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Application of the Cloud Point Method to the Study of the Interaction of Polyvinylpyrrolidone with Some Organic Compounds in Aqueous Solution

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Polyvinylpyrrolidone (PVP) aqueous solution exhibited coacervation by addition of some inorganic salts and organic compounds. The cloud point of PVP K-90 solution appeared by addition of the least amount of ammonium sulfate, among PVP K-15, K-30 and K-90. Effect of some aromatic compounds on the cloud point of PVP aqueous solution containing ammonium sulfate was investigated. The extent of the aromatic compounds to lower the cloud point of PVP solution differed depending upon their functional groups, number of their substituents, and mutual position of the substituents. Urea derivatives elevated the cloud point, its extent being increased with the number of substituted methyl groups. Thiourea derivatives, on the other hand, lowered the cloud point. The order of the compounds to lower the cloud point was in parallel with the order of extent of interaction studied by the equilibrium dialysis method.

Keywords——polyvinylpyrrolidone; cloud point; interaction; coacervation; urea derivatives; phenolic compounds; benzoic acid derivatives

Macromolecular additives are often used in various pharmaceutical dosage forms. Consequently, the interaction between the macromolecular additives and drugs are of importance in physicochemical and biopharmaceutical aspects; *i.e.*, effect of these additives on the solubilities or dissolution rates of drugs,[2] on the stabilities of unstable drugs,[3] and on the gastrointestinal absorption of drugs.[4] Since the interactions between the macromolecular additives and drugs are of great importance, serious consideration has to be given to them during the formulation of dosage forms when macromolecular additives are to be used.

The studies of the interaction between the macromolecular additives and drugs have been carried out by equilibrium dialysis,[5] solubility measurement,[2a,b] and viscometry,[6] *etc.*, but, these methods require much time before the equilibrium is reached, or tend to give much error in the measurements. The cloud point method was therefore examined to evaluate the interaction between polyvinylpyrrolidone (PVP) and some compounds.

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2) *a)* D. Guttman and T. Higuchi, *J. Pharm. Sci.*, **50**, 441 (1961); *b)* E.G. Rippie, D.J. Lamb, and P.W. Romig, *ibid.*, **53**, 1346 (1964); *c)* A.P. Simonelli, S.C. Mehta, and W.I. Higuchi, *ibid.*, **58**, 538 (1969); *d)* E.I. Stupak, H.A. Rosenberg, and T.R. Bates, *J. Pharmacokin. Biopharm.*, **2**, 511 (1974); *e)* H. Sekikawa, M. Nakano, and T. Arita, *Yakugaku Zasshi*, **98**, 62 (1978).

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Though PVP has a hydrophobic structure, its solubility is extremely high in water. When PVP is dissolved in water, the polymer molecule is expected to be hydrated, and the hydrated polymer chain spreads out in aqueous media. PVP aqueous solution is therefore considered to be hydrophilic colloid.[7)]

Hydrophilic colloids are more stable than hydrophobic colloids, and need high concentration of the electrolytes before they are flocculated. For instance, ovalbumin and hemoglobin are coagulated by addition of some sulfates and tartrates,[8)] and this process is termed "salting out." The aqueous solution of polymethacrylic acid is geled by raising the temperature of the solution.[9)] By addition of electrolytes or elevation of temperature, hydrophilic colloids lose their water, and dehydrated colloids begin to precipitate. The phenomenon is known as coacervation.[10)]

PVP aqueous solution is coacervated by addition of ammonium sulfate,[11)] but effect of drugs or organic compounds on coacervation of PVP aqueous solution is not fully studied, with an exception of phenolic compounds.[6,12)]

The authors investigated the coacervation of PVP aqueous solution by some inorganic salts, then they studied the effect of some organic compounds on the coacervation of PVP aqueous solution containing ammonium sulfate, and evaluated the interaction between PVP and these compounds by comparing the tendency of the compounds to cause the coacervation of PVP aqueous solution. When the coacervation begins to take place, PVP aqueous solution develops "microcoacervate," and the solution becomes cloudy. The authors applied this cloud point measurement to the study of interaction of the macromolecule and organic molecules in this study.

Experimental

Materials PVP——PVP K-15, K-30 and K-90 were obtained from Daiichi Pure Chemicals Co., Tokyo. In each PVP sample, monomer and oligomer fraction were extracted with ether using Soxhlet's extractor. The intrinsic viscosity listed in Table I was measured by Ostwald viscometer at 30.0°.

TABLE I. Polyvinylpyrrolidone

Grade	Intrinsic viscosity in water at 30° (dl/g)	Labeled average molecular weight
K-15	0.0769	10000
K-30	0.189	40000
K-90	1.72	360000

Salts——All of the inorganic salts were of reagent grade.

Organic Compounds——Benzoic acid and salicylic acid were of J.P. IX grade. Phenol, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, hydroquinone, catechol, phloroglucinol, aniline, nitrobenzene, *p*-nitrophenol, 2,4-dinitrophenol, picric acid, urea, methylurea, 1,3-dimethylurea, tetramethylurea, thiourea, and 1,3-dimethylthiourea were of reagent grade and were purified by recrystallization if necessary.

Apparatus for the Measurement of Cloud Point of PVP Solution——The measurement of cloud point of PVP solution was carried out in 50 ml water-jacketted beaker (35 mm in diameter) thermoregulated by water from the thermobath (Haake Model KT 33) connected to the programmer (Haake Model TP 32 and

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7) *a*) H.H.G. Jellinek and S.Y. Fok, *Kolloid-Z. Z. Polym.*, **220**, 122 (1967); *b*) N. Ise and T. Okubo, *J. Phys. Chem.*, **73**, 1488 (1969).

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12) D. Guttman and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 659 (1956).

Model PG 11) to raise temperature of the bath at a constant rate (0.5°/min, initially, then, 0.1°/min near the cloud point temperature). The solution was stirred by Teflon magnetic stirring bar (Fig. 1). The process was reversible, *i.e.*, the cloud in the solution disappeared upon lowering the temperature of the solution, and the clear solution resulted. Reproducibility of the cloud point measurement was excellent, within the range ±0.1°.

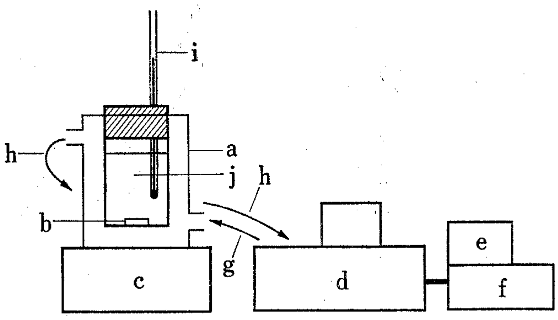

Fig. 1. Apparatus for Cloud Point Measurements

a: jacketted beaker, b: stirring bar, c: magnetic stirrer d: water bath, e: thermoelement, f: programmer, g: inlet for circulating water, h: outlet for water, i: thermometer, j: test solution.

Equilibrium Dialysis——Equilibrium dialysis method was used to evaluate the interactions between organic compounds and PVP in aqueous solution and to compare them with the results obtained by the cloud point method. Dialysis cells consisted of a pair of cylindrial half grass chambers with an O-ring and the membrane in-between. The capacity of each half cell was 20 ml and the surface area of the membrane was 5.3 cm^2. Vertical tubes in both half cells served as filling and sampling ports.[13] Possible preservatives in Visking Cellulose Membrane (18/32 type, Union Carbide Corp., Chicago) was leached by frequently changing water at about 60° for at least three days. Twenty ml of PVP solution (solution I) was pipetted into one compartment and 20 ml of the drug solution (solution II) into the other compartment separated by Visking membrane. As a reference, 20 ml of the solution without PVP (solution III), and 20 ml of the drug solution (solution IV) were pipetted into each compartment of another cell. The solution I was consisted of 5% PVP K-90 in 0.1 M hydrochloric acid. The solution II and IV were consisted of 10 mM benzoic acid derivatives in 0.1 M hydrochloric acid at pH 1.2. The solution III was 0.1 M hydrochloric acid. To take the effect of ammonium sulfate into consideration, ammonium sulfate was added to all the systems (0.5 M). pH of the solution was 1.3. Dialysis cells were set in a shaking water-bath (Taiyo M-1 N) maintained at 37.0±0.1°, and shaken for five days to ensure the equilibrium. Sample solution was analysed by means of Hitachi-Perkin Elmer 139 Spectrophotometer. Cellulose membrane did not show any measurable degree of binding or interaction with any of the solutes. After dialysis equilibrium, the concentration of the drug in each compartment was determined. Drug bound (%) to PVP was calculated as follows.

$$\text{Bound}(\%) = \frac{[C_I]-[C_{II}]}{[C_I]} \times 100$$

Where $[C_I]$ and $[C_{II}]$ are the equilibrium drug concentrations in the PVP solution and PVP-free solution, respectively.

Results and Discussion

Cloud Point of PVP Aqueous Solution

Hydrophilic colloids of polysaccharides and other synthetic polymers have been reported to be coacervated by addition of ammonium sulfate or other inorganic salts.[14] In this case, the coacervation tended to be caused easily at the high concentration of the hydrophilic colloids. Temperature of coacervation at which the coacervation takes place is correlated with the average molecular weight of the polymer. Jirgensons reported that PVP polymers of average molecular weight about 100000 and 20000 were coacervated in 0.76 M and 0.93 M ammonium sulfate, respectively at room temperature.[11]

The effects of the molecular weight and the concentration of PVP on the cloud point were investigated. Results are shown in Fig. 2a and 2b.

Figure 2a shows the effect of concentration of ammonium sulfate on the cloud point of PVP K-15, K-30 and K-90 solution. The cloud points of PVP aqueous solutions lowered linearly with increasing concentration of ammonium sulfate. PVP K-90, with the highest molecular weight among three types of PVP, was coacervated by addition of the least amount of ammonium sulfate at a given temperature. The amount of ammonium sulfate needed to

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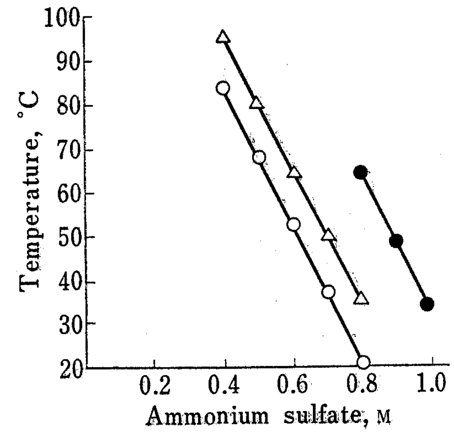
14) H. Denel and J. Solm, *Kolloid-Z.*, 124, 1 (1951).



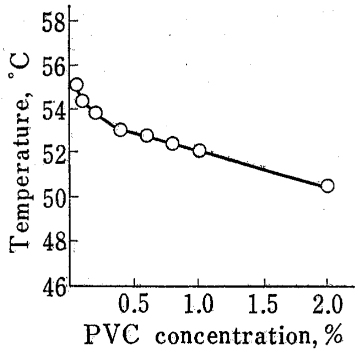


Fig. 2a. Effect of Molecular Weight of PVP and Concentration of Ammonium Sulfate on the Cloud Point of 1% PVP Solution

—●—; K-15 —△—; K-30 —○—: K-90

Fig. 2b. Effect of the Concentration of PVP K-90 Containing 0.6 M Ammonium Sulfate on the Cloud Point

coacervate the solution at a given temperature was in the following order; PVP K-90<K-30 <K-15. The cloud point in PVP K-90 appeared most sharply among three types of PVP.

Figure 2b shows the effect of PVP K-90 concentration on the cloud point. Cloud points appeared at lower temperature with higher concentration of PVP. When the concentration of PVP was below 0.4%, the slope in the graph was greater than that at higher concentration range. Between 0.5% and 2.0%, the cloud point-concentration relationship was linear. Aqueous solution containing 1.0% PVP K-90 was thus employed for further studies.

Effect of Inorganic Salts on the Cloud Point of PVP Aqueous Solution

The cloud point of 1% PVP aqueous solution was measured with addition of various inorganic salts. Fig. 3 shows the effects of sodium and potassium chloride.

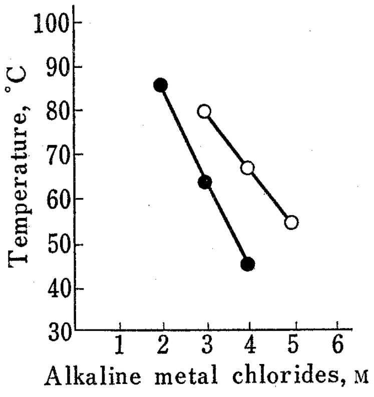


Fig. 3. Effect of Concentration of Sodium (○), and Potassium (●) Chloride on the Cloud Point of 1% PVP K-90 Solution

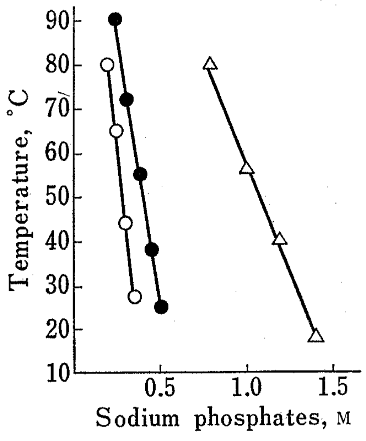


Fig. 4. Effect of Concentration of Mono (△) Di, (●) and Tri (○) Basic Sodium Phosphates on the Cloud Point of 1% PVP K-90 Solution

The ability of the potassium chloride to cause the coacervation was greater than that of sodium chloride. The addition of lithium chloride did not cause the coacervation under the experimental conditions (to 95°), even the salt concentration was over 5 M. This result is in accord with expectation that the ability to cause the coacervation of PVP aqueous solution is greater with the cation of larger periodic number. On the other hand, in the case of PVP solutions containing either potassium bromide or potassium iodide, no coacervation was observed under the experimental conditions. This suggests that the ability to cause the coacervation of PVP solution may be greater with the anion of smaller periodic number. This result shows that the effect of the cation on cloud point is opposite to Hofmeister's series, but that of anion is accordance with it.

Fig. 4 shows the effect of concentrations of monobasic, dibasic and tribasic sodium phosphates on the cloud point. The ability of the sodium phosphates to cause the coacervation increased with the number of negative charge on phosphate ion. The PVP K-90 solution containing 0.35 M of tribasic sodium phosphate became cloudy at as low as 27.5°. Only small difference was found in the effect of concentration of sodium and potassium carbonate on the cloud point of PVP aqueous solution (Fig. 5). Difference among the anions in the monovalent cationic-divalent anionic salts were not so great as monovalent cationic-monovalent anionic salts. Cloud point was observed in the presence of lithium ion when sulfate salts were used (Fig. 6). Lithium sulfate, however, lowered the cloud point to the smaller extent than others. The effect of the alkaline metal cation was in the following order; potassium> sodium>lithium. The effect of cupric sulfate, which is divalent cationic-divalent anionic, was not so great as ammonium, sodium, and potassium sulfates.

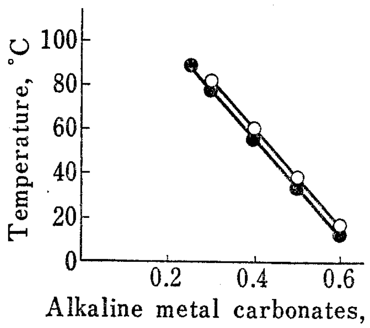


Fig. 5. Effect of Concentration of Sodium (○), and Potassium (●) Carbonates on the Cloud Point of 1% PVP K-90 Solution

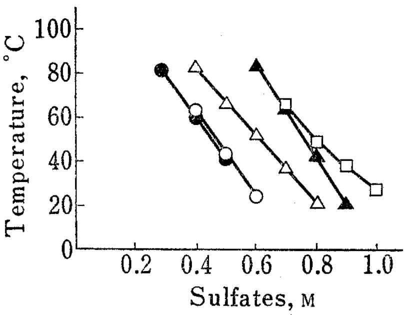


Fig. 6. Effect of Concentration of Lithium (□), Ammonium (△), Sodium (○), Potassium (●), and Cupric (▲) Sulfates on the Cloud Point of 1% PVP K-90 Solution

Effect of Aromatic Compounds on the Cloud Point of PVP Aqueous Solution

Some of the aromatic compounds; benzoic acid, acetaminophen, phenol, *etc.*, caused the coacervation of 1% PVP K-90 aqueous solution.[15] Guttman *et al.* reported on the similar clouding phenomenon in the interaction of some phenolic compounds with polyethylene glycols, polypropylene glycols and PVP by titration method.[12] Bandyopadhyay reported on the cloud point of PVP aqueous solution by addition of hydroquinone.[6] The concentration of these organic compounds to cause the coacervation of the PVP solution was much smaller (*e.g.* order of $\text{M} \times 10^2$) than that of the inorganic salts. Some of the organic compounds, however, do not have enough solubility to cause the coacervation of PVP aqueous solution. PVP aqueous solution did not show coacervation itself over the experimental temperature range (0—95°). In order to ascertain the effect of these organic compounds at the lower concentration below their solubilities, an inorganic salt in a given concentration was added into PVP aqueous solution beforehand to lower the cloud point to a temperature range where measurement can be made easily.

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[15] H. Sekikawa, K. Hanada, R. Hori, and T. Arita, 92nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April, 1972.

The selection of the salt was dominated by the desired pH of the solution. In this study ammonium sulfate was used. The solution consisted of 1.0% (w/v) PVP K-90, 0.5 M ammonium sulfate and compound to be tested. The solution exhibited pH around 5.4.

PVP has been reported to interact with aromatic compounds in aqueous solution.[5,6] Effect of some simple functional groups, $-OH$, $-NH_2$, and $-NO_2$, attached to benzene ring on the cloud point of PVP aqueous solution containing ammonium sulfate was investigated. Results are shown in Fig. 7. The ability to lower the cloud point of the solution was in the following order; phenol>nitrobenzene>aniline.

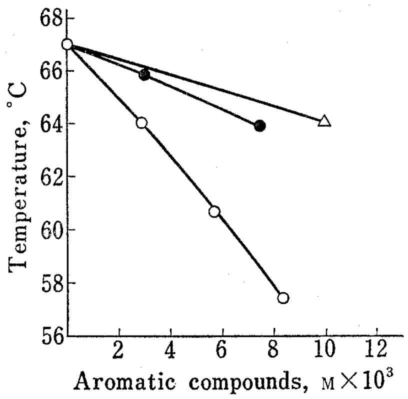

Fig. 7. Effect of Concentrations of Aniline (△), Nitrobenzene (●), and Phenol (○) on the Cloud Point of 1% PVP K-90 Solution Containing 0.5 M Ammonium Sulfate

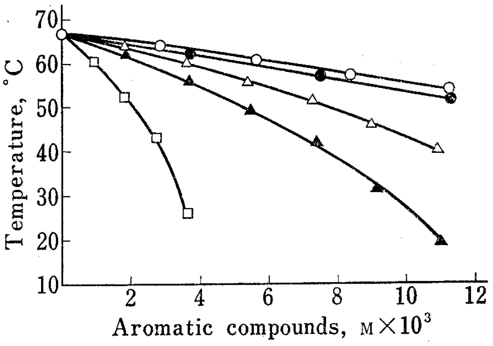

Fig. 8. Effect of Concentrations of Phenol (○), Catechol (●), Hydroquinone (△), Resorcinol (▲), and Phloroglucinol (□) on the Cloud Point of 1% PVP K-90 Solution Containing 0.5 M Ammonium Sulfate

Figure. 8 shows the effect of phenol, catechol, resorcinol, hydroquinone, and phloroglucinol on the cloud point of the PVP solution. The ability to lower the cloud point is related to the number of the hydroxyl group, that is, phloroglucinol having three hydroxyl groups, lowered the cloud point most extensively, and phenol having only one hydroxyl group, least among them. The ability is also related to the mutual position of two hydroxyl groups. Catechol lowered the cloud point least among the compounds having two hydroxyl groups. Two hydroxyl groups of catechol at *ortho*-position might interact with each other to form

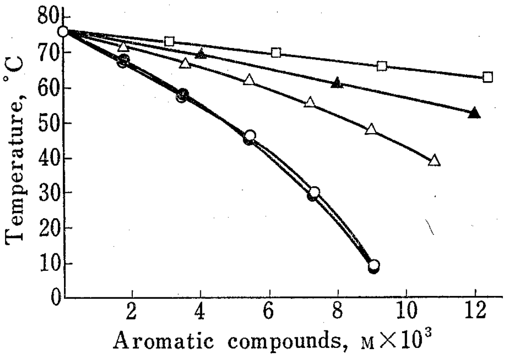

Fig. 9. Effect of Concentrations of Phenol (□), Benzoic Acid (▲), *o*- (△), *m*- (●), and *p*- (○) Hydroxybenzoic Acid on the Cloud Point of 1% PVP K-90 Solution Containing 0.5 M Ammonium Sulfate and 0.1 N Hydrochloric Acid

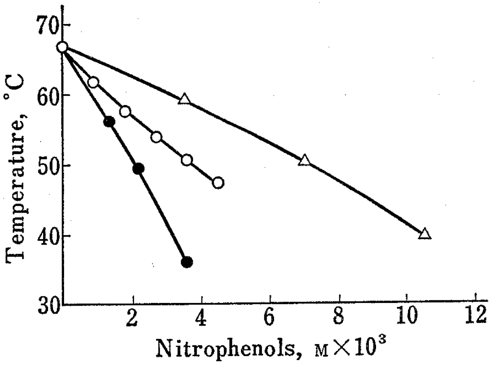

Fig. 10. Effect of Concentration of 4-Nitrophenol (△), 2,4-Dinitrophenol (○), and Picric Acid (●) on the Cloud Point of 1% PVP K-90 Solution Containing 0.5 M Ammonium Sulfate

intramolecular hydrogen bond, and a part of hydroxyl groups might not be available for interaction with PVP molecule.[16]

Figure 9 shows the effects of benzoic acid derivatives having hydroxyl group on the cloud point of the PVP solution. In this case, hydrochloric acid was added to the sample solution to suppress any dissociation of benzoic acid derivatives. The pH of the solution was 1.3.

The ability of benzoic acid to lower the cloud point was greater than phenol. Hydroxybenzoic acids were more effective than benzoic acid in lowering the cloud point. Salicylic acid (*o*-hydroxybenzoic acid) was not so effective as *m*-, or *p*-hydroxybenzoic acid. It may be considered that carboxyl group and hydroxyl group at *ortho*-position might interact with each other by intramolecular hydrogen bond, and a part of these groups is not available for interaction with PVP molecule.[16]

Figure 10 shows the effects of nitrophenols on the cloud point of the PVP solution. The ability to lower the cloud point was increased with the increase in the number of nitro group.

The ability to lower the cloud point of PVP aqueous solution containing ammonium sulfate was now shown to differ among the aromatic compounds depending upon the kind of functional groups, number of the functional groups, and the mutual positions.

Dialysis Studies

Studies with equilibrium dialysis method were compared with the results with the cloud point method to evaluate the interaction between the organic compounds and PVP in aqueous solution. Results of the dialysis studies are shown in Table II. The order of the compounds bound to PVP was essentially the same as that obtained by the cloud point measurement. When ammonium sulfate was present in each system, percent bound was larger than that in the absence of ammonium sulfate, but the order was the same as obtained when ammonium sulfate was absent. This tendency was similar to that for the other compounds reported in the interaction with PVP obtained by dialysis method[5] or viscosity method.[6]

TABLE II. Binding of Benzoic Acids to 5% PVP K-90 in 0.1 M Hydrochloric Acid in the Presence and Absence of 0.5 M Ammonium Sulfate Studied by Dialysis Technique at 37°

Additive	Percent bound	
	without $(NH_4)_2SO_4$	with $(NH_4)_2SO_4$
Benzoic acid	33.2	39.7
o-Hydroxybenzoic acid	37.9	50.5
m-Hydroxybenzoic acid	57.4	65.5
p-Hydroxybenzoic acid	54.1	64.7

Effect of Urea and Thiourea Derivatives on the Cloud Point of PVP Aqueous Solution

Urea is used as the denaturant of protein and it is known to inhibit the interaction between low molecular weight compound and macromolecule. Klotz, *et al.* reported that the interaction between methyl orange and bovine serum albumin was inhibited by urea.[17] They reported on the same inhibitory effect of urea on the interaction between methyl orange and PVP.[17] Feldman and Gibaldi noted the increase in the solubilities of benzoic acid and salicylic acid by addition of urea derivatives.[18] The effects of these compounds on the cloud point of PVP aqueous solution are shown in Fig. 11.

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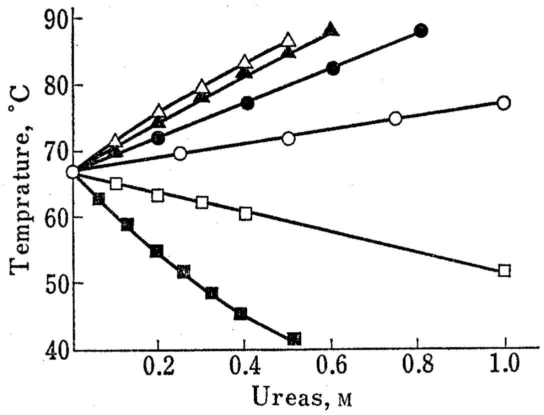

Fig. 11. Effect of Concentrations of Urea (○), Methylurea (●), 1,3-Dimethyl Urea (▲), Tetramethylurea (△), Thiourea (□), and 1,3-Dimethylthiourea (■) on the Cloud Point of 1% PVP K-90 Solution Containing 0.5M Ammonium Sulfate

Contrary to the effect of aromatic compounds on the cloud point, urea derivatives caused to elevate the cloud point of the PVP solution. The extent of elevation was increased with the number of methyl group in urea molecule. The tendency was similar to the effect on the denaturation of protein,[19] stability of micelles,[20] or solubilization of drugs.[18]

On the other hand, thiourea and 1,3-dimethylthiourea brought about lowering of the cloud point of the PVP solution. Urea derivatives have oxygen atom in the molecules which can engage in hydrogen bonding. Sulfur atom in thiourea derivatives, on the other hand, does not serve as a good proton acceptor. Substituted methylgroup might play a role of increasing the hydrophobicity of the molecule.[18]

General Discussion

Coacervation of PVP aqueous solution has now been shown to be caused by addition of some inorganic salts and some organic compounds. Ise, *et al.* proposed that PVP molecule might be present in the aqueous solution with hydrophobic hydration around its polymer chain.[7b] Inorganic salt might compete with PVP molecules in hydration, and PVP molecule start to precipitate from the solution and thus the solution becomes cloudy.

Bandyopadhyay, *et al.* reported that PVP aqueous solution exhibited the cloud point by addition of hydroquinone.[6] They pointed out that hydroquinone played a role of θ-solvent. They also reported the decrease in the viscosity of PVP solution by addition of some phenolic compounds.

Compounds which interact with PVP molecule in aqueous solution, might release water which is hydrating PVP molecule. The configuration of PVP polymer chain may shrink when these compounds and PVP molecule interact.[21] Thus the degree of interaction between a compound and PVP may be evaluated by measuring the effect of the compound on the cloud point of PVP solution. Addition of inorganic salt in PVP solution allowed the determination of the cloud point at lower temperature.

Advantages of the present cloud point method lie in a) good reproducibility, b) easy control of the desired pH of the solution by addition of suitable salts, c) no neccessity of the chemical analysis, and d) determination in short time. The cloud point method will therefore be useful and be widely applicable to the study of interaction between low molecular weight compounds and other water-soluble macromolecules.

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