Preparation of Sustained Release Granules of Bumetanide[1)]

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Sustained release granules of bumetanide (BN) were prepared to obtain mild and sustained diuresis. BN was dispersed in combined matrixes of ethylcellulose (EC) with hydroxypropylmethylcellulose phthalate (HPMCP), povidone (PVP) or cyclodextrins (CDs). Release of BN from these preparations was evaluated by the JP XIII dissolution test in JP disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8) at 37 °C. The interaction of BN with CDs in buffer solutions with different pHs was also investigated, and BN from the preparation with the component of BN : γ-CD : EC = 1 : 4 : 2 (weight ratio) showed the most favorable sustained release pattern. The apparent stability constant of the γ-CD system on the BN solubility was greater than those of the α- or β-CD systems. Crystalline BN was not observed in this system in studies using powder X-ray diffraction spectra and a differential scanning calorimeter.

Key words bumetanide; sustained release; dissolution; cyclodextrin; ethylcellulose; povidone

Bumetanide (BN), 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid, is one of the most potent loop diuretics used to treat edema associated with congestive heart failure, hepatic and renal diseases, including the nephrotic syndrome.[2,3)] The diuretic effect of BN 1 mg is equal to that of furosemide 40 mg.[2)] The available dosage forms of BN today are tablets and injections. Following oral administration of those diuretic agents, diuresis occurs rapidly and the effects are short. Strong diuresis sometimes causes dehydration and a feeling of lassitude or fatigue. The authors reported the *in vitro* dissolution behavior and the relation of bioavailability and diuresis of furosemide tablets (Lasix® tablets) and furosemide retarded capsules (Eutensin® capsules) in human subjects.[4)] All of the subjects felt lassitude or fatigue caused by rapid decrease of electrolyte within one hour post-administration of Lasix® tablets. They did not experience those feelings following administration of Eutensin® capsules, however. As the granules in Eutensin® capsules were the enteric-coated type, their dissolution behavior was extremely restricted and they rapidly dissolved in the JP XIII disintegration medium No. 1 and No. 2, respectively. The diuretic effect was delayed only a few hours and lasted for a short period. The mean area under the plasma concentration–time curve (*AUC*) following administration of Eutensin® capsules was almost half that of Lasix® tablets, but diuresis was obtained sufficiently and mildly for three to four hours after administration of the capsules. A possible reason for the low bioavailability of these capsules might be low absorption of furosemide in the absorption site. After dissolution of furosemide from enteric-coated granules, most furosemide molecules might exist in an ionized form (pK_a 3.8[5)]) which is poorly absorbed. BN is a weak acid (pK_a 3.6[5)]) similar to furosemide. Dissolution of furosemide from Eutensin® capsules was only restricted in medium No. 1. In this report, we prepared granules that possessed a sustained release in gastrointestinal fluids within a few hours in order to obtain mild diuresis and to avoid its occurrence during the night.

Materials and Methods

Materials BN tablets (Lunetoron® Tablets, 1 mg/tablet, lot No. T110P) were obtained from Sankyo Co., Tokyo, Japan. BN powders (lot No. 74H0942, 50.5 ± 1.2 μm, mean ± S.D., Green diameter, $n = 100$), were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. Piretanide (lot No. 03 L035), supplied by Hoechst Marion Roussel, Tokyo, was dissolved in phosphate buffer at pH 7.4 and used as an internal standard. Alpha (α), β and γ-cyclodextrins (CDs) and povidone (PVP) K 30 (average molecular weight: 40000) were obtained from Nacalai Tesque Inc., Kyoto, Japan. Ethylcellulose (EC) with an ethoxy content of about 49% (lot No. TWL7800) was obtained from Wako Pure Chemical Ind., Osaka, Japan. Hydroxypropylmethylcellulose phthalate 200731 (HPMCP, lot No. 905517) was obtained from Shin-etsu Chemical Co., Toyama, Japan. Acetonitrile was HPLC grade from Kanto Chemicals Co., Inc., Tokyo. All other chemicals were of reagent grade.

Preparation of Sustained Release Granules of BN The preparation of sustained release granules of BN was done as follows: BN/HPMCP/EC system: An appropriate weight ratio of BN and additives were dissolved in a mixture of acetone and ethyl alcohol (1 : 4). BN/PVP/EC system: An appropriate weight ratio of BN and additives were dissolved in ethyl alcohol. BN/CD/EC system: An appropriate weight ratio of BN and CDs were dissolved in a minimum volume of water and mixed in EC ethanolic solution. These solvents were removed *in vacuo* using a rotary evaporator at 40 °C. The residue was ground in a motor, and dried *in vacuo* at room temperature for 24 h. The granules obtained by passing through a JP XIII No. 12 sieve (1400 μm) and which remained in a No. 42 sieve (355 μm) were used for the study. Granules were stored in tight light-resistant containers at room temperature. No characteristic changes in those were observed during the study.

Powder X-Ray Diffraction Spectra Powder X-ray diffraction spectra were obtained with a Geigerflex, model 2013 diffractometer, Rigaku Denki Co., Kyoto. The conditions of measurement were: Ni filter, CuK_α ray, 30 kV, 20 mA, scanning rate 1°/min, count range 2000 cps.

Thermal Analysis A differential scanning calorimeter (DSC, DT-40, Shimadzu Co., Kyoto) was used under N_2 gas flow at a scanning rate of 10 °C/min.

Dissolution and Release Studies Dissolution and release profiles of BN from powders, tablets and granules in JP XIII disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8) were obtained by a paddle method of JP XIII dissolution test apparatus (Toyama Sangyo Co., Ltd., Osaka). Powders, tablets or granules containing 1 mg of BN were suspended in the medium (500 ml, 37 ± 0.5 °C) and the paddle was rotated at 100 rpm. Two milliliters was withdrawn with a syringe at predetermined time intervals, then filtered immediately through a membrane filter (0.5 μm, HPLC sample Prep LCR 13-LH, Nihon Millipore Kogyo Co., Yonezawa, Japan). Two milliliters of fresh fluid at the same temperature was added to the container in order to maintain the volume of the medium. When medium No. 1 was used, the filtrate was added to 0.5 ml of 0.37 mol/l sodium hydroxide to prevent the recrystallization of BN. The sample solutions were diluted appropriately, added to the internal

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standard (0.5 ml, 10 μg/ml of piretanide) and analyzed for BN by the HPLC method.

Phase Solubility Studies Phase solubilities of BN were carried out according to the method by Higuchi and Connors.[6] Excess amounts of BN (10 mg in the buffer solutions at pHs 2.0 and 4.0, 100 mg in the buffer solution at pH 7.0) were dispersed in buffer solutions (20 ml) which contained three kinds of CDs (0.88 to 24.7 $\times 10^{-3}$ mol/l) and each mixture was shaken (60 strokes/min) at 37 $\pm$ 0.1 °C. The buffer systems used were 0.2 N hydrochloride $-$ 0.2 mol/l potassium chloride (pH 2.0) and McIlvain buffer (pHs 4.0 and 7.0). After the equilibrium (7 d), an aliquot was filtered through the membrane filter described above. One milliliter of sample solution appropriately diluted was added to 0.5 ml of the internal standard, and analyzed for BN by the HPLC method.

Analytical Procedure for BN The analytical procedure for BN was performed by the HPLC method based on the previous study.[7] HPLC was performed on a Shim-pack CLC-ODS reversed-phase column (150 $\times$ 6 mm i.d.) and a Shim-pack G-ODS guard column (10 $\times$ 4 mm i.d.) (Shimadzu). The columns were maintained at 40 $\pm$ 0.1 °C in the column oven (CTO-10A, Shimadzu). A spectrofluorometric detector (RF-550, Shimadzu) was used. Excitation and emission wavelengths were 335 and 415 nm, respectively. The mobile phases were 20% acetonitrile containing 0.2% acetic acid (A) and 80% acetonitrile containing 0.2% acetic acid (B). From 0 to 5 min, the mobile phases consisted of 75% A and 25% B; 5 to 8 min, 50% A and 50% B; 8 to 15 min, 40% A and 60% B; and 15 to 20 min, 75% A and 25% B. The flow rate was 1.0 ml/min. Sample solutions were automatically injected into the HPLC using a SIL-10A autoinjector (Shimadzu) and a LC-10AD pump (Shimadzu) using a system controller (SCL-10A, Shimadzu) with a run time of 20 min. A C-R6A computing integrator (Shimadzu) was used to calculate the area of the peaks of the chromatogram. In this chromatogram, peaks of BN and the internal standard were observed at 17.8 and 15.9 min, respectively. The detection limits of BN in media No. 1 and No. 2 were 1 and 0.5 ng/ml, respectively.

Protection from Photodegradation During the study, all processes were protected carefully from photodegradation of BN in a darkened room.[8]

Results and Discussion

Release Patterns of BN from Powders and Tablets Figure 1 shows the dissolution or the release pattern of BN from powders and tablets (Lunetron® tablets). The dissolution of BN from powders in medium No. 1 was low (7% at 4 h). The solubility of BN was 0.14 μg/ml (3.8×10^{-7} mol/l) at pH 1.2. In medium No. 2, the dissolution of BN powders was fast and complete. As BN is a weak acid with a pK_a of 3.6,[5] the solubility in medium No. 1 was considered to be that of an unionized form of BN. In contrast, BN dissolved rapidly from Lunetron® tablets in both media. Improvement in the dissolution characteristics of BN such as the solid dispersion system[9] might be made during the manufacturing process of Lunetron® tablets. In medium No. 1, the concentration of BN decreased gradually after 60 min, indicating that BN was in a hypersaturated state in the medium and its recrystallization occurred after dissolution from the tablets.

Release of BN from HPMCP/EC System Figure 2 shows the release pattern of BN from the HPMCP/EC system. When the weight ratio of BN : HPMCP : EC was 1 : 1 : 1, release of BN was negligible (0.2% at 4 h) in medium No. 1. Thirty five% of BN was released in medium No. 2 at 4 h. When the weight ratio of BN : HPMCP : EC was 1 : 2 : 1, the amount of BN released increased (50%) in medium No. 2; however, no release was observed in medium No. 1. EC itself is practically insoluble in water. HPMCP is insoluble in medium No. 1 and soluble in medium No. 2. BN in the BN/HPMCP/EC system might be released by the dissolution of HPMCP from the system. The HPMCP/EC system might be useful for other drugs such as weak basic drugs, or drugs those are unstable in the stomach. A sustained release formulation of BN might be achieved by adding additives such as water-soluble polymer to obtain continuous release in the intestinal tract.

Release of BN from PVP/EC System Figure 3 shows the release patterns of BN from the PVP/EC system. When the weight ratio of PVP to BN was more than 3 : 1, PVP/BN coprecipitate was formed. The concentration of BN exceeded the solubility in medium No. 1, and BN dissolved completely in medium No. 2. As PVP is a water-soluble polymer, the release of BN was believed to be followed by the dissolution of PVP from the PVP/EC system. When the weight ratio of PVP was larger, greater release and higher concentrations of BN were observed in medium No. 1. Released PVP in medium No. 1 might prevent BN recrystallization.[10] The PVP/EC system thus might be useful for a sustained release form of BN by adjusting the weight ratio of PVP and EC.

Release of BN from CDs/EC System Figure 4 shows the release patterns of BN from the β-CD/EC system

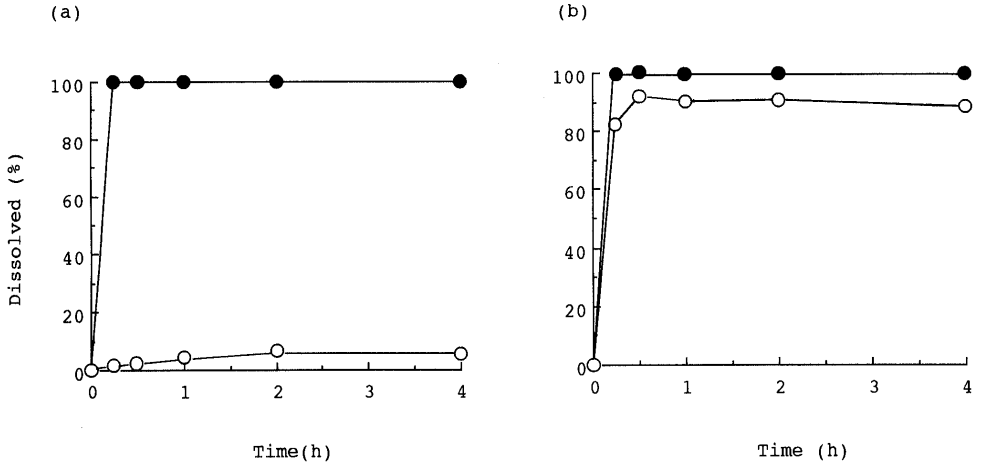

Fig. 1. Dissolution Profiles of BN from BN Powders (a) and BN Tablets (b) in JP XIII Disintegration Media No. 1 and No. 2 at 37 °C
○, medium No. 1; ●, medium No. 2. Each point represents the mean of three experiments. All standard errors were within 4%.

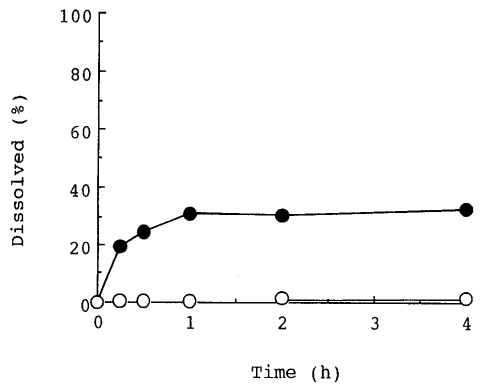

Fig. 2. Dissolution Profiles of BN from BN/EC/HPMCP (1:1:1, weight ratio) System in JP XIII Disintegration Media No. 1 and No. 2 at 37 °C

Symbols are the same as Fig. 1. Each point represents the mean of three experiments. All standard errors were within 4%.

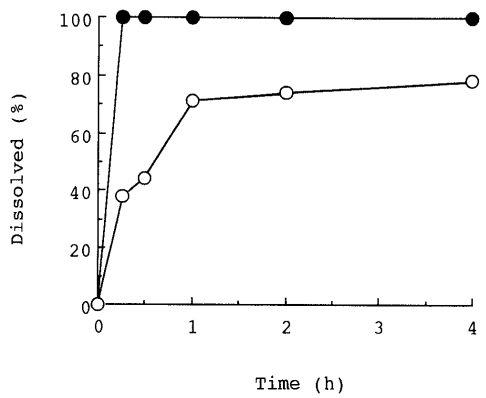

Fig. 3. Dissolution Profiles of BN from BN/PVP/EC (1:3:1) System in JP XIII Disintegration Media No. 1 and No. 2 at 37 °C

Symbols are the same as Fig. 1. Each point represents the mean of three experiments. All standard errors were within 4%.

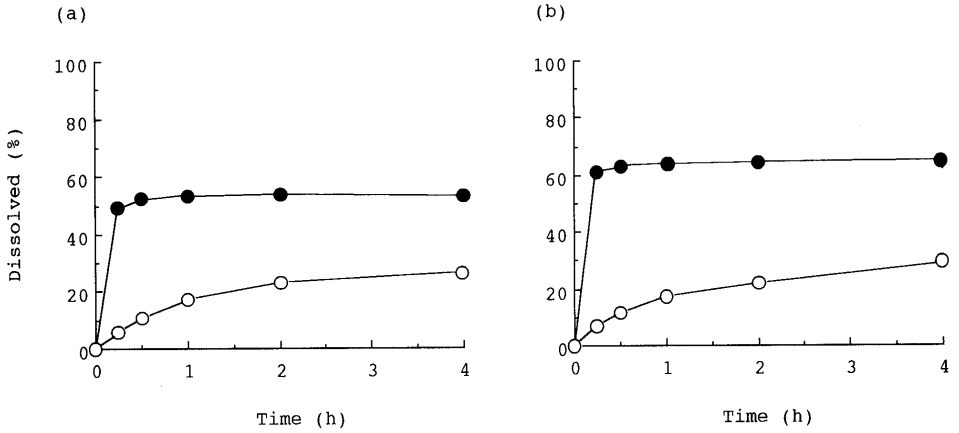

Fig. 4. Dissolution Profiles of BN from BN/CDs/EC (1:4:2) Systems in JP XIII Disintegration Media No. 1 and No. 2 at 37 °C

(a) BN/β-CD/EC system; (b) BN/γ-CD/EC system. Symbols are the same as Fig. 1. Each point represents the mean of three experiments. All standard errors were within 3%.

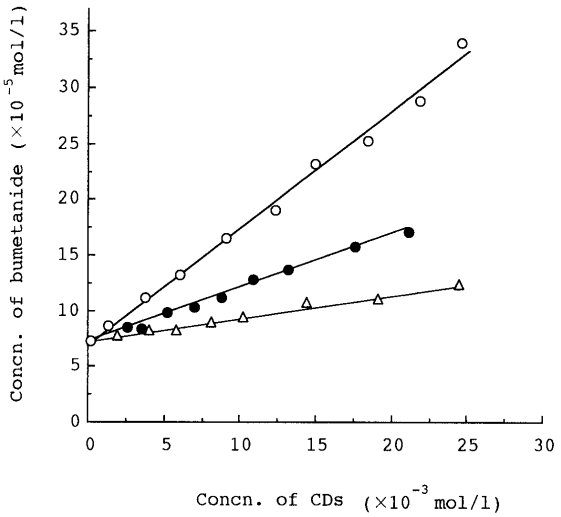

Fig. 5. Phase Solubility Diagrams of BN/CDs in pH 4.0 Buffer Solution at 37 °C

△, α-CD; ●, β-CD; ○, γ-CD.

and the γ-CD/EC system. When the weight ratio of BN:β-CD:EC was 1:4:2, sustained release of BN was observed in medium No. 1 (26%); the concentrations of BN exceeded the solubility. These concentrations decreased at 24 h (18%), however, the hypersaturation of BN continued. The release of BN was fast in medium No. 2, and was 52%. Sustained release of BN was also observed from the γ-CD/EC system in medium No. 1 (32%). The hypersaturation of BN continued without decrease in concentration after 24 h (38%). The dissolution behavior of BN from the physical mixture of BN/γ-CD/EC in media No. 1 and No. 2, on the other hand, was almost the same as that of BN powders. Other physical mixtures of BN and additives were also the same as those dissolution profiles of BN powders in both media. When the ratio of β or γ-CD was larger, the rate of release of BN was larger in both media. Study of the weight ratio of BN:α-CD:EC at 1:4:2 showed BN release to be small in medium No. 1 (10% at 4 h).

Phase Solubility Studies Figure 5 shows a phase solubility diagram of BN and three kinds of CDs in the buffer solution (pH 4.0) at 37 °C; all three diagrams were A_L-type.[6] BN showed the same type above described in buffer solutions at pHs 2.0 and 7.0. The BN molecule is ionized about 80% at pH 4.0. CD has strong interaction with an unionized drug,[11,12] however, γ-CD showed strong interaction with BN at pH 4.0.

Table 1 summarizes the stability constants from the

Table 1. Apparent Stability Constants of BN with CDs at 37 °C

Buffer solution	Apparent stability constants (l/mol)		
	α-CD	β-CD	γ-CD
pH 2.0	29.4	71.4	314
pH 4.0	30.4	72.8	156
pH 7.0	73.3	157	162

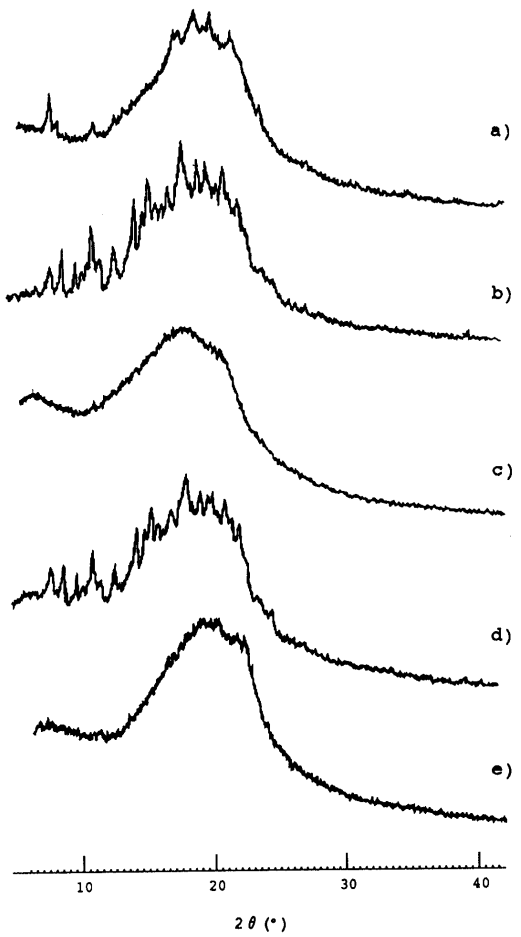

Fig. 6. Powder X-Ray Diffraction Spectra of BN/γ-CD/EC (1:4:2) System

(a) BN; (b) γ-CD; (c) EC; (d) physical mixture; (e) solid dispersion system.

phase solubility of BN by CDs in buffer solutions at pHs 2.0, 4.0 and 7.0. The result showed that γ-CD solubilized BN most. The stability constant with γ-CD was largest at pH 2.0, suggesting that unionized BN interacted with γ-CD more strongly than its ionized form. The effect of α-CD was the smallest among them. The release of BN from CD/EC systems depended on the solubility of BN by CDs. BN concentrations in medium No. 1 exceeded the solubility by β or γ-CDs.

Crystallinity of BN in the Granules Figure 6 shows the powder X-ray diffraction spectra of the granules and the physical mixture of the BN/γ-CD/EC system at a ratio of 1:4:2. Sharp diffraction peaks due to the crystalline form of BN and γ-CD disappeared in the granules, but remained in the physical mixture.

Figure 7 shows their DSC curves. An endothermic peak due to the melting of BN (233 °C) disappeared in the

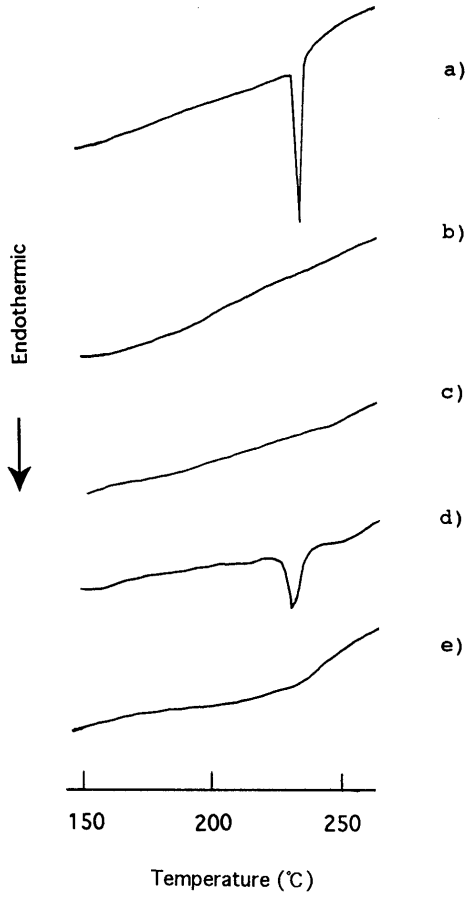

Fig. 7. Differential Scanning Calorimetry (DSC) Curves of BN/γ-CD/EC (1:4:2) System

(a) BN; (b) γ-CD; (c) EC; (d) physical mixture; (e) solid dispersion system.

granules, but remained in the physical mixture. From the above analysis, solid dispersion systems of BN, γ-CD and EC at 1:4:2 weight ratios were formed by the co-evaporation.

The granules of BN/γ-CD/EC (1:4:2 weight ratio) increased the concentrations of BN in medium No. 1 and restrained them in medium No. 2 compared to that of Lunetron® tablets of which had a rapid effect. Considering storage of the prepared granules, the CD/EC system may be better than the PVP/EC system because of the hygroscopicity of PVP.[13,14] Based on these results, the granules of the BN/γ-CD/EC system are anticipated to be a sustained release form of BN.

References and Notes

1) A part of this study was presented at the 115th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, March 1995.
2) Cook J. A., Smith D. E., Cornish L. A., Tankanow R. M., Nicklas J. M., *Clin. Pharmacol. Ther.*, **44**, 487—500 (1988).
3) Handler B., Dhingra R. C., Rosen K. M., *J. Clin. Pharmacol.*, **21**, 691—696 (1981).
4) Yagi N., Kiuchi T., Satoh H., Kenmotsu H., Sekikawa H., Takada M., *Biol. Pharm. Bull.*, **19**, 616—622 (1996).
5) Orita Y., Ando A., Urakabe S., Abe H., *Arzneim.-Forsch.*, **26**, 11—13 (1976).
6) Higuchi T., Connors K. A., *Adv. Anal. Chem. Instr.*, **4**, 117—212 (1965).
7) Yagi N., Kenmotsu H., Shimode Y., Oda K., Sekikawa H., Takada M., *Biol. Pharm. Bull.*, **16**, 263—267 (1993).
8) Yagi N., Kenmotsu H., Sekikawa H., Takada M., *Chem. Pharm.*

Bull., **39**, 454—457 (1991).
9) Yamamoto K., Nakano M., Arita T., Takayama Y., Nakai Y., *J. Pharm. Sci.*, **65**, 1484—1488 (1976).
10) Sekikawa H., Nakano M., Arita T., *Chem. Pharm. Bull.*, **27**, 1223—1230 (1979).
11) Uekama K., *Yakugaku Zasshi*, **101**, 857—873 (1981).
12) Hirayama F., Kurihara M., Uekama K., *Int. J. Pharm.*, **35**, 193—199 (1987).
13) Sugimoto I., Sasaki K., Kuchiki A., Ishihara T., Nakagawa H., *Chem. Pharm. Bull.*, **30**, 4479—4488(1982).
14) Suzuki H., Miyamoto N., Masada T., Hayakawa E., Ito K., *Chem. Pharm. Bull.*, **44**, 364—371 (1996).