Water Absorption and Elution of Ointment Five grams of the ointment sample were applied to the cellulose membrane mounted on the Franz diffusion cell and 50 ml of the medium was introduced into the receiver cell. The system was placed in a water bath thermally controlled at 37 °C. After 3 h, the ointment absorbed water was removed from the membrane and weighed (W_1). The ointment completely desiccated with silica gel was weighed (W_2). The eluted ointment base (E) and absorbed water (A) were calculated from the following equations.

$$E = 5.0 - W_2 \quad (1)$$

$$A = W_1 - W_2 \quad (2)$$

Measurement of Surface Tension The surface tensions of the solution of 0–10% HCO-60 containing 5% β -CyD or DM- β -CyD, and the solution of 0–10% β -CyD or DM- β -CyD containing 10% HCO-60 were measured by a surfactensionmeter (A3 type, Wilhelmy surfactensionmeter, Kyowa Japan).

Stability of Emulsion Physical stability of emulsion of fused ointment base containing 5% β -CyD was examined with the microscope after standing at cooled or room temperature. The drug release behavior from the ointment base fused with β -CyD was investigated. The release rate constant was calculated by fitting the data to the Higuchi model.¹²⁾

Results and Discussion

Drug Release from Ointment Base In our previous study, it was found that a mixed base of HO and PL at the formulating ratio of 7:3 showed a good high drug release rate and sufficient water absorption capacity. Therefore, we selected this formulation as a reference formulation to examine the influence of various CyDs or dextrin on the release behavior of the drug, MH. As shown in Fig. 1, a linear correlation was observed between the amount of released MH from the ointment base with various CyDs and the square root of time plotted according to the Higuchi model.¹²⁾ This finding suggested that the drug release rate from the ointment base was determined by the diffusion of the drug in the matrix base. Table 1 lists the apparent release rate constant (k) of MH from the ointment base calculated from the slope of straight line determined by the least squares method in Fig. 1. The release rate varied with the type of CyD and the preparation of the ointment base. The order of drug release

rate constants of fused ointment base according to the type of CyD formulated was as follows: dextrin < TM- β -CyD < DM- β -CyD < γ -CyD < none < α -CyD < β -CyD. The release of MH was promoted by adding β -CyD ($p < 0.05$), whereas it was retarded by adding γ -CyD ($p < 0.05$), DM- β -CyD ($p < 0.01$), TM- β -CyD ($p < 0.05$) and dextrin ($p < 0.05$). However, the CyDs admixed physically in the ointment base did not affect or reduce the drug release rate. There was a significant difference in the release rate constant of drug between the physical mixture of CyDs and ointment base and the fused mixture. In the fused mixture of ointment base with α -CyD ($p < 0.01$) or β -CyD ($p < 0.01$), a higher release rate than in the physical mixture was found. There was little difference in the cases of γ -CyD, DM- β -CyD and TM- β -CyD.

Physicochemical Properties (Water Absorption, Elution, Apparent Viscosity) The changes in viscosity, elution volume and water absorption of the ointment base by adding the CyDs were investigated to correlate the drug release rate with those changes. The apparent viscosity of the fused mixture of CyDs is listed in Table 1. The effect of addition of CyDs on the apparent viscosity was found and the order was β -CyD < α -CyD < none < γ -CyD < DM- β -CyD < TM- β -CyD < dextrin. The diffusion rate constant of drug in the ointment base was reduced with increasing the apparent viscosity of the ointment base as shown in Fig. 2. By adding DM- β -CyD, TM- β -CyD and dextrin to the ointment base,

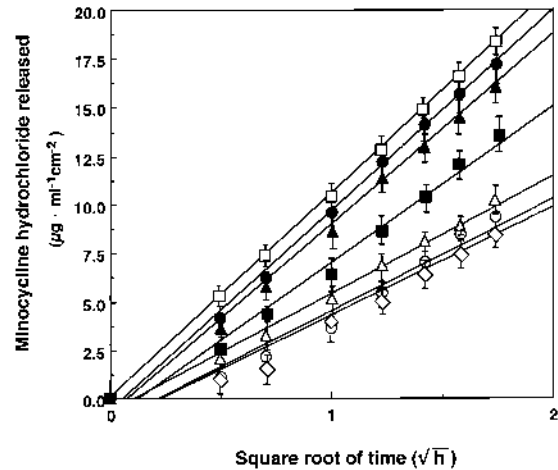


Fig. 1. Higuchi Plots of Release Profiles of Minocycline Hydrochloride from Ointment Containing Various Cyclodextrins Prepared by Fusing Method

●, α -CyD; □, β -CyD; ■, γ -CyD; △, DM- β -CyD; ○, TM- β -CyD; ◇, dextrin; ▲, none. Data represent the mean \pm S.E. of three experiments.

Table 1. Apparent Release Rate Constant and Viscosity of MH Ointment Containing Various Additives

Additives	k^a	k^b	ν^c	Solubility (g/100 ml) ^{13,14)}
None	9.72 \pm 0.53	9.38 \pm 0.49	0.93 \pm 0.11	—
α -CyD	10.16 \pm 0.22	7.12 \pm 0.18 ^{d)}	0.90 \pm 0.18	14.5
β -CyD	10.93 \pm 0.31 ^{e)}	5.22 \pm 0.35 ^{d)}	0.89 \pm 0.11	2.0
γ -CyD	8.05 \pm 0.49 ^{e)}	7.93 \pm 0.39	1.02 \pm 0.22	23.2
DM- β -CyD	6.06 \pm 0.59 ^{f)}	6.19 \pm 0.46	1.09 \pm 0.16	57
TM- β -CyD	5.79 \pm 0.67 ^{e)}	5.86 \pm 0.62	1.18 \pm 0.13	31
Dextrin	5.37 \pm 0.69 ^{e)}	5.31 \pm 0.67	1.66 \pm 0.23	—

a) k , apparent release rate constant (fused mixture) ($\mu\text{g} \cdot \text{ml}^{-1} \text{cm}^{-2} \text{h}^{-1/2}$). b) k , apparent release rate constant (physical mixture) ($\mu\text{g} \cdot \text{ml}^{-1} \text{cm}^{-2} \text{h}^{-1/2}$). c) ν , viscosity (Pa · s) of fused mixture. Composition of ointment base: HO 70: PL 30. Data represent the mean \pm S.E. of three experiments. d) $p < 0.01$ vs. fused mixture. e) $p < 0.05$ vs. none. f) $p < 0.01$ vs. none.

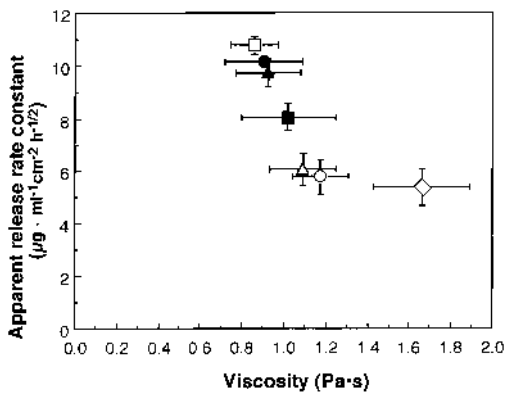


Fig. 2. Relationship between Viscosity and Release Rate
 ●, α -CyD; □, β -CyD; ■, γ -CyD; △, DM- β -CyD; ○, TM- β -CyD; ◇, dextrin; ▲, none. Data represent the mean \pm S.E. of three experiments.

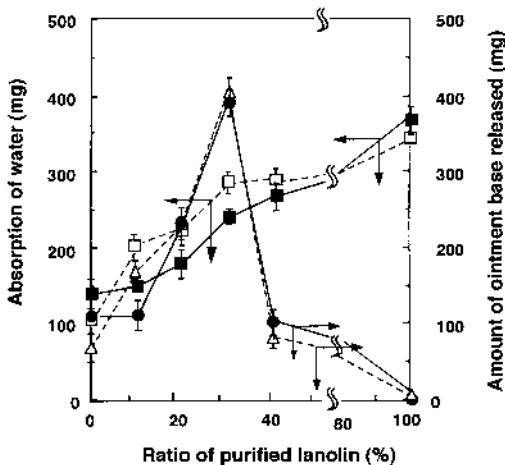


Fig. 3a. Effect of Concentration of Purified Lanolin on Absorption of Water and Amount of Released Ointment Base with DM- β -CyD
 ●, DM- β -CyD; ■, DM- β -CyD (— fused mixture); △, DM- β -CyD; □, DM- β -CyD (--- Physical mixture). Data represent the mean \pm S.E. of three experiments.

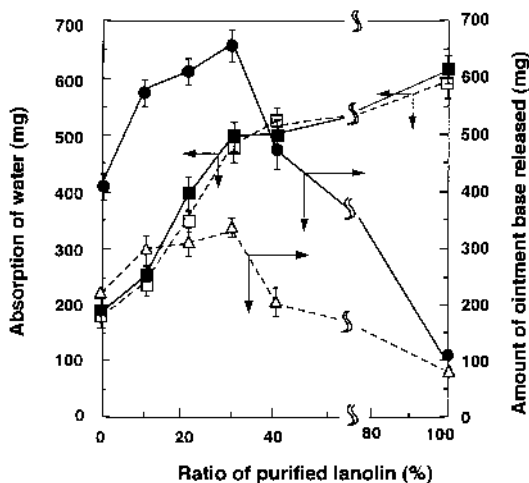


Fig. 3b. Effect of Concentration of Purified Lanolin on Absorption of Water and Amount of Released Ointment Base with β -CyD
 ●, β -CyD; ■, β -CyD (— fused mixture); △, β -CyD; □, β -CyD (--- physical mixture). Data represent the mean \pm S.E. of three experiments.

the drug release was reduced because of decreasing the diffusion coefficient of drug in the matrix base due to their higher viscosity. On the contrary, β -CyD promoted the drug release rate because of reduced viscosity. It was confirmed that the solubility of MH in water at 37 °C was not changed with addition of β -CyD. This finding indicated that there was no possibility of complex formation between the drug and β -CyD in the ointment base to promote the drug release rate. The amounts of elution and water absorption of the bases with fused mixture and physical mixture of β -CyD or DM- β -CyD were investigated by the elution test. With the DM- β -CyD added ointment base, there was no significant difference between the fused mixture and the physical mixture, as shown in Fig. 3a. However, with the β -CyD added ointment base, there was a significant difference of the elution of the base between the fused mixture and the physical mixture, as shown in Fig. 3b. The elution amount of base was maximum when the base contained 30% PL as found in the previous study. The absorption of water in ointment increased gradually with increasing concentration of PL in the ointment base. However, the amount of elution of ointment base decreased when the concentration was greater than 30% PL. Therefore, the water absorbed with PL in the ointment base swelled the matrix structure of the base, promoting the elution of water soluble ointment base from the base as well as the diffusion of drug. The water absorption capacity of ointment base increased with increasing content of purified lanolin without discrimination between the preparation methods. The reduced viscosity and increased elution and water absorption capacities of fused ointment base with 30% PL and β -CyD might be preferable for better treatment of bedsore because it reduces the stimulation of the skin and is easily washable.

Ointment Structure Structural changes in the ointment base prepared by the fusing method modified with CyDs were investigated to clarify the mechanism of the resultant physicochemical modification by using an optical microscopy. As shown in Fig. 4, coarse liquid droplets were found in the fused ointment base with α -CyD or β -CyD promoting the drug release rates, whereas no visible structural changes with DM- β -CyD or TM- β -CyD were found. In a coloring examination with the water soluble coloring Amalance and hydrophobic coloring sudan III, the drops were distinguished as oil droplets. Therefore, the fused mixture with α -CyD and β -CyD liberated oil droplets, and they coalesced. As a result of this change in structure, it was assumed that the viscosity of the ointment was lowered, due to the decreased interfacial area of ointment base. The schematic representation of the presence or absence of oil droplets is summarized in Fig. 5. In the fused mixture base of 70% HO and 30% PL with α -CyD or β -CyD and without CyD, the oil droplets were produced, whereas DM- β -CyD, TM- β -CyD and dextrin did not produce droplets. In the case of the physical mixture of CyDs and dextrin added to the ointment base, oil droplets were not produced in any ointment. These findings suggest that during the fusion process the emulsifying agent, HCO-60, might interact with α -CyD and β -CyD. Therefore, the interfacial tension of the oil droplets in the fused ointment is reduced, leading to liberation of oil droplets from the system. DM- β -CyD and TM- β -CyD were much more hydrophilic as suggested by their

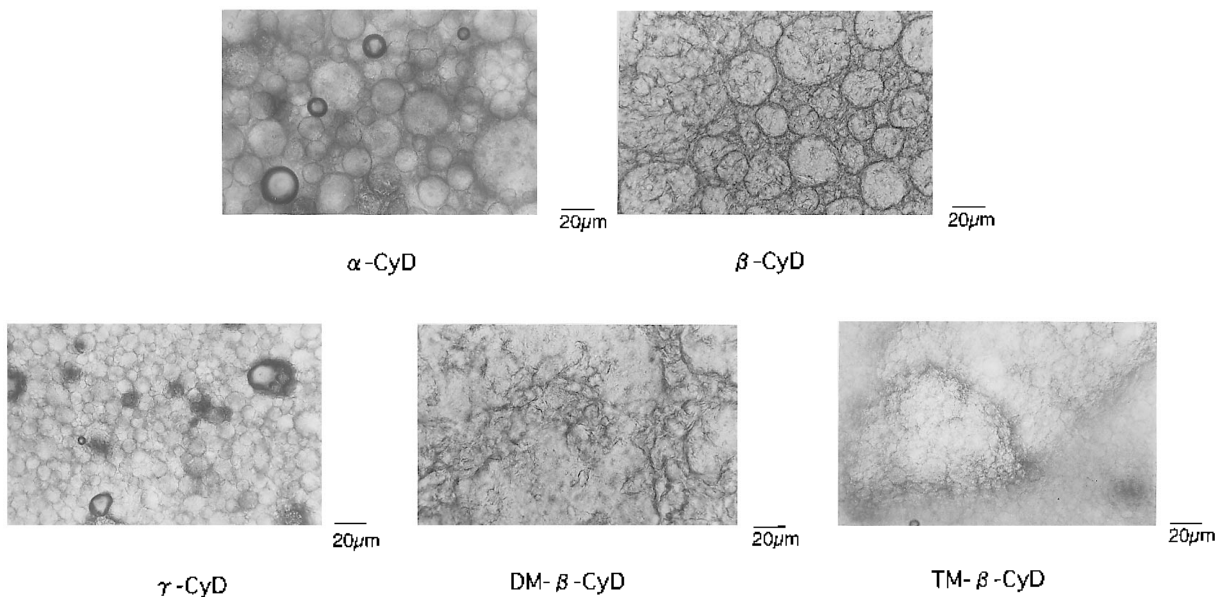


Fig. 4. Photomicrograph of Ointments
Fused mixture: 5% additives, 1% MH. Ointment base: HO70: PL30.

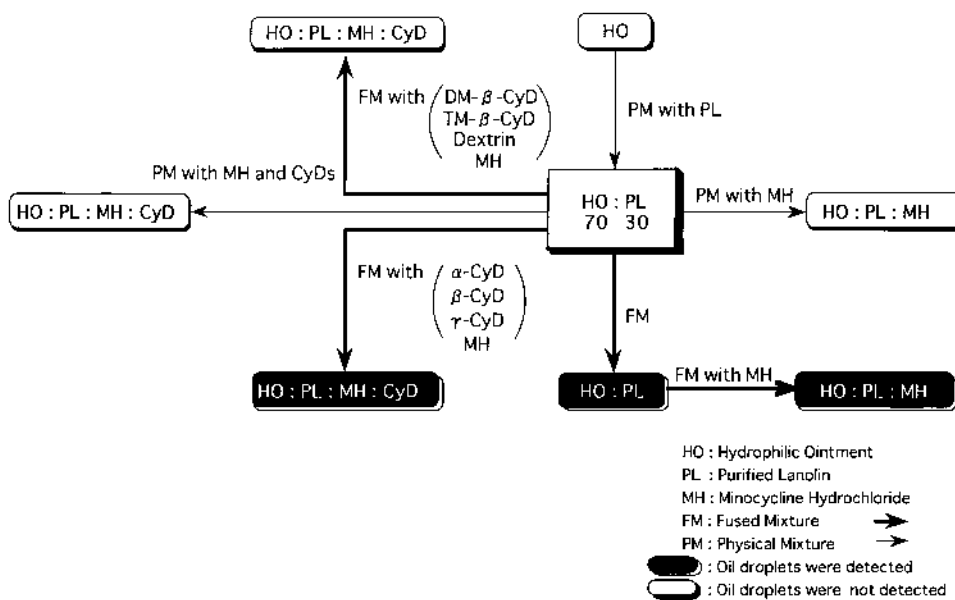


Fig. 5. Schematic Diagram of Structural Conversion of Ointment

water solubility (Table 1), distributing preferentially in the aqueous phase rather than at the interface of oil droplet. Dextrin admixed did not affect the physicochemical properties of the resultant mixture, because it has no binding cavity to interact with the surfactant. To prove this conjecture, the change in surface tension of an aqueous solution of HCO-60 was investigated by mixing CyD as shown in Fig. 6a. The addition of DM-β-CyD little affected the surface tension of the HCO-60 solution with increasing concentration in the mixed solution, while the surface tension increased with increasing β-CyD. This finding indicated an interaction of β-CyD with HCO-60 at the interface of oil droplet in the ointment base. The higher surface activating properties of TM-β-CyD and DM-β-CyD, as shown in Fig. 6b, over β-CyD prevented the coalescence of oil droplets in the ointment base keeping the

viscosity of ointment higher than the β-CyD containing ointment. Therefore, the oil droplets coalesced in the case of fused mixture of β-CyD, attributed to a decrease in the viscosity and to an increase in the drug release rate of the resultant mixed ointment base.

Stability of Ointment Base The structural change of ointment base after storage at ambient or cooled place (4 °C in refrigerator) was investigated by microscopy. It was found that some coalescence of oil droplets occurred after one week storage at room temperature, whereas no significant change was found after storing at cooled temperature as shown in Fig. 7. This difference reflected on the drug release rate change after storage as shown in Fig. 8. The stability of fused ointment base with β-CyD was confirmed up to 7 and 3 d when stored at cooled and room temperatures, respec-

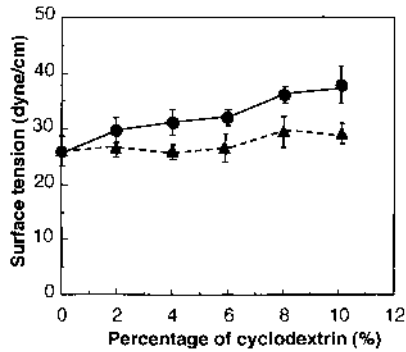


Fig. 6a. Effect of Concentration of Cyclodextrin on Surface Tension
 ●, β -CyD; ▲, DM- β -CyD. Concentration of HCO-60, 10%. Data represent the mean \pm S.E. of three experiments.

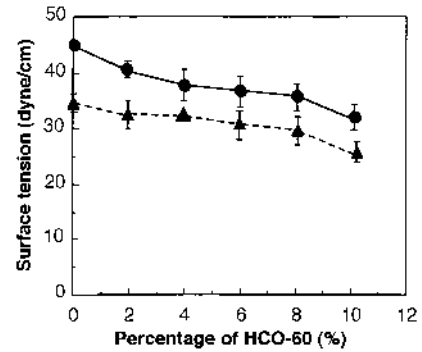


Fig. 6b. Effect of Concentration of HCO-60 on Surface Tension
 ●, β -CyD; ▲, DM- β -yD. Concentration of cyclodextrins, 5%. Data represent the mean \pm S.E. of three experiments.

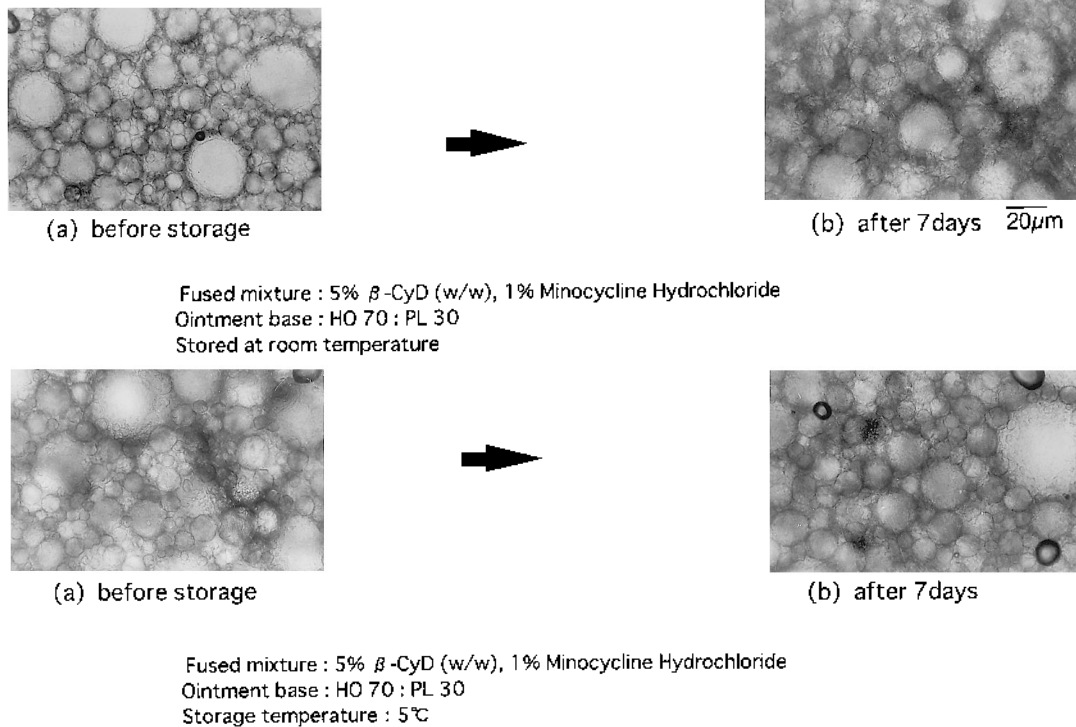


Fig. 7. Photomicrographs of (a) Freshly Prepared Ointment and (b) Stored Ointment for 7 d

tively. This stability is satisfactory for clinical usage, because they are usually used immediately after preparation in the hospital pharmacy.

Conclusion

The drug (MH) release rate from the HO (70%) and PL (30%) mixed ointment base for treatment of bedsore was desirably improved by coformulating with β -CyD by the fusing method.

The viscosity and the elution amount of the resultant ointment base was reduced and increased, respectively, for better treatment because it is less stimulating to the skin and is easily washable on application. The modification of the physico-chemical properties of the ointment base with β -CyD was explained by the interaction of β -CyD with the emulsifying agent (HCO-60) at the interface of oil droplets in the ointment base, leading to the liberation and coalescence of oil droplets in the ointment base. This phenomenon was con-

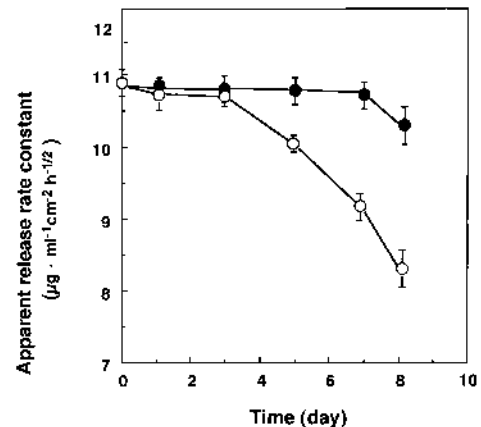


Fig. 8. Changes in Apparent Release Rate Constant of Fused Ointments with β -CyD during Storage
 Storage temperature: ●, 5°C; ○, room temperature. Data represent the mean \pm S.E. of three experiments.

firmed by an increase in the surface tension of HCO-60 solution with increasing concentration of β -CyD admixed. The reproducible drug release rate after storage of the ointment base at room temperature for 3 d was confirmed for the clinical usage. In conclusion, the ointment base was preferably modified with β -CyD for better treatment of bedsore and supplied to test clinically.

References and Notes

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