Chem. Pharm. Bull. 26(12)3836—3842(1978)

UDC 615.276.015.2 : 615.451.014.076.9

Interaction of Several Nonsteroidal Antiinflammatory Drugs with Pectin in Aqueous Solution and in Solid State[1,2]

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(Received July 20, 1978)

Interaction between several nonsteroidal antiinflammatory drugs and pectin was studied by the centrifugation method, the equilibrium dialysis method and the solubility method. Benzydamine hydrochloride (BZM) and ketoprofen (KPF) formed coprecipitates with pectin by the centrifugation method, and the binding isotherms of BZM and pectin showed a multi-layer type. On the other hand, the binding isotherm of BZM to pectin by the equilibrium dialysis method showed a Langmuir type in low equilibrium concentration range and the saturated amount bound decreased with an increase of ion concentration of the solution. The solubility of all drugs used decreased with an increase of concentration of pectin. IR absorption spectroscopy, X-ray diffraction pattern and differential scanning calorimetry of the BZN/pectin coprecipitate showed clear difference from the physical mixture.

The dissolution rate of BZM/pectin coprecipitate and physical mixture of BZM and pectin was very slow in comparison with intact BZM and physical mixture of BZM and galacturonic acid. Pectin forms water insoluble complexes with these nonsteroidal antiinflammatory drugs, suggesting a usefulness as an additive for sustained-release preparations, and this might afford a mean for reducing adverse reactions of nonsteroidal antiinflammatory drugs to stomach after oral administration.

Keywords——pectin; nonsteroidal antiinflammatory drugs; water insoluble complex; centrifugation method; equilibrium dialysis method; solubility; binding isotherm; decrease of dissolution rate

Pectin is one of conjugated polysaccharides present in cell walls of all kinds of plant tissues, especially, in lemon or orange rind, being widely used as a food additive in preparation of jellies. In veterinary field, pectin is used as an antidiarrheic agent. Experimentally, it is recognized that pectin is effective in cleaning up intestine or detoxication in human.[4,5] In pharmaceutical field, it has been reported that pectin has influence on solubilities of drugs,[6,7,8] and also is effective in reducing an adverse reaction of aspirin to stomach.[9] Additionally, pectin is known to form complexes with several drugs, such as phenylbutazone,[10] aminophenazone,[11] and sorbic acid,[12] and an interaction with penicillines.[13]

1) This paper forms Part XII of "Pharmaceutical Interaction in Dosage Forms and Processing." The preceding paper, Part XI: N. Nambu, K. Kikuchi, T. Kikuchi, Y. Takahashi, H. Ueda, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), **26**, 3609 (1978).
2) This work was partly presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1976.
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Nonsteroidal antiinflammatory drugs are generally very slightly soluble in water and also sometimes cause adverse reaction due to their stimulant property to stomach after oral administration. Expecting to reduce such adverse reactions of nonsteroidal antiinflammatory drugs pharmaceutically by a combination with pectin, the present study was attempted to investigate the interaction between several nonsteroidal antiinflammatory drugs and pectin in aqueous solution and in solid state.

Experimental

Materials——Low methoxyl pectin of Sunkist Growers Inc. was used in this experiment, containing 9% methoxyl group and 57% galacturonic acid when determined by the method described in N.F. XIV. The loss on drying under reduced pressure over anhydrous phosphoric acid in a desiccator at 80° was 10%. The molecular weight was found to be 120000.[14]

Very pure compounds of seven nonsteroidal antiinflammatory drugs, which all conformed to the registered standards, were as follows: azapropazone (APZ), mp 236—238°; benzydamine hydrochloride (BZM), mp 160°; flufenamic acid (FFA), mp 133—136°; indomethacin (IMC), mp 153—154°; ketoprofen (KPF), mp 93°; mepirizole (MPZ), mp 90—92°; phenylbutazone (PBZ), mp 105°.

Determination of Drugs and Pectin——All nonsteroidal antiinflammatory drugs used were determined according to ultraviolet absorption method using a Hitachi 124 spectrophotometer. BZM in BZM/pectin coprecipitate was determined as follows: The coprecipitate was dissolved in a small amount of dilute hydrochloric acid and was diluted with 1/30 M phosphate buffer of pH 7.0 and the absorbance at 306 nm was measured. Reaction in the effluent sclution of gel chromatography was determined by calorimetric method using carbazole.[15]

Centrifugation Method for Determination of the Amount of Drugs Bound to Pectin——According to the method reported by Graham,[7] 5 ml of 1% aqueous solution of pectin and 5 ml of aqueous solution of drugs were put into a centrifugation tube and shaken gently. Concentration ranges of drugs were as follows: APZ, 1.31×10^{-4}—1.31×10^{-3} M; BZM and MPZ, 8×10^{-3}—8×10^{-2} M; KPF, 1.36×10^{-3}—1.36×10^{-2} M; PBZ, 5.6×10^{-4}—5.6×10^{-3} M. The mixed solution was warmed and kept at 45° for 15 min and after cooling to room temperature the solution was centrifuged by using a Kubota swinging type centrifugation apparatus at 3000 rpm for 10 min. The concentration of drugs in the supernatant was determined, and the amount of drugs bound to pectin was calculated.

Equilibrium Dialysis Method for Determination of the Amount of Drugs Bound to Pectin——According to the method reported by Meyer,[16] a Visking cellulose tubing containing 10 ml of drugs in water or buffer solution was immersed in 30 ml of water or buffer solution in a Nessler tube, being kept at 10° and the concentration of drugs in a Nessler tube determined at appropriate time intervals and the amount drugs bound to pectin was calculated.

Solubility Method for Determination of the Amount of Drugs Bound to Pectin——According to the method reported by Higuchi,[17] solubility of drugs with various concentration of pectin was determined at 10°.

Preparative Method of Coprecipitate (Complex) of BZM and Pectin——A coprecipitate (complex) of BZM and pectin was prepared according to method described by Graham.[18] Five ml of 30% aqueous solution of BZM was added to 50 ml of 2% aqueous solution of pectin, and the solution was agitated at room temperature for 5 min, then warmed and kept at 45° for 15 min. The solution was kept overnight at 5°, and a coprecipitate formed was filtered and washed with both 30 ml water and 30 ml methanol and dried under reduced pressure over anhydrous phosphoric acid in a desiccator at 60° for 3 hours.

Infrared (IR) Absorption Spectroscopy——IR absorption spectroscopy was measured using a Shimadzu Model IR-400 infrared spectrophotometer according to the KBr disk method.

X-Ray Diffraction Studies——Powder X-ray diffractometry was carried out using a Rigaku Denki Geigerflex Model D-2 diffractometer by Ni-filtered Cu-Kα radiation.

Differential Scanning Calorimetry (DSC)——Differential scanning calorimetry was carried out using a Perkin-Elmer Model DSC 1B differential calorimeter, in the sample pan for solid sample at the scanning speed of 4°/min.

14) This was determined by gel chromatography method using Sephadex G-100 under the condition as follows: Column length, 89 cm; inner diameter of column, 1.7 cm; effluent solution, 1/30 M Clark–Lubs buffer solution of pH 7.0, referring to bovine serum albumin (69000), pepsin (35000) and ribonuclease A (14000) as standards.
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16) M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).
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18) H.D. Graham, Y.M. Baker, and A.N. Njoku-Obi, *J. Pharm. Sci.*, **52**, 192 (1963).

Determination of Dissolution Rate——The dissolution rate was determined by a stationary disk method, using the apparatus described in the previous paper.[19] Three-tenths grams of powder sample, which passed 100 mesh, was compressed in a cylindrical die by a Shimadzu hydraulic press for KBr tablets for infrared spectroscopy. The compressed disk was not ejected out of the die, and the cavity was stoppered. The die wearing the compressed disk was set on the dissolution apparatus so as to make the disk face to the stirrer. Every experiment was carried out under the following conditions: 200 ml of distilled water as the dissolution medium; at 37°; 300 rpm of rotating velocity of the stirrer; and 1.5 cm diameter of the disk of the drug compressed under 200 kg/cm^2 for 3 min. One ml of the solution was sampled out at appropriate time intervals, the resultant want of volume was compensated by adding the dissolution medium of the same temperature.

Results and Discussion

Interaction between Drugs and Pectin observed by Centrifugation Method

According to the centrifugation method, binding isotherms of BZM, KPF and MPZ were obtained as shown in Fig. 1. A coprecipitate was formed between BZM and pectin in an equilibrium concentration range between 1.2—3.2$\times10^{-2}$ M, and the binding isotherms showed a multi-layer binding type. KPF bound a little to pectin and formed a coprecipitate. On the other hand, APZ and PBZ did neither form coprecipitates with pectin nor bind to pectin. Graham[18] reported that promazine hydrochloride, a cationic drug, formed precipitates with carboxylic acid type polyelectrolytes such as carboxymethylcellulose or pectin showing binding isotherms of Langmuir type. In this study, BZM, also a cationic drug, easily formed a coprecipitate with pectin, while its binding isotherm was not of Langmuir type. The reason of this difference may be explained by considering that the equilibrium concentration of promazine of Graham's experiment was lower as compared with that of BZM of our experiment.

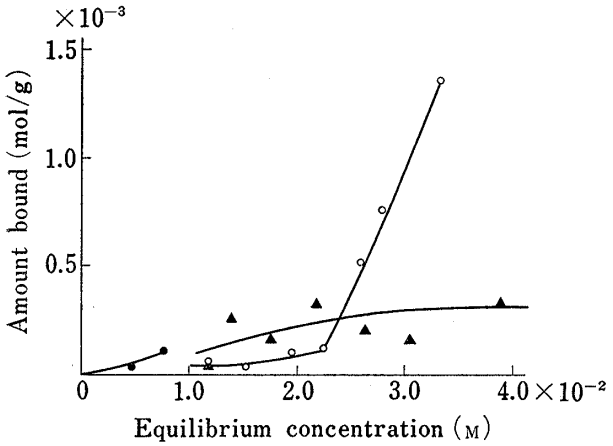

Fig. 1. Binding Isotherms of BZM, KPF and MPZ to Pectin by Centrifugation Method in Distilled Water at 10°

○: BZM; ●: KPF; ▲: MPZ.

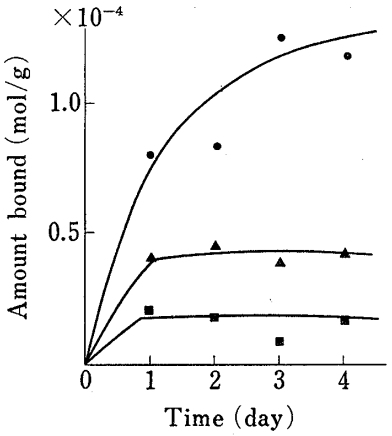

Fig. 2. Time Course of Binding of BZM to 1% Pectin at 10° by Equilibrium Dialysis Method at Initial Concentration of BZM 1.0$\times10^{-3}$ M

●: in distilled water.
▲: in 1/30 M Clark-Lubs buffer solution of pH 7.0.
■: in 1/10 M Clark-Lubs buffer solution of pH 7.0.

Interaction between BZM and Pectin observed by Equilibrium Dialysis Method

As a result observed by the centrifugation method mentioned already it was shown that an interaction between BZM and pectin was strong. Therefore, an investigation was carried

19) Y. Hamada, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.* (Tokyo), 23, 1205 (1975).

out by the equilibrium dialysis method in low equilibrium concentration range, where no coprecipitate was formed. As shown in Fig. 2, the binding of BZM to pectin reached equilibrium after 3 days by this method, and sampling was made after 3 days. The amount bound was the highest in distilled water, decreasing with an increase of the concentration of the buffer solution. This result suggested that cationic ions in the buffer solution bound to an anionic polyelectrolyte inhibiting the binding of BZM.

Figure 3 shows the binding isotherms of BZM in distilled water and in 1/30 M Clark–Lubs buffer solution of pH 7.0, and no coprecipitate was observed in equilibrium concentration range from zero to 7×10^{-4} M. Similarly to the result for BZM mentioned above, the amount bound decreased with an increase of the concentration of the buffer solution. Both the results obtained by the centrifugation method and the equilibrium dialysis method showed that the binding isotherms were in Langmuir type in lower equilibrium concentration range where no coprecipitate was observed and in multi-layer binding type in higher equilibrium concentration range where a coprecipitate was observed.

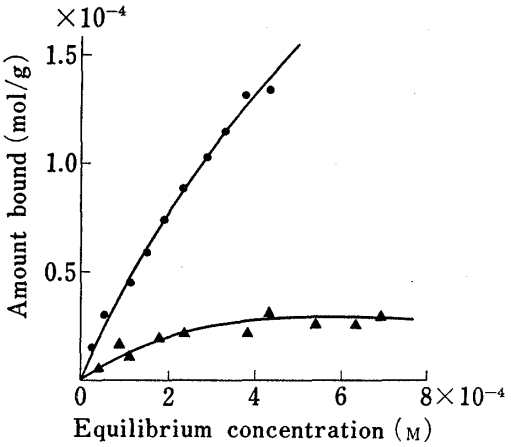

Fig. 3. Binding Isotherms of BZM to Pectin at 10° in Distilled Water and in 1/30 M Clark-Lubs Buffer Solution of pH 7.0

●: in distilled water.
▲: in 1/30 M Clark-Lubs buffer solution of pH 7.0.

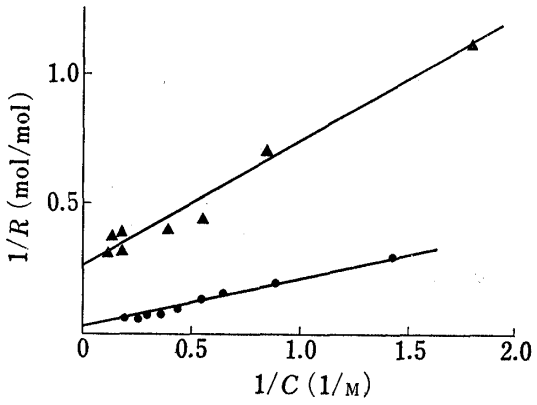

Fig. 4. Scatchard Plots of Binding of BZM to Pectin in Distilled Water and in 1/30 M Clark-Lubs Buffer Solution of pH 7.0 at 10°

●: in distilled water.
▲: in 1/30 N Clark-Lubs buffer solution of pH 7.0.
C: equilibrium concentration (M).
R: moles of BZM bound to a mole of pectin.

Saturated amount bound per mol of pectin when an entire surface was covered with a monolayer of BZM molecule, A, was obtained from Langmuir plots as follows: 57.6 mol/mol in distilled water and 2.8 mol/mol in the buffer solution. The value A in distilled water was about 20 times larger than that in the buffer solution. This result suggested that the pectin preferentially bound to cationic ions in the buffer solution than BZM. The numbers of binding sites in one molecule of pectin, n, obtained from Scatchard plots shown in Fig. 4 were 50.0 and 4.0 mol/mol in distilled water and in the buffer solution, respectively. The value n in distilled water was 12.5 times larger than that of in the buffer solution. This difference may also be explained in a similar way as that for A mentioned above.

Interaction between Drugs and Pectin observed by Solubility Method

From the results of the centrifugation method and the equilibrium dialysis method, an interaction between BZM and pectin was recognized in aqueous solution, then the solubility method was carried out to observe an interaction between other antiinflammatory drugs and pectin. As shown in Fig. 5, the solubility of APZ and IMC reached equilibrium after 3 days in 1/30 M phosphate buffer solution both with and without 0.5% pectin.

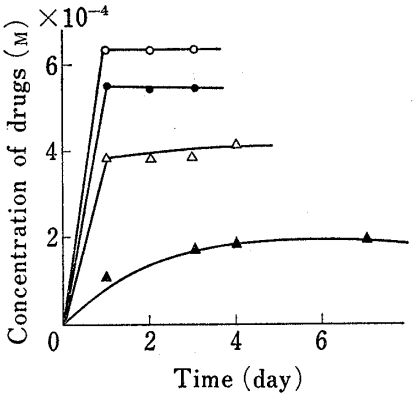

Fig. 5. Time Course of Solubility of APZ and IMC in 1/30 M Phosphate Buffer Solution of pH 7.0 with and without 0.5% Pectin at 10°

●: APZ with pectin.
○: APZ without pectin
▲: IMC with pectin.
△: IMC without pectin.

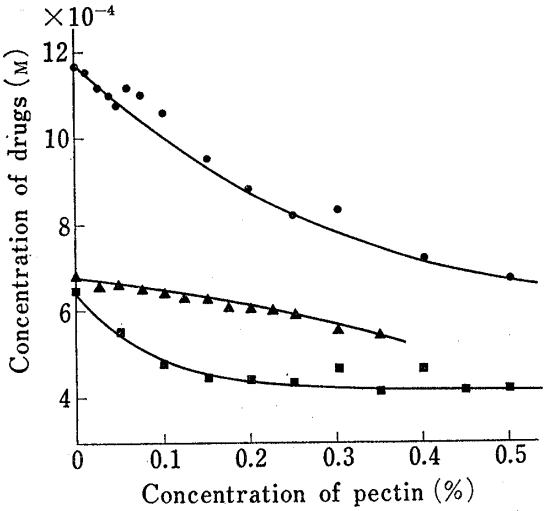

Fig. 6. Effect of Pectin on Solubility of APZ, FFA and IMC in 1/30 M Phosphate Buffer Solution of pH 7.0 at 10°

●: APZ; ▲: FFA; ■: IMC.

The solubility of other drugs such as FFA, KPF and PBZ also reached equilibrium after 3 days. Then the samples were taken at after 3 days. The solubility of APZ, FFA and IMC in Fig. 6 and also KPF and PBZ in Fig.7 decreased with an increase of the concentration of pectin. The similar phenomenon was reported by Higuchi.[17] As a result it is evident that pectin forms water insoluble complex with APZ, FFA, IMC, KPF and PBZ.

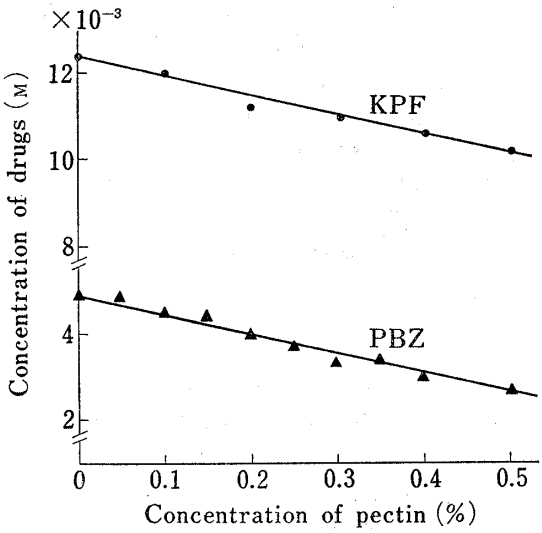

Fig. 7. Effect of Pectin on Solubility of KPF and PBZ in 1/30M Phosphate Buffer Solution of pH 7.0 at 10°

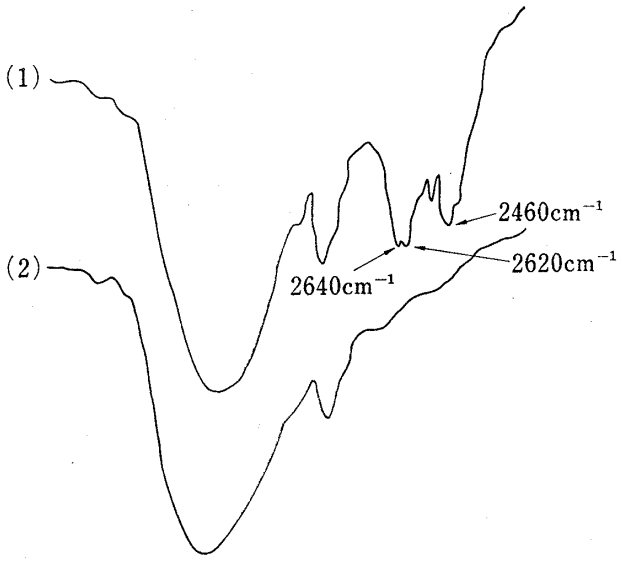

Fig. 8. IR Absorption Spectra of BZM-Pectin System According to the KBr Disk Method

(1): physical mixture of BZM and pectin.
(2): BZM/pectin coprecipitate (content of BZM 37 w/w%).

IR Absorption Spectroscopy, X-Ray Diffraction Studies and DSC of BZM/Pectin Coprecipitate

BZM formed a coprecipitate with pectin in aqueous solution. The content of BZM in the coprecipitate determined by ultraviolet absorption method was 26 and 37 w/w %. Figure 8 shows IR absorption spectra of the coprecipitate in comparison with the physical mixture. Absorption bands at 2460, 2620 and 2640 cm^{-1}, which are considered due to the hydrochloride

of the tertiary amines of BZM and observed for the physical mixture, disappeared in the coprecipitate. This result suggested that the tertiary amines of BZM bound strongly to carboxyl residues of galacturonic acid in pectin molecule. Figure 9 shows the powder X-ray diffraction patterns of the coprecipitate and the physical mixture of BZM and pectin. The physical mixture showed several steep peaks in the diffraction pattern but the coprecipitate showed no steep peak and was found to be amorphous. Also the coprecipitate had no endothermic peak in DSC curve, while intact BZM, pectin and the physical mixture had it, as shown in Fig. 10. This seems to have relation to the amorphous nature of the coprecipitate. These results

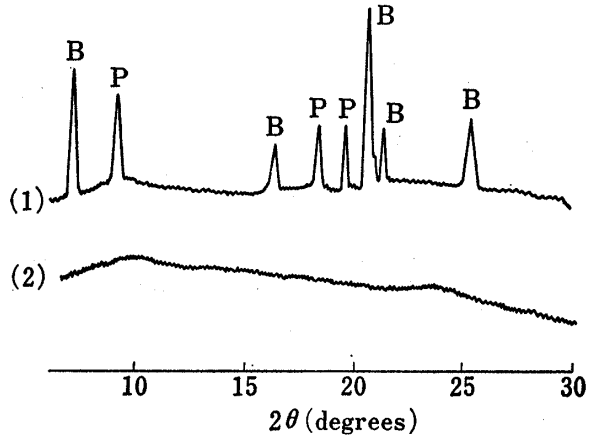

Fig. 9. Powder X-Ray Diffraction Pattern of BZM–Pectin System by Cu-Kα Radiation

(1): physical mixture of BZM and pectin.
(2): BZM/pectin coprecipitate (content of BZM 37 w/w%).
B: diffraction peak attributable to BZM.
P: diffraction peak attributable to pectin.

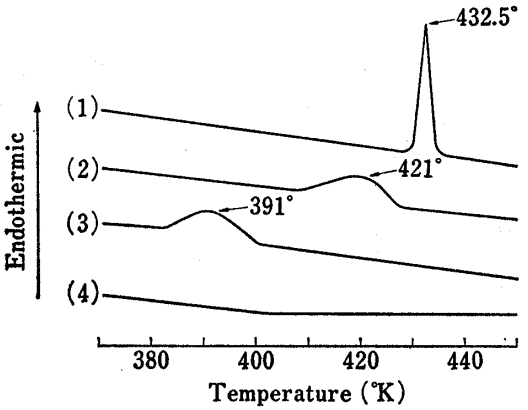

Fig. 10. DSC Curves of BZM–Pectin Systems at Scanning Speed of 4°/min

(1): intact BZM.
(2): intact pectin.
(3): physical mixture of BZM and pectin.
(4): BZM/pectin coprecipitate (content of BZM 37 w/w%).

showed that pectin inhibited the recrystallization of BZM by forming a complex and that the coprecipitate was different in physicochemical properties from the physical mixture of BZM and pectin.

Dissolution Rate of BZM/Pectin Coprecipitate

The dissolution rate of BZM/pectin coprecipitate was measured in comparison with the physical mixture of BZM and pectin or galacturonic acid, a main monomer component of pectin. The dissolution rate of the physical mixture with galacturonic acid or intact powder of BZM was too high to obtain a very accurate dissolution parameter, and thus the apparent initial dissolution rate K' was obtained from dissolution curves in Fig. 11, as shown in Table I. The relative ratio of K' of the physical mixture of BZM and pectin against K' of the coprecipitate was 0.90 and both K' were almost same, but those of the physical mixture of BZM and galacturonic acid was 106 and intact BZM was 98.7 and both K' were almost same. These results indicate that a water insoluble complex between BZM and pectin was rapidly formed, and there seemed no interaction between BZM and galacturonic acid.

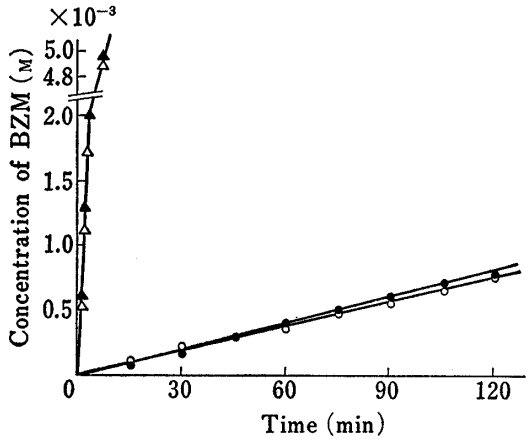

Fig. 11. Dissolution Curves of BZM, Pectin and Galacturonic Acid Systems in Distilled Water at 37° by Stationary Disk Method

●: BZM/pectin coprecipitate (content of BZM 26 w/w%).
○: physical mixture of BZM and pectin (content of BZM 26 w/w%).
▲: physical mixture of BZM and galacturonic acid) content of BZM 26 w/w%).
△: intact BZM.

Table I. Initial Dissolution Rate K'

Samples	K' (M/min)	Ratio[a]
BZM/pectin coprecipitate	6.82×10^{-6}	1.00
Physical mixture of BZM and pectin	6.15×10^{-6}	0.90
Physical mixture of BZM and galacturonic acid	7.23×10^{-4}	106
Intact BZM	6.73×10^{-4}	98.7

a) Against K' of BZM/pectin coprecipitate.

From pharmaceutical point of view, this result gave a suggestion of usefulness for preparing sustained-release preparations of BZM by using pectin, lowing an adverse reaction or toxicity because of the slow dissolution rate of BZM/pectin system. Also adverse reactions of other nonsteroidal antiinflammatory drugs to stomach after oral administration might be reduced by this kind of sustained-release preparations.

Acknowledgement The authors are very grateful to Mr. Toshio Kuroda and Mr. Yoshiaki Saito of Wakamoto Pharmaceutical Company for their various supports in this work. Thanks are also given to Mrs. Miyuki Hirai (née Inada) and Mr. Tatuo Kobayashi for their assistance in the experimental work.