CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 18, No. 6 June 1970

Regular Articles

[Chem. Pharm. Bull. 18(6)1083—1090(1970)]

UDC 615.356.011.5 : 577.164.11

Interaction of Drugs with Polymers. III.[1] Phase Separation of Polyacids by O-Benzoylthiamine Disulfide Hydrochloride and Gastrointestinal Absorption of O-Benzoylthiamine Disulfide-Polyacid Complexes[2]

Nobuo Tanaka, Goichi Hirata and Isamu Utsumi

Pharmaceutical Research Laboratory, Tanabe Seiyaku Co., Ltd.[3]

(Received June 2, 1969)

The precipitation reaction of polyacrylic acid (PAA), polymethacrylic acid (PMA), polyvinyl alcohol sulfate (PVS), and polyvinyl alcohol phosphate (PVP) with O-benzoylthiamine disulfide (BTDS) has been investigated as a function of BTDS concentration, molecular weight, acid strength, and neutralization degree of them in aqueous solution. This precipitation reaction was found to be independent of molecular weight as long as the molecular weight is in the high polymer range, but remarkably to vary with BTDS concentration, acid strength, and neutralization degree. The extent of BTDS cation binding increases in the order PMA<PAA<PVP<PVS. On the other hand, the rate of release of BTDS from the polyacid complexes in 0.1N HCl aqueous solution decreases in the order BTDS–PVP>BTDS–PMA>BTDS–PAA>BTDS–PVS. The blood total thiamine level after oral administration of the BTDS-polyacid complexes into rabbits has also been investigated. The BTDS–PVS complex exhibited sustained blood level, but with the other complexes, sustained blood levels were not observed.

In the previous parts[1,4] of this series it has shown that the presence of inorganic or organic cations affects the phase separations of polyelectrolytes.

In the present study to examine the effects of molecular weights (MW), acid groups, and neutralization degrees (α) of polyacids on precipitation reactions in aqueous solution O-benzoylthiamine disulfide hydrochloride (BTDS·HCl) which showed the strongest precipitating action dealt in part II[1] was used. These effects were considered in terms of the precipitation temperature (T_p) and the analysis of the precipitated complexes from reaction systems. Moreover, *in vivo* sustained or timed release of O-benzoylthiamine disulfide (BTDS) from the BTDS-polyacid complexes formed through precipitation reactions of polyacids with BTDS·HCl in aqueous solution was investigated, since such complexes may be of value as the incor-

1) Part II: N. Tanaka, G. Hirata and I. Utsumi, *Chem. Pharm. Bull.* (Tokyo), **14**, 414 (1966).
2) Presented at the 86th Annual Meeting of Pharmaceutical Society of Japan, Toyama, April 1966.
3) Location: *Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) N. Tanaka, G. Hirata and I. Utsumi, *Yakugaku Zasshi*, **85**, 799 (1965).

poration of polymer in sustained release preparations which have been receiving much attention in industrial pharmaceutical research recently.[5]

As the polyacids for this study polyacrylic acid (PAA), polymethacrylic acid (PMA), polyvinyl alcohol phosphate (PVP), and polyvinyl alcohol sulfate (PVS) were chosen whose structures of polymers are simple and similar in most respects except acid groups.

Results and Discussion

Precipitation Equilibrium of the BTDS-Polyacid Systems

The BTDS concentrations in the supernatant solutions resulting from the reaction of various concentrations of BTDS·HCl in 0.1 eq./liter[6] PAA ($\alpha=0$, mol.wt=208000) aqueous solution at 3° are given in order to be compared with the precipitation temperatures (T_p) of those reaction systems in Fig. 1. The BTDS concentration curve exhibits the straight line of the slope of about 1, having an inflection point at about 0.01N of added BTDS·HCl concentration. On the other hand, the T_p curve also shows a distinct maximum at the same concentration (0.01N) of BTDS·HCl as the inflection point of the BTDS concentration curve. This coincidence suggests that the BTDS–PAA complex formed by reaction between aqueous solution of 0.01N BTDS·HCl and 0.1 eq./liter PAA is most hydrophobic, since generally the hydration of polyelectrolyte decreases with increasing T_p.

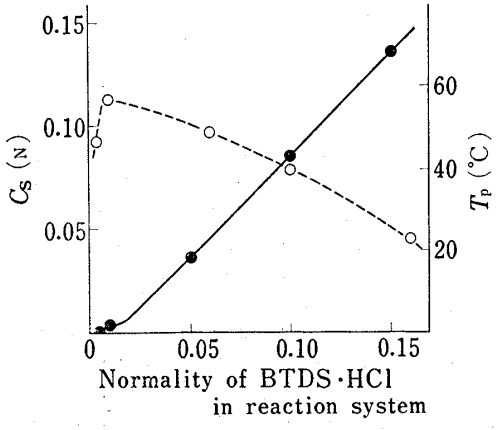

Fig. 1. The BTDS Concentration (C_S) in the Supernatant Solutions and the Precipitation Temperatures (T_p), at 0.1 eq./liter of PAA ($\alpha=0$), as a Function of BTDS Concentration

○: T_p ●: C_S

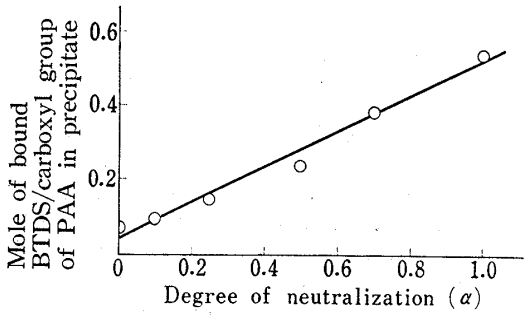

Fig. 2. BTDS–PAA Ratios in the Precipitates as a Function of Degree of Neutralization

Compositions of the precipitates resulting from the above mentioned reaction systems were also investigated and shown in Table I. In the BTDS·HCl concentration range of 0.005 to 0.15N, almost all the PAA are precipitated, while at the BTDS·HCl concentration of 0.001N the amount of the precipitated PAA is not obtained enough to be measurable. The number of mole of bound BTDS per carboxyl group of PAA in the precipitated complex increases 0.024 to 0.076 with increasing added BTDS·HCl concentrations and at that of 0.15N decreases reversely to 0.067.

5) H.D. Graham and Y.M. Baker, *J. Pharm. Sci.*, **52**, 964 (1963); H.D. Graham, Y.M. Baker and A.N.N. Obi, *ibid.*, **52**, 192 (1963); B.B. Wolfson and G.S. Banker, *ibid.*, **54**, 195 (1965); S.N. Ushakov and E.F. Panarin, *Dokl. Akad. Nauk. SSSR.*, **147**, 1102 (1962) [*C.A.*, **58**, 11168 g (1963)].

6) All concentrations of polyacids were expressed in terms of gram equivalent monomer per liter (eq./liter).

Table I. Compositions in the Precipitates and the Supernatant Solutions from the Reaction of Varying Amounts of BTDS·HCl in 6 ml of 0.1 eq./liter PAA (α=0) Solutions at 3°

Original BTDS·HCl concn. (N)	Supernatant soln. BTDS (mg)	Precipitate		
		BTDS (mg)	PAA (mg)	Mole of bound BTDS per carboxyl group of PAA
0.150	316.6	30.3	42	0.067
0.100	197.8	31.8	39	0.076
0.050	85.4	30.2	39	0.072
0.010	8.0	15.1	38	0.037
0.005	1.9	9.6	38	0.024
0.001	1.9	0.3	—	—

Ikegami, *et al.*[7] have stated that a mechanism of the precipitation of PAA by divalent cations (M^{2+}) is based on at least two kinds of typical binding forms; one form is (COO–Me^{+} +COOH), accompanied by a small amount of dehydration, and the other form is (COO–Me–OOC), accompanied by a large amount of dehydration. On the analogy of this assumption, the precipitation of PAA by BTDS cation (B^{2+}) may qualita tively be interpretated in the following way.

In the case of the above precipitation at α=0, because the fraction of bound B^{2+} in the form of (COO–B–OOC) increases with increasing added B^{2+} concentrations (0.001 to 0.01N), and by this ion binding the hydration of COOH groups on a polymer chain is directly depressed, consequently the T_p rises. However, above the added B^{2+} concentration of 0.01N, the T_p falls with B^{2+} concentrations, because as the concentration of B^{2+} increases, not only the uncharged complex (COO–B–OOC) but also a more hydrate complex (COO–B^{+}+COOH) appears correspondingly.

Table II. Compositions in the Precipitates and the Supernatant Solutions from the Reaction of Varying Degrees of Neutralization of PAA (0.1 eq./liter) in 6 ml of 0.1N BTDS·HCl Solutions at 3°

α	T_p (°C)	Supernatant soln. BTDS (mg)	Precipitate		
			BTDS (mg)	PAA (mg)	Mole of bound BTDS per carboxyl group of PAA
0.00	39.3	197.8	31.8	39	0.076
0.10	>100	190.9	40.4	39	0.097
0.25	>100	169.5	61.8	40	0.14
0.50	>100	137.4	93.9	39	0.23
0.75	>100	64.8	166.4	40	0.39
1.00	>100	6.1	225.2	39	0.54

In equimole mixtures (0.1 eq./liter) of BTDS·HCl and various neutralization degrees (α) of PAA in aqueous solutions, although almost all the PAA are precipitated regardless of α, the number of mole of bound BTDS in complexes increases linearly with increasing α (Fig. 2, Table II). Above α=0.1 all the mixtures remained insoluble up to 100°. From these results, it is seen that the probability of binding of B^{2+} with two dissociated carboxyl groups (A^{-}) will be favored over its binding with two undissociated carboxyl groups (AH),

7) A. Ikegami and N. Imai, *J. Polymer Sci.*, **56**, 133 (1962).

and be proportional to α. Therefore, on the assumption proposed by Ikegami, *et al.*,[7] the difference of T_p at $\alpha=0$ and above $\alpha=0.1$ may be interpreted as the difference of the hydration extent of the carboxyl group-cation complex; at $\alpha=0$, B^{2+} are bound to carboxyl groups in the form of AB^+ and of A_2B, but above $\alpha=0.1$, since the fraction of bound B^{2+} in the form of A_2B which is more hydrophobic than that of AB^+ becomes more than at $\alpha=0$, the T_p rises shaply above 100°.

Table III. Compositions in the Precipitates and the Supernatant Solutions from the Reaction of Varying Ionizable Groups of Polyacid (0.1 eq./liter, $\alpha=0$) in 6 ml of 0.1N BTDS·HCl Solutions at 3°

Polymer	MW×10^{-3}	T_p (°C)	Supernatant soln. BTDS (mg)	Precipitate		
				BTDS (mg)	Polymer (mg)	Mole of bound BTDS per ionizable group of polymer
PAA	208	39.3	197.8	31.8	39	0.076
PAA	400	37.8	197.1	32.5	39	0.078
PAA	3000	38.0	196.8	34.5	41	0.079
PMA[a)]	110	83.3	151.3	80.0	50	0.18
PVP	500[b)]	82.5	204.5	26.8	15	0.32
PVS	92	100	34.3	197.0	121	0.50

a) This reaction system contains 0.1N BTDS and 0.3N H_2SO_4.
b) This figure is degree of polymerization of polyvinyl alcohol used for the phosphorylation.

The data on the effects of various molecular weights (MW) and acid groups of polymer ($\alpha=0$) on the precipitation are listed in Table III. In the PAA system the T_p and the bound BTDS remaines essentially constant, and are independent of MW of PAA. According to the results of Costantino, *et al.*[8] the same phenomenon is found for PMA precipitation by divalent cations (Mg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}). Therefore, these results may be explained by the fact that the apparent dissociation constant is almost independent of molecular weights.

In the series of polyacids, the precipitation temperatures and the mole number of bound BTDS per acid group of polymer decrease in the order PVS>PVP>PMA>PAA (Table III). Among these systems, the mole number of bound BTDS in the PVP system was calculated as a monobasic acid, since the second dissociation constant of PVP is negligibly small ($pK_1=2.5$, $pK_2=6.8$), and it is not valid that the PMA system containing 0.1N BTDS·H_2SO_4 and 0.2N H_2SO_4, is compared with other systems. In fact, according to preliminary experiments, in the 0.1N BTDS·HCl–0.1 eq./liter PMA system no precipitation occurred until added concentration of hydrochloric acid amounted approximately to 0.3N, and also in the 0.1N BTDS·H_2SO_4–0.1 eq./liter PMA system excess addition of 0.2N sulfuric acid was needed to bring about the precipitation. From this experimental fact, it may be concluded that the BTDS cation being more strongly bound to polyacrylic acid than to the weaker polymethacrylic acid, the mole number of bound BTDS increases in the order PMA<PAA<PVP<PVS with increasing acid strength of the acid groups on the polymer chain.

Elucidation of Complex

In Fig. 3, the IR spectrum of the dry BTDS–PAA complex in KBr was compared with reference spectra of PAA, and the mechanical mixture which has the same composition as that of the BTDS–PAA complex (BTDS: 31.8 mg, PAA:39 mg) formed by precipitation reaction of 0.1 eq./liter PAA with 0.1N BTDS·HCl.

The characteristic NH_3^+ stretching absorption band of BTDS in the BTDS–PAA complex is obscured by a strong PAA band at 2500—3500 cm^{-1}, and no shifts are observed in the

8) L. Costantino, V. Crescenzi, F. Quandrifoglio and V. Vitagliano, *J. Polymer Sci.*, **A2**, **5**, 771 (1967).

bands of the BTDS–PAA complex. However, many characteristic absorption bands of BTDS are exhibited at 1600—1720, 1280, 1120, and 710 cm^{-1}. From these results no conclusions can be drawn with respect to the interaction of the carboxyl groups (or hydrocarbon segments) on PAA with amino groups (or the aromatic π-electrons) on BTDS. On the other hand, as seen in Fig. 4, the UV spectrum of the BTDS–PAA complex dose not exhibit the absorption maximum at 275 mμ, and also are almost the same as that of BTDS·HCl which is brought about by the NH_3^+ of BTDS molecule, (the UV spectrum of the uncharged BTDS have two absorption maxima at 231 and 275 mμ). Therefore, from this result and the absence of Cl^- by the $AgNO_3$ test, it may be concluded that the precipitated complexes are formed by the electrostatic binding between the COOH (or COO^-) on PAA and the NH^+ on BTDS.

Release of the BTDS from the Complex *in Vitro*

Fig. 5 shows the results of elution experiments of the dry BTDS–polyacid complexes (particle diameter:below 44 μ) listed in Table III with 0.1N HCl as eluant. When the per-

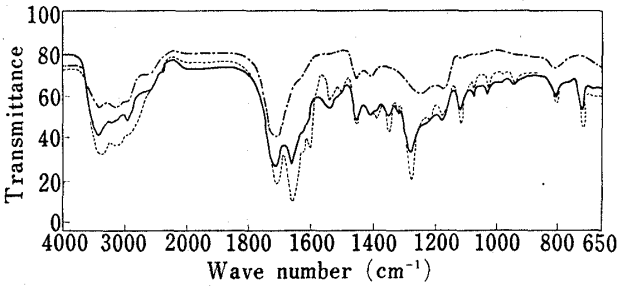

Fig. 3. Infrared Absorption Spectra of BTDS-PAA Systems (BTDS:31.8 mg, PAA:39 mg) in KBr

———: BTDS–PAA complex
----------: mechanical mixture
– – – – –: PAA

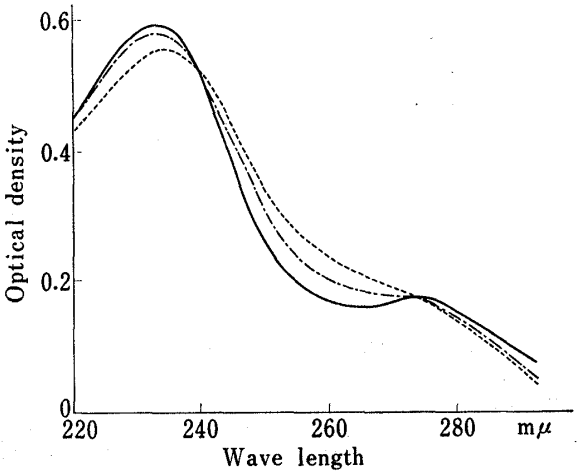

Fig. 4. Absorption Spectra of BTDS–PAA Complexes, BTDS, and BTDS·HCl

———: BTDS in 2.7% MeOH aqueous solution
– – – – –: BTDS–HCl in 2.7% MeOH aqueous solution
----------: BTDS–PAA complex in 2.7% MeOH aqueous solution

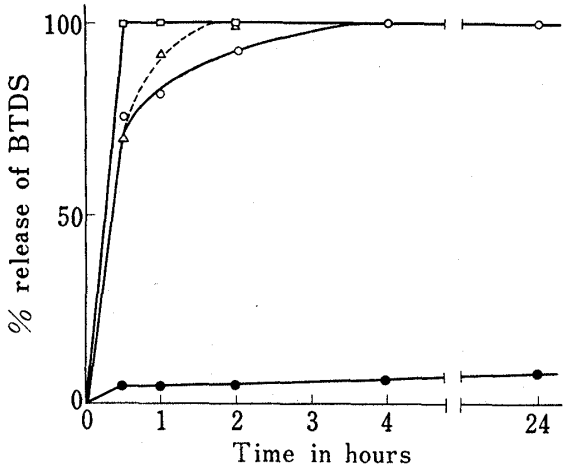

Fig. 5. Release of BTDS from BTDS–Polyacids Complexes (Particle Diameter; below 44μ) in 0.1N HCl at 30°C

○: BTDS–PAA complex (mol. wt=400000)
△: BTDS–PMA complex
□: BTDS–PVP complex
●: BTDS–PVS complex

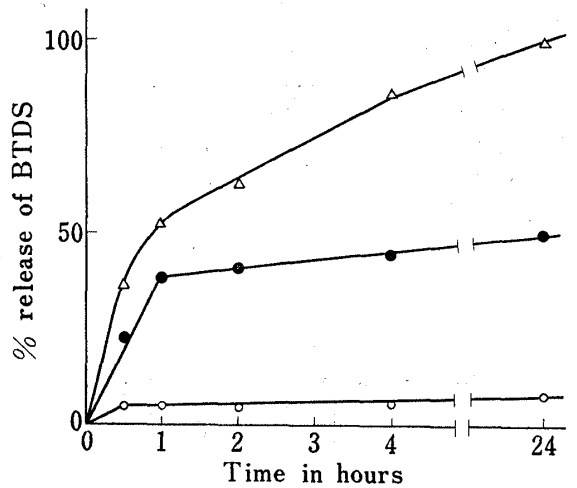

Fig. 6. Effect of Various Concentration of HCl on the Release of BTDS from BTDS–PVS Complex (Particle Diameter; below 44 μ) at 30°C

○: 0.1N HCl ●: 1N HCl △: 2N HCl

centage release of BTDS from the complexes is plotted against time of contact with 0.1N HCl, it is seen that the rate of release of BTDS from the complex decreases in the order BTDS-PVP>BTDS-PMA>BTDS-PAA>BTDS-PVS. Among them, the BTDS-PVS complex whose T_p is highest is almost insoluble in 0.1N HCl, but the percentage release of BTDS after 24 hours increases gradually 8 to 100% with increasing concentration of HCl 0.1 to 2N (Fig. 6).

Release of the BTDS from the Complex *in Vivo*

In order to ascertain if sustained release of the BTDS from the BTDS-polyacid complexes could be achieved, the complexes of an equivalent dose of 10 mg/kg body weight as thiamine hydrochloride were orally administered to five male rabbits.

The influence of particle sizes of BTDS-PAA (MW=208000) complexes on the blood total thiamine levels is shown in Fig. 7. Although the complexes with particle diameter of 1000—1410, 297—590, and below 44 μ were used, no great difference was observed between the blood level curves. However, the time needed to arrive at a maximum blood level was retarted about two hours as compared with BTDS alone. Next, as seen in Fig. 8, this behaviour of the blood level also holds true in the case where the complexes of MW=208000 and 400000 were administered. This is due to the fact that particle sizes of complexes and MW of PAA have not a great effect upon the dissolution rate of complexes in digestive juice. Last, the effects of acid groups of polyacids on the blood level were investigated in order to obtain sustained release form of the BTDS-polyacid complexes.

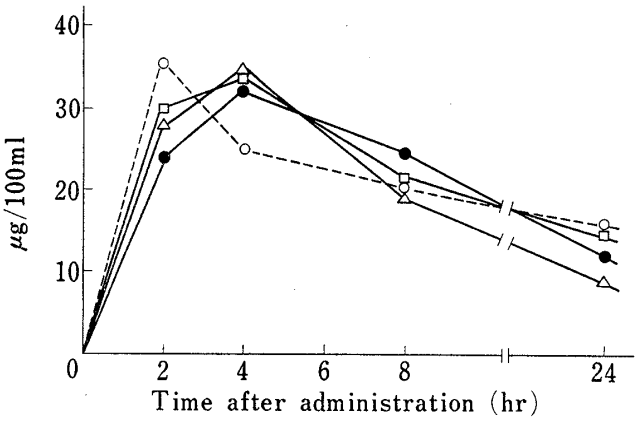

Fig. 7. Effect of Particle Sizes of BTDS-PAA Complex on the Blood Total Thiamine Levels after Oral Administration of it 10 mg/kg Body Weight as Thiamine Hydrochloride (One Group: Five Rabbits)

○: BTDS alone
□: below 44 μ diameter
△: 297—590 μ diameter
●: 1000—1410 μ diameter

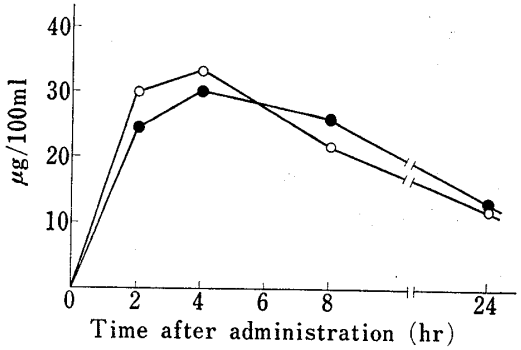

Fig. 8. The Effect of Molecular Weights of PAA on the Blood Total Thiamine Levels after Oral Administration of BTDS-PAA Complexes (Particle Diameter: below 44 μ) 10 mg/kg Body Weight as Thiamine Hydrochloride (One Group: Five Rabbits)

○: mol. wt=208000 ●: mol. wt=400000

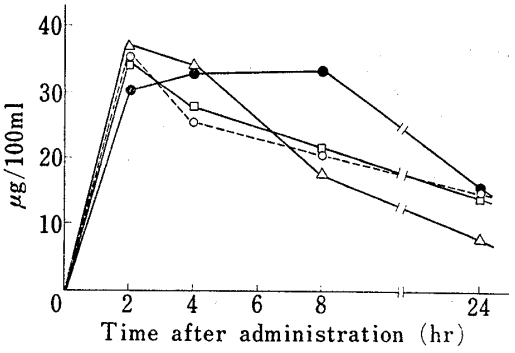

Fig. 9. The Effect of Ionizable Groups of Pfolyacid on the Blood Total Thiamine Levels after Oral Administration of a Series of BTDS-Polyacids Complexes (Particle Diameter: below 44 μ) 10 mg/kg Body Weight as Thiamine Hydrochloride (One Group: Five Rabbits)

○: BTDS alone
●: BTDS-PVS complex
△: BTDS-PMA complex
□: BTDS-PVP complex

From Fig. 9 the blood level curves for the BTDS–PMA and –PVP complex were almost the same as that for BTDS alone. However, only the BTDS–PVS complex exhibited sustained blood level as might be expected from the precipitation temperature and the strong ion binding.

From the above results, it is found that *in vivo* the gastrointestinal absorption of BTDS from the complexes is closely related to the rate of release of BTDS from them in HCl solution.

Experimental

Drugs——O-Benzoylthiamine disulfide and its hydrochloride were prepared by our laboratory.

Polyacrylic Acid (PAA)——Three PAA of different average molecular weight (MW) were used in this investigation. One polymer (MW=400000) was polymerized by reacting the purified glacial acrylic acid in 25% solutions of the monomer in dioxane using benzoyl peroxide catalyst for 6 hours at 40° under continuous nitrogen stirring. After stopping the reaction, the polymer precipitated from the reaction mixture using benzene as the precipitant was purified by dioxane–benzene reprecipitation, and dried in a vacuum oven at 60°. The two other polymers (MW=208000 and 3000000) were the commercial products carefully purified as described in part II.

The MW of the polymers was determined from the viscosity measurement in 2N NaOH solutions.[9]

The polymer concentration (equivalent per liter) of these solutions was standardized with KOH in the presence of 0.1M KCl using phenolphthalein as the indicator. A fully neutralized solution of $\alpha=1$ was obtained by adding a calculated amount of NaOH into a given volume of the stocked solution of $\alpha=0$. On the other hand, solutions of intermediated degrees of neutralization were obtained by mixing solutions of $\alpha=0$ and $\alpha=1$.

Polymethacrylic Acid (PMA)——Glacial methacrylic acid, purified by distillation at reduced pressure, was polymerized in aqueous solution at 50° with hydrogen peroxide as initiator. The polymer was precipitated from the reaction mixture by adding HCl, and then purified by water–HCl reprecipitation. Finally, the polymer was vacuum-dried over P_2O_5 and NaOH.

The MW of the polymers was found to be 110000 by the viscosity measurement in 0.002N HCl solutions.[10] The polymer concentration of these solutions was determined by titration with NaOH in the presence of 1M $NaNO_3$.

Polyvinyl Alcohol Sulfate (PVS)——PVS was prepared by passing commercial K-PVS, through Amberlite IR–120 and IR–4B columns at 5°, having the degree of esterification of 28%. Its MW was found to be 92000 by the viscosity measurement in 0.5M NaCl solutions.[11]

Polyvinyl Alcohol Phosphate (PVP)——The sample of PVP was prepared according to the method described by Daul, *et al.*[12] To a mixture of 50 g of 85% orthophosphoric acid and 29.2 g of urea was added 16.7 g of polyvinyl alcohol (degree of polymerization of 500) dissolved in 50 ml of water. This mixture was heated at 110° for 3 hours in an air oven with occasional stirring, and then heated at 160° for 30 minutes in an oil bath. The reaction mass was purified by water–alcohol, and then its aqueous solution was deionized by passages through Amberlite IR–120 and Amberlite IR–4B columns. Its degree of esterification (74%) were determined potentiometrically using NaOH.

Precipitation Temperature (T_p) Measurements——The T_p measurements were performed by the method described in Part II.

Analysises of Precipitation Systems——BTDS·HCl solutions and polymer solutions at appropriate concentrations were mixed to make 6 ml of mixtures at 50° in centrifuge tubes of 10 ml capacity. After allowed to stand at 3° for 24 hours, the mixture was centrifuged, and the supernatant carefully poured off. The BTDS-polyacid complexes precipitate was washed five times with 2 ml of distilled water, and dried in vacuum over P_2O_5 at 60°. From the weight of the dry precipitate and the BTDS concentration in the supernatant (or in the precipitate), the composition of the BTDS-polyacid precipitate system was determined (the BTDS concentrations were little affected by this H_2O-washing). In order to analyze the concentration of BTDS spectrophotometrically, the supernatant solutions (or the precipitates) were dissolved in mixed solvents of HCl–H_2O–MeOH (or DMF), and suitably diluted with water to give an absorbance of 0.3 to 0.6 at isobestic point ($\lambda=274$ mμ).

Elution Experiments——Each Complex equivalent to 30 mg of BTDS was transferred to a stoppered L-type glass tube and 15 ml of eluting solution was added. The tubes were clamped to arms attached to a shaft which caused them to oscillate at 15 rpm in a water bath at 30°. After each interval, a tube was removed and a sample of the liquid in it was spectrophotometrically analyzed for BTDS.

9) H. Ito, S. Shimizu and S. Suzuki, *Kogyo Kagaku Zasshi*, **59**, 930 (1956); A. Takahashi, Y. Hayashi and I. Kagawa, *ibid.*, **60**, 1059 (1957).
10) A. Katchalsky and H. Eisenberg, *J. Polymer Sci.*, **6**, 145 (1955).
11) A. Takahashi, M. Nagasawa and I. Kagawa, *Kōgyō Kagaku Zasshi*, **61**, 1614 (1958).
12) G.C. Daul, J.D. Reid and R.M. Reinhardt, *Ind. Eng. Chem.*, **46**, 1042 (1954).

***In Vivo* Experiments**——Five female rabbits, weighing 2.0 to 2.5 kg, were starved for 24 hours before the administration. The gelatin capsule containing BTDS-polyacid complexes listed in Table III were administered orally to these rabbits in a dose equivalent to 10 mg of thiamine hydrochloride per kg body weight, blood samples of 2.5 ml were taken by heart puncture of rabbits before the administration, and 2, 4, 8 and 24 hours after that.

The blood samples were assayed for total thiamine levels by the thiochrome method.[13]

Acknowledgement The authors wish to express their deep gratitude to the late Professor M. Aoki and Assistant Professor A. Kamata, Department of Pharmaceutical Science, Osaka University for their helpful suggestions and encouragement.

13) I. Utsumi, K. Harada, Y. Kondo and H. Hirao, *Vitamin*, **25**, 74 (1962).