In Vitro Stability and *in Vivo* Absorption Studies of Colloidal Particles Formed from a Solid Dispersion System

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We investigated the physicochemical and pharmaceutical properties of a solid dispersion (SD) derived from a solution of the poorly water-soluble drug (*R*)-1-[2,3-dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (YM022), hydroxypropylmethylcellulose 2910 (TC-5E) and polyoxyethylene hydrogenated caster oil 60 (HCO-60). Colloidal particles produced when the SD was dispersed into water had a mean diameter of 160 nm and contained 67—77% YM022. Powder X-ray diffractometry showed that YM022 existed in the colloidal particles in an amorphous state. The particle diameter and YM022 content remained unchanged during storage for 7 d, confirming that the colloidal solution was stable. On oral administration to rats, good absorption was observed for both the colloidal solution prepared immediately before and the sample stored for 7 d before administration. Thus, the stability of this colloidal solution of SD was confirmed by *in vitro* storage tests and by *in vivo* absorption experiments in rats.

Key words poorly water-soluble drug; solid dispersion; colloidal particle; colloidal solution; water soluble polymer; bioavailability

(*R*)-1-[2,3-Dihydro-1-(2′-methylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)-urea (YM022) is a compound originally synthesized by Yamanouchi Pharmaceutical Co., Ltd., which inhibits acid secretion. Although it may exist in one of three forms, namely, crystalline α, crystalline β or an amorphous state, the β-form has been developed for clinical use due to its ease of production. It has been found[1] that crystalline YM022 (α and β forms) is exceedingly insoluble in water, at less than 0.1 μg/ml at 20 °C, and is not absorbed on oral administration in rats. In our previous study,[1] to improve the solubility and absorption of YM022, we prepared a solid dispersion (SD) of YM022, hydroxypropylmethylcellulose 2910 (TC-5E) and polyoxyethylene hydrogenated caster oil (HCO-60) by a solvent method. This formulation showed good results on oral administration in dogs. In this study, we found that the amount of YM022 dissolved was remarkably low compared to the amount added when SD was dispersed into purified water. The reason for the large increase in bioavailability in dogs was assumed to be due to the effect of colloid formation in the dispersed solution.

Tachibana *et al.*[2–4] reported a number of characteristics of the behavior of SD in water, noting that SD might display a supersaturated condition and that microparticulate crystals of a drug might be formed after the dispersal of SD into water.

Considering that the colloid formation of SD dispersed into water is due to a different phenomena from that described by Tachibana *et al.*, we investigated the *in vitro* stability of colloidal particles formed from SD under different conditions and storage, and also examined the influence of storage on *in vivo* absorption characteristics in rats.

Experimental

Reagents YM022 and 1-[2,3-dihydro-1-(4′-methoxylphenacyl)-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (NGA-9) were synthesized at the Chemical Technology Laboratory, Yamanouchi Pharmaceutical Co., Ltd. (Takahagi, Japan). TC-5E and HCO-60 were purchased from Shin-etsu Kagaku Co. (Tokyo, Japan) and Nikko Chemicals (Tokyo, Japan), respectively. Both were of commercial grade, whereas sodium cholate (Wako-junyaku Kogyo Co., Osaka, Japan) was of biochemical grade. Other chemicals used in this study met the specifications of the Japanese Pharmacopoeia 12 (JP12).

Preparation of SD A SD was prepared by dissolving 10 g of YM022, 35 g of TC-5E and 5.0 g of HCO-60 in 450 g of a methylene chloride–methanol (4 : 1) solvent mixture. A fine powder was produced using a spray-dryer (DL-41,Yamato Kagaku Co., Tokyo, Japan) with an inlet temperature of 120 °C.

Determination of Particle Size SD of 50 mg was dispersed into 200 ml of purified water and stirred for 30 min. The diameter of the particles formed in the dispersed solution was determined using an LA-910 laser diffraction scattering particle analyser (Horiba Co., Kyoto, Japan).

Measurement of Solubility The solubility of SD in purified water at 20 °C was determined. SD of 500 mg (equivalent to 100 mg of YM022) was added to 40 ml of purified water and the mixture was shaken in an incubator for 1 h. The obtained suspension was centrifuged (L-70, Beckman, California, U.S.A.) at 50000 rpm for 30 min and the YM022 content in the supernatant was determined using HPLC[1] (LC-6A, Shimadzu Co., Kyoto, Japan). Elution peaks were monitored at 240 nm using a YMC A-302 ODS column (4.6 mm i.d. × 150 mm). The mobile phase used was phosphate buffer (pH 5.5)–acetonitrile (10 : 15) at a flow rate of 1.0 ml/min and a column temperature of 40 °C. Pyrene was used as an internal standard.

Powder X-Ray Diffraction A powder X-ray diffractometric study was performed using a Rint 1400 (Rigaku, Tokyo, Japan) with Ni-filtered Cu$K\alpha$ radiation at 40 kV, 40 mA. The samples were scanned from 5° to 30° (2θ) at the rate of 3°/min.

Differential Scanning Calorimetry (DSC) A differential scanning calorimetric study was performed using a DSC2910 (TA Instruments, Tokyo, Japan) at a heating rate of 10 °C/min under a nitrogen gas stream (50 ml/min). Open pans were used to allow the escape of volatile gases.

Formation and Accumulation of Microparticles Regulation of Rotation Number of Centrifugation[5]: SD of 750 mg was added to 20 ml of purified water and the mixture was shaken in an incubator (M-100D, Taiyo Industry, Tokyo, Japan) at 20 °C for 30 min. The suspension was then centrifuged according to the following conditions: a) 50000 rpm for

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30 min b) 10000 rpm for 30 min c) storage at 20 °C for 7 d followed by centrifugation at 3000 rpm for 30 min. The obtained sediment was dried at 40 °C for 15 h.

Regulation of the Number of Washings with Purified Water for Microparticle Sediment: SD of 750 mg was added to 20 ml of purified water and the mixture was shaken at 20 °C for 30 min. The SD suspension was centrifuged (L-70, Beckman) at 50000 rpm for 30 min to obtain a microparticle sediment. The sediment was then repeatedly washed with purified water as follows. The sediment was dispersed into 20 ml of purified water and shaken at 20 °C for 30 min. After centrifugation at 50000 rpm for 30 min, the sediment was dried at 40 °C for 15 h.

Regulation of Concentration of the Suspension: Fifty, 250, 500 or 750 mg of SD was dispersed into 20 ml of purified water, and the mixture was shaken for 30 min and centrifuged at 50000 rpm for 30 min to obtain a microparticle sediment. The sediment was then dried at 40 °C for 15 h.

Regulation of the Temperature of Purified Water: SD of 750 mg was dispersed into 20 ml of purified water at 20, 30, 40 and 50 °C, shaken for 30 min and centrifuged at 50000 rpm for 30 min to obtain a microparticle sediment. The sediment was then dried at 40 °C for 15 h.

Determination of Dissolved Amount and UV Spectroscopic Studies of Colloidal Solution Dissolution tests of 1 ml of 0.50%, 5.0% and 6.25% SD in purified water were carried out at 37 °C using a dissolution apparatus, NTR-VS6 (Toyama Sangyo Co., Osaka, Japan). Paddle speed was 100 rpm. The test solution used was 500 ml of JP 12 1st disintegration test fluid. UV spectroscopic studies (200—400 nm) were carried out in a test solution after 10-min of stirring, and the amount of YM022 dissolved at λ_{max} = 251—254 nm was calculated.

***In Vivo* Absorption Studies** Male F344 rats aged 9—10 weeks were used for the *in vivo* study. A 6.25% SD aqueous suspension prepared by dispersing SD of 625 mg into 10 ml of purified water was given by a single oral administration (YM022 125 mg/kg) to the rats. Blood samples were collected in heparinized tubes at 0.5, 1, 2 and 8 h after administration and centrifuged at 3000 rpm for 15 min. The plasma was stored in a refrigerator.

Analysis of YM022 in Plasma Analysis was done as previously described.[1] Briefly, an internal standard solution of 200 μl (1 μg NGA-9/ml in methanol solution) was evaporated in a test tube under reduced pressure. To this was added 1 ml of plasma, 1 ml of purified water, 1 ml of saturated sodium bicarbonate aqueous solution and 5 ml of a diethylether–*n*-hexane (1 : 1) solvent mixture. The sample solution was pretreated by shaking for 15 min and then centrifugation at 2000 rpm for 10 min to separate the organic layer. The organic layer was then distilled under reduced pressure, and the residue was dissolved in 200 μl of 99.5% ethanol. The unchanged YM022 content in the treated sample was determined by HPLC using an LC-9A (Shimadzu Co.) with a UV detector (250 nm, SPD-6A, Shimadzu Co.) with a TSL-gel ODS-80-Tm column (4.6 mm i.d. × 150 mm). A 0.05 M phosphoric acid–acetonitrile (40 : 60) solvent system was used as a mobile phase with a flow rate of 1.0 ml/min at room temperature. The detection limit of YM022 was 5 ng/ml.

Results and Discussion

SD Dissolution Behavior and Formation of Colloidal Particles A previous dissolution test of SD prepared from slightly soluble YM022, water-soluble macromolecular TC-5E and non-ionic surfactant HCO-60 using JP12 1st disintegration test fluid showed that the supersaturation state of YM022 was well maintained.[1] When SD was dispersed into purified water, a blue-white semi-transparent colloidal solution[6,7] was immediately formed. The particle size distribution was determined and the average diameter was calculated to be about 160 nm (Fig. 1). The dissolution behavior of YM022 in SD was investigated by dispersing SD of 500 mg (equivalent to 100 mg of YM022) into 40 ml of purified water at 20 °C to obtain a blue-white semi-transparent colloidal solution. The YM022 content in this supernatant was then determined to be 4.4 μg/ml. Although solubility increased more than 40 times less-than-0.1 μg/ml solubility of the β-form in purified water at 20 °C, the amount dissolved was still very small, at less than 1% of the total amount added. The remaining 99% of YM022 was thought to exist as colloidal particles.

Physicochemical and Pharmaceutical Properties of Colloidal Particles SD was dispersed into purified water to obtain colloidal particles. After centrifugation, the sediment was dried at 40 °C and measured by powder X-ray diffraction and DSC measurement. Figure 2 shows the powder X-ray diffraction pattern of dried sediment. No sharp peaks due to crystals were found. In addition, the DSC scan showed no remarkable thermal changes (Fig. 3). These data suggested that YM022 in colloidal particles exists in an amorphous state. The YM022 content in dried sediment was determined by HPLC to be 66.7% of the total amount. Whereas the initial composition of SD was YM022 : TC-5E : HCO-60 = 1.0 : 3.5 : 0.5, the colloidal particles might be composed of YM022 and both TC-5E and HCO-60 or YM022 and either TC-5E or HCO-60.

The influence of the conditions for colloid formation (concentration and temperature of dispersed solution) and

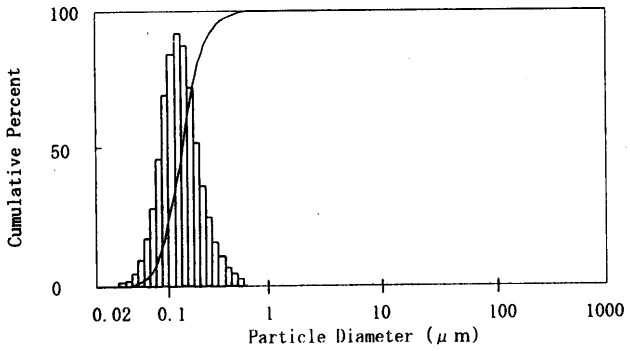

Fig. 1. Particle Size Distribution of Colloidal Particles Obtained from SD Dispersed into Water

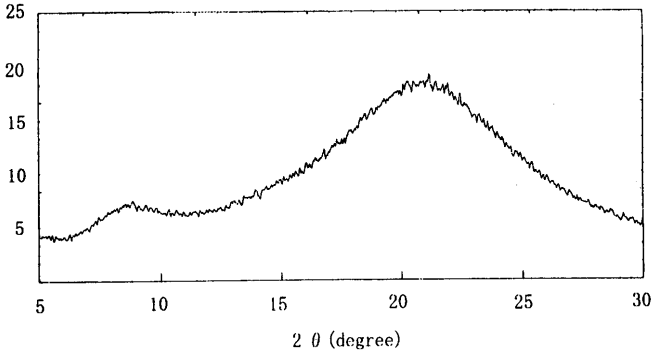

Fig. 2. Powder X-Ray Diffraction Pattern of Dried Sediment Obtained from Colloidal Solution

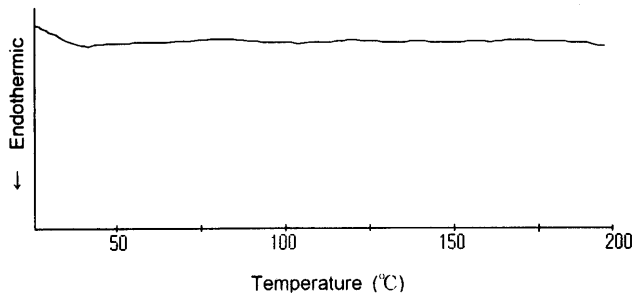

Fig. 3. DSC Curve of Dried Sediment Obtained from Colloidal Solution

sedimentation (rotation speed in centrifugation and number of water washings of the sediment) on the composition of the colloidal particles was then investigated. Centrifugation speed varied from 3000 to 50000 rpm, corresponding to a variation in gravity from 2000 to 250000 × *g*. In the case of 3000 rpm, 7-d storage before centrifugation was necessary to complete the sedimentation. The weight ratios of the total amount of colloidal particles and YM022 are shown in Table 1. The total amount of colloidal particles obtained after centrifugation at 3000, 10000 and 50000 rpm was 1.33, 1.34 and 1.35 times the amount of YM022, respectively.

The effect of washing with purified water was then studied. After centrifugation at 50000 rpm, the colloidal particles were washed with purified water at 20 °C 1, 2 and 3 times. The weight ratio of the total colloidal particles and YM022 before washing was 1.37. In contrast, the values after washing 1, 2 and 3 times were 1.32, 1.32 and 1.31, respectively. Some of the water-soluble TC-5E and HCO-60 was eluted by washing with purified water, but as no marked difference in composition was observed with the number of washings, it was assumed that there might be some interaction between the ingredients which resulted in the formation of water-insoluble particles.

The influence of the concentration of dispersed suspension was also investigated by determining the YM022 content in the accumulated colloidal particles formed from 0.25%, 1.25%, 2.5% and 3.75% SD suspensions (Table 2). The weight ratio of YM022 to the total colloidal particles for 0.25%, 1.25%, 2.5% and 3.75% SD suspensions was 1.35, 1.35, 1.38 and 1.39, respectively. It was expected that the amount of YM022 taken up into both TC-5E and HCO-60 or either TC-5E or HCO-60 might depend on the ratio of SD and purified water in the SD suspension prepared, but the results showed no difference in colloidal particle composition among the 0.25—3.75% SD suspensions.

Futhermore, the effect of the dispersion medium temperature was investigated by adding SD into purified water at 20—50 °C, followed by centrifugation of the obtained colloidal particles at 50000 rpm. The results (Table 3) showed that colloidal particle composition showed almost no change with dispersion medium temperature. The results of these determinations of colloidal particle composition obtained under various formation and accumulation conditions showed that the values of (total weight of colloidal particles)/(weight of YM022) varied from 1.3 to 1.5, that is, 66.7—76.9% YM022 in the colloidal particles. These data suggest that colloidal particles were formed from YM022 and either or both TC-5E and HCO-60, and that there might be some interaction such as hydrogen bonding among the ingredients resulting in the formation of a complex.

In order to evaluate the physicochemical stability of the colloidal particles, the particle size and the dissolved amount for the 0.5%, 5.0% and 6.25% colloidal solutions after storage at 20 °C for 7 d were determined and UV spectroscopic studies were performed. The variation of average particle size determined by laser diffraction and the changes in absorbance at 240 nm are shown in Table 4. The amount of YM022 dissolved tended to decrease slightly with storage time. However, no significant change in particle size was observed, with the average particle size in all solutions being less than 200 nm. No significant changes were observed among the UV spectra of colloidal solutions before or after storage (Fig. 4). These results indicate that the colloidal solutions were highly physicochemically stable. When SD systems are kept under humidified conditions, drug molecules in an amorphous

Table 1. Weight Ratio of YM022 and Total Amount of Colloidal Particles after Centrifugation

Centrifuge conditions	Total weight of particles / Weight of YM022
50000 rpm (250000 × *g*), 30 min	1.35
10000 rpm (10000 × *g*), 30 min	1.34
After standing for 7 d	1.33
3000 rpm (2000 × *g*), 30 min	

Table 2. Weight Ratio of YM022 and Total Amount of Colloidal Particles as a Function of Dispersion Concentration

Concentration	SD: purified water	Total weight of particles / Weight of YM022
0.25%	50 mg : 20 ml	1.35
1.25%	250 mg : 20 ml	1.35
2.5%	500 mg : 20 ml	1.38
3.75%	750 mg : 20 ml	1.39

Table 3. Weight Ratio of YM022 and Total Amount of Colloidal Particles by Water washing at Different Temperatures

Temperature (°C)	Total weight of particles / Weight of YM022
20	1.43
30	1.43
40	1.35
50	1.31

Table 4. Physicochemical Stability of Colloidal Solutions at 20 °C

Dispersion concentration	0.50%		5.0%		6.25%	
	Absorbance at 240 nm	Mean particle diameter (nm)	Absorbance at 240 nm	Mean particle diameter (nm)	Absorbance at 240 nm	Mean particle diameter (nm)
Initial	1.69	146	1.68	141	2.15	124
After storage for 3 d	1.65	173	1.61	190	2.04	148
After storage for 7 d	1.58	168	1.49	175	1.84	184

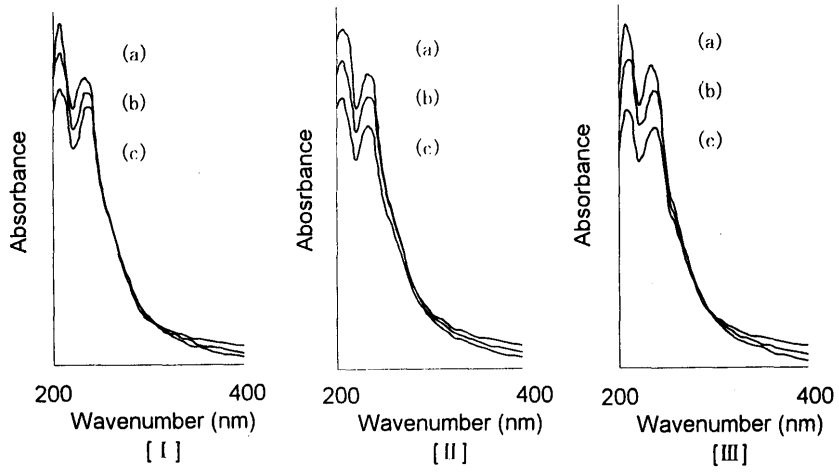

Fig. 4. UV Spectra of Colloidal Solution Obtained from SD Dispersed into Water

Dispersion concentrations were [I] 0.50%, [II] 5.0% or [III] 6.25%. Values are (a) initial, (b) after storage for 3 d and (c) after storage for 7 d.

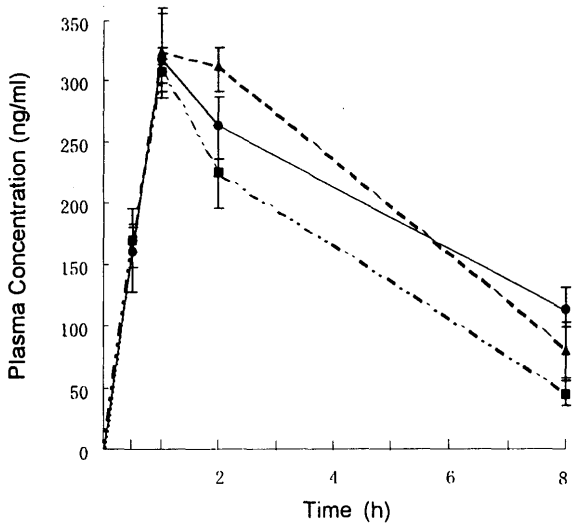

Fig. 5. Plasma Concentration of YM022 in Rats after Oral Administration of Colloidal Solution at a Dose of YM022 125 mg/kg

●, administered immediately after preparation; ■, administered 3 d after preparation; ▲, administered 7 d after preparation ($n=3$, mean ± S.E.).

Table 5. Pharmacokinetic Parameters of YM022 in Rats after Oral Administration of Solution at a Dose of YM022 125 mg/kg

Time of dosing	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-8} (ng·h/ml)
Immediately after preparation	319.1 ± 38.7	1	1579
3 d after preparation	325.4 ± 29.8	1	1673
7 d after preparation	306.9 ± 20.0	1	1242

$n=3$, mean ± S.E.

Table 6. Solubility Behavior of Colloidal Particles in Different Dispersion Solutions

Medium	Additive	
1st fluid/JP12 (pH 1.2)	0.2% Tween 80	○
	0.3% Tween 20	○
	0.1% SLS	○
	7.5% PEG400	×
	0.5% HCO-60	×
Purified water	0.3% SLS	○
2nd fluid/JP12 (pH 6.8)	0.2% Tween 80	○
	1.7% Sodium cholate	×

○: Clear solution was obtained with the addition of additive. ×: No change was observed.

state usually crystallize into a crystalline form.[8] To confirm the molecular state of YM022 in microparticles after storage, the colloidal solution stored for 7 d was either freeze-dried, or centrifuged and dried at 40 °C. Powder X-ray diffraction and DSC measurements of both the freeze-dried product and the dried sediment were carried out. Both powder X-ray patterns showed a halo pattern, and the DSC curves showed no endothermic peak resulting from the melting phenomena of YM022. Furthermore, no disappearance of polarized light on polarization microscopy was observed. These data suggest that the SD prepared in this study was extremely stable.

Investigation of Absorption Characteristics of Colloidal Solution in Rats The *in vivo* absorption characteristics of the colloidal solution were investigated using rats. A 6.25% colloidal solution of SD, which was dispersed into purified water which showed a blue-white colloidal color, was stored for 3 and 7 d. Freshly prepared 3- and 7-d stored solutions were administered to rats (125 mg/kg), and the blood concentration of YM022 was determined (Fig. 5). For all samples, YM022 was found to distribute rapidly to blood. The maximum plasma concentration (C_{max}) and area under the plasma concentration–time curves (AUC_{0-8h}) of 3 samples are shown in Table 5. No significant difference in C_{max} or AUC among the 3 samples was observed. The colloidal solution of SD dispersed in purified water revealed no change in oral absorption characteristics in rats, even after storage for 7 d.

The finding that colloidal particles grown from SD of 50 mg dispersed in 500 ml of purified water, JP12 1st or JP12 2nd disintegration test fluids could be dissolved by the addition of various surfactants (Table 6) suggests that the colloidal particles might also be dissolved in the gastrointestinal tract, depending on the presence of surfactants here.

Hasegawa *et al.*[9] reported that when a solid dispersion system was dispersed into water, the resulting supersaturated state was not stable. Our study revealed,

however, that the absorption characteristics of the present dispersed suspension did not differ before and after storage, indicating the remarkable stability of this SD suspension.

References

1) Yano K., Takamatsu N., Yamazaki S., Sako K., Nagura S., Tomizawa S., Shimaya J., Yamamoto K., *Yakugaku Zasshi*, **116**, 639—646 (1996).
2) Hasegawa A., Taguchi M., Suzuki R., Miyata T., Nakagawa H., Sugimoto I., *Chem. Pharm. Bull.*, **36**, 4941—4950 (1988).
3) Tachibana T., Nakamura A., "Kolloid-Zeitschrift und Zeitschrift fur Polymere," Band 203-Heft 2, 1965, pp. 130—133.
4) Yoshitomi Pharmaceutical Co., Ltd., Japan. Patent No. Hei 6-16556 (1994).
5) Kondo N., Iwao T., Kikuchi M., Shu H., Yamanouchi K., Yokoyama K., Ohyama K., Ogyu S., *Biol. Pharm. Bull.*, **16**, 796—800 (1993).
6) Tsutsumi H., Arai H., *Yukagaku*, **26**, 662—668 (1977).
7) Jirgensons B., Straumanis M. E., "Colloid Chemistry," Baihukan, 1962, p. 5,.
8) Sugimoto I., Kuchiki A., Nakagawa H., *Chem. Pharm. Bull.*, **29**, 1715—1723 (1981).
9) Hasegawa A., Kawamura R., Nakagawa H., Sugimoto I., *Yakugaku Zasshi*, **105**, 586—592 (1985).