# Interaction of Povidone with Aromatic Compounds II: Evaluation of Ionic Strength, Buffer Concentration, Temperature, and pH by Factorial Analysis

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**Abstract** □ The interaction of a series of ligand molecules, all consisting of substituted benzoic and nicotinic acid derivatives, and povidone was studied. The influence of ionic strength, buffer concentration, and temperature was evaluated using factorial analysis. Complex formation was not affected at low ionic strength, but increased considerably at higher values due to dehydration of the macromolecule. Complex formation was enhanced in phosphate solutions, particularly in the presence of dibasic phosphate ions. A linear relationship was found between the logarithm of the percentage of bound ligand and ionic strength and buffer capacity.

Increasing the temperature lowered complex formation. Although dehydration of the macromolecule also occurred, the decrease in complex formation could be attributed to the solubility increase of the ligand molecules. The influence of the degree of dissociation of the ligand molecules was investigated by factorial analysis. The compounds mainly interacted to a lesser extent in the dissociated than in the nondissociated state. In addition, a negative effect of a pyridine ring with respect to a phenyl ring was observed. The binding tendency was markedly increased by substituting the aromatic ring structure with hydroxyl functions and by esterification of the carboxyl function attached to the ring. The results suggested that lipophilicity and hydrogen bonding played a predominant role in povidone complexation.

**Keyphrases** □ Povidone—interaction with aromatic compounds, evaluation of complex formation by factorial analysis □ Complexation—evaluation of povidone–aromatic compound interaction by factorial analysis □ Factorial analysis—evaluation of complex formation, povidone–aromatic compound interaction □ Aromatic compounds—interaction with povidone, evaluation of complex formation by factorial analysis

The influence of parameters such as ionic strength, temperature, and buffer concentration and composition on the complex formation between small ligand molecules and macromolecules such as povidone has not yet been investigated systematically (1–9). However, the influence of these parameters is important for meaningful comparisons between the complexing tendencies of related ligand molecules (10) and for understanding the binding and thermodynamic characteristics involved (11–16).

The present report deals with a factorial analysis of these parameters.

## EXPERIMENTAL

**Reagents**—Povidone[1] with a molecular weight of 700,000 was used as the macromolecule and was oven dried at 50° until a constant weight was reached. The following ligand molecules were investigated: benzoic acid[2], nicotinic acid[3], isonicotinic acid[2], salicylic acid[4], nicotinamide[2], salicylamide[2], benzoic acid hydrazide[5], nicotinic acid hydrazide[6], isonicotinic acid hydrazide[4], iproniazide phosphate[7], salicylic acid hydrazide[6], 4-hydroxysalicylic acid[8], 5-hydroxysalicylic acid[4], 5-nitrosalicylic acid[8], methyl salicylate[4], and ethyl salicylate[2].

The following buffer solutions were used: hydrochloric acid–potassium chloride buffers (17) at pH 1.0 and 1.93, McIlvaine buffers (18) at pH 2.20–5.60, a phosphate buffer (17) at pH 7.00, a boric acid–disodium tetraborate buffer at pH 7.20 (19), and a boric acid–sodium hydroxide buffer (17) at pH 9.20. The solutions were brought to a determined ionic strength with sodium chloride. [Sodium chloride does not complex with povidone (6).] The pH of the solutions was controlled with a potentiometric pH measurement[9] and adjusted if necessary.

To control the influence of buffer ions two kinds of buffer solutions were used: the normal buffers are denoted by 1 and those with half the normal capacity are indicated as 0.5.

**Methods**—*Complex Formation Using Factorial Analysis*—Complex formation of the ligand molecules and povidone was investigated by ultrafiltration as previously described (10). The povidone concentration was 4.0%, and the ligand concentrations varied from $1.33 \times 10^{-3}$ to $1.00 \times 10^{-2}$ *M*, depending on their solubility at the pH values used. The complexing tendency expressed as percent of bound ligand (*B%*) was, in the ranges used, independent of the ligand concentration. The concentration of free ligand in the filtrate was determined, after appropriate dilution, with a double-beam spectrophotometer[10] at the respective $\lambda_{max}$ of the ligands. Corrections were made for possible membrane adsorption, and the percentage of bound ligand was calculated as a measure of the complexing tendency. For each sample, these values were obtained at two levels of the three parameters studied (*i.e.*, ionic strength, buffer composition, and temperature) and investigated by factorial analysis (10, 20–22).

For the binding of ligand molecules at pH 1.0, only ionic strength and buffer composition were investigated. At pH 7.00, for 4-hydroxysalicylic acid and 5-nitrosalicylic acid, only the temperature and buffer composition were investigated.

*Complex Formation at Varying Ionic Strengths*—The effect of ionic strength was determined in a series of solutions containing $1.00 \times 10^{-2}$ *M* salicylic acid and 4.0% povidone in McIlvaine buffer (18) (pH 2.20). Ionic strength varied from 0.15 to 6.00.

*Complex Formation at Varying Buffer Concentrations*—The effect of phosphate buffer (pH 7.00) was investigated for three ligand molecules by varying the buffer concentration from 0.0500 to 0.00834 *M*, expressed as dibasic phosphate ions.

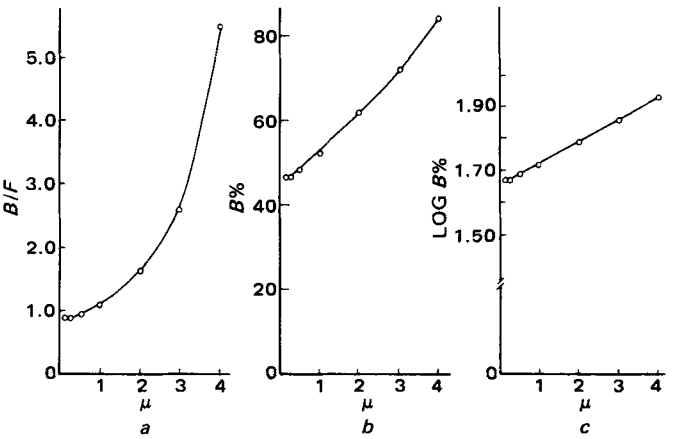


**Figure 1**—*Effect of ionic strength on the complexing tendency of salicylic acid ($1.00 \times 10^{-2}$ M) onto povidone (4.0%) at 25° and pH 2.20*

[1] Kollidon K90, BASF, Brussels, Belgium.
[2] U.C.B., Brussels, Belgium.
[3] B. D. H., Poole, England.
[4] Merck, Darmstadt, West Germany.
[5] Schuchardt, München, West Germany.
[6] Aldrich, Beerse, Belgium.
[7] Serva, Heidelberg, West Germany.
[8] Merck-Schuchardt, München, West Germany.
[9] Radiometer, Copenhagen, Denmark.
[10] Perkin-Elmer model 124.



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**Table I—Complex Formation of Various Cosolutes with Povidone (4.0%) as a Function of Ionic Strength at 25° and 0.5 Buffer**

| Cosolute | pH | Ionic Strength: Low Level μ | Low Level Bound Ligand, % | High Level μ | High Level Bound Ligand, % | Significant Difference |
|---|---|---|---|---|---|---|
| Benzoic acid | 1.0[a] | 0.15 | 34.3 | 0.30 | 34.7 | 0 |
| | 3.40[b] | 0.25 | 30.0 | 0.50 | 30.1 | 0 |
| | 5.00[b] | 0.25 | 11.9 | 0.50 | 11.8 | 0 |
| Nicotinic acid | 5.60[b] | 0.32 | 6.3 | 0.50 | 6.3 | 0 |
| Isonicotinic acid | 5.60[b] | 0.32 | 8.3 | 0.50 | 8.1 | 0 |
| Salicylic acid | 1.0[a] | 0.15 | 47.7 | 0.30 | 48.1 | 0 |
| | 2.20[b] | 0.15 | 46.8 | 1.00 | 52.9 | + |
| | 3.80[b] | 0.15 | 42.7 | 1.00 | 41.8 | 0 |
| | 7.00[c] | 0.25 | 44.0 | 0.50 | 44.1 | 0 |
| Salicylamide | 5.00[b] | 0.25 | 34.6 | 0.50 | 34.8 | 0 |
| | 7.20[d] | 0.15 | 32.7 | 0.30 | 32.7 | 0 |
| | 9.20[e] | 0.15 | 10.7 | 0.30 | 10.9 | 0 |
| Salicylic acid hydrazide | 7.00[c] | 0.25 | 33.1 | 0.50 | 33.9 | 0 |
| 4-Hydroxysalicylic acid | 1.0[a] | 0.15 | 72.2 | 0.30 | 72.4 | 0 |
| | 2.22[b] | 0.25 | 71.4 | 0.50 | 71.3 | 0 |
| | 4.22[b] | 0.25 | 61.9 | 0.50 | 62.0 | 0 |
| 5-Hydroxysalicylic acid | 1.0[a] | 0.15 | 59.0 | 0.30 | 59.0 | 0 |
| | 1.93[a] | 0.25 | 58.2 | 0.50 | 58.2 | 0 |
| | 3.93[b] | 0.25 | 51.3 | 0.50 | 51.3 | 0 |
| 5-Nitrosalicylic acid | 1.30[a] | 0.15 | 25.5 | 0.30 | 25.5 | 0 |
| | 3.30[b] | 0.25 | 38.1 | 0.50 | 38.1 | 0 |

[a] Hydrochloric acid–potassium chloride buffer. [b] MacIlvaine buffer. [c] Phosphate buffer. [d] Boric acid–disodium tetraborate buffer. [e] Boric acid–sodium hydroxide buffer.

*Desolvating Effect of Temperature on Macromolecule*—Solutions of povidone in different solvents were slowly heated, and the cloud point was determined.

## RESULTS AND DISCUSSION

**Factorial Analysis**—Factorial analysis was carried out as described previously (10). The three parameters were each investigated at a low and high level: temperature, 25 and 50°; buffer concentration, 0.5 and 1.0 and low or high ionic strength, depending on the buffers used.

With the data resulting from the ultrafiltration experiments, i.e., the percentage of bound ligand, analyses of variance were carried out and the significance of the individual parameters was evaluated with an *F* test (10). Results are summarized in Tables I–III.

Interaction between parameters were not observed except for salicylic acid at pH 2.20 where a significant relative positive interaction was observed between temperature and ionic strength.

In Tables I–III, the significance value for a 99% certainty level was indicated as 0, +, or −, corresponding with a zero, positive, or negative significant influence on complex formation when the respective factor was changed from a low to a high level.

Preliminary studies found that some derivatives showed no interaction with povidone. They are included in Table IV (the effects of the parameters were not investigated with them).

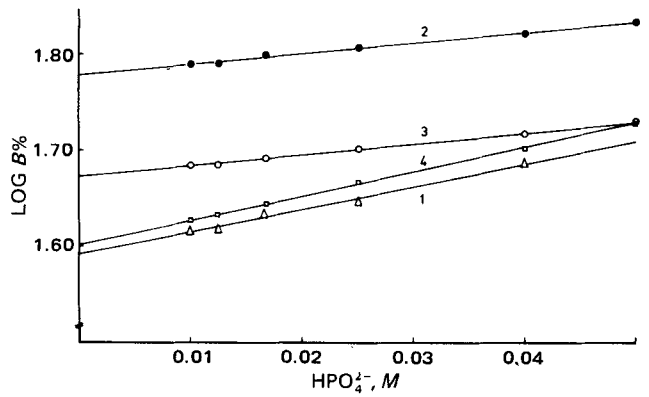


**Figure 2**—*Effect of phosphate ions (pH 7.00) on the complexing tendency of various cosolutes onto povidone (4.0%). Key: 1, salicylic acid (5.00 × 10⁻³ M), 25°, μ = 0.25; 2, 4-hydroxysalicylic acid (4.81 × 10⁻³ M), 25°, μ = 0.25; 3, 4-hydroxysalicylic acid (4.81 × 10⁻³ M), 50°, μ = 0.25; and 4, 5-nitrosalicylic acid (4.81 × 10⁻³ M), 25°, μ = 0.25.*

**Table II—Complex Formation of Various Cosolutes with Povidone (4.0%) as a Function of the Buffer Concentration Using Low Ionic Strength**

| Cosolute | Temperature | pH | Percent Bound Ligand, Buffer 0.5 | Buffer 1.0 | Significant Difference | Percent Difference |
|---|---|---|---|---|---|---|
| Benzoic acid | 25° | 1.0[a] | 34.3 | 34.2 | 0 | |
| | 25° | 3.40[b] | 30.0 | 30.0 | 0 | |
| | 50° | 3.40[b] | 31.7 | 31.7 | 0 | |
| | 25° | 5.00[b] | 11.9 | 11.8 | 0 | |
| | 50° | 5.00[b] | 10.2 | 10.2 | 0 | |
| Nicotinic acid | 25° | 5.60[b] | 6.3 | 6.7 | 0 | |
| | 50° | 5.60[b] | 4.2 | 4.4 | 0 | |
| Isonicotinic acid | 25° | 5.60[b] | 8.3 | 8.0 | 0 | |
| | 50° | 5.60[b] | 6.3 | 6.3 | 0 | |
| Salicylic acid | 25° | 1.0[a] | 47.7 | 48.3 | 0 | |
| | 25° | 2.20[b] | 46.8 | 47.0 | 0 | |
| | 50° | 2.20[b] | 43.9 | 45.0 | 0 | |
| | 25° | 3.80[b] | 42.7 | 42.6 | 0 | |
| | 50° | 3.80[b] | 36.3 | 36.4 | 0 | |
| | 25° | 7.00[c] | 44.0 | 51.2 | + | 16.4 |
| | 50° | 7.00[c] | 36.9 | 42.1 | + | 14.1 |
| Salicylamide | 25° | 5.00[b] | 34.6 | 35.1 | 0 | |
| | 50° | 5.00[b] | 32.5 | 32.6 | 0 | |
| | 25° | 7.20[d] | 32.7 | 33.0 | 0 | |
| | 50° | 7.20[d] | 27.3 | 27.3 | 0 | |
| | 25° | 9.20[e] | 10.7 | 10.7 | 0 | |
| | 50° | 9.20[e] | 7.5 | 7.6 | 0 | |
| Salicylic acid hydrazide | 25° | 7.00[c] | 33.1 | 34.0 | + | 2.7 |
| | 50° | 7.00[c] | 30.6 | 31.5 | + | 2.9 |
| 4-Hydroxysalicylic acid | 25° | 1.0[a] | 72.2 | 72.6 | 0 | |
| | 25° | 2.22[b] | 71.4 | 71.4 | 0 | |
| | 50° | 2.22[b] | 61.6 | 61.9 | 0 | |
| | 25° | 4.22[b] | 61.9 | 62.0 | 0 | |
| | 50° | 4.22[b] | 51.4 | 51.5 | 0 | |
| | 25° | 7.00[c] | 64.5 | 68.6 | + | 6.4 |
| | 50° | 7.00[c] | 49.7 | 54.2 | + | 9.1 |
| 5-Hydroxysalicylic acid | 25° | 1.0[a] | 59.0 | 59.5 | 0 | |
| | 25° | 1.93[a] | 58.2 | 58.2 | 0 | |
| | 50° | 1.93[a] | 52.3 | 52.4 | 0 | |
| | 25° | 3.93[b] | 51.3 | 51.4 | 0 | |
| | 50° | 3.93[b] | 39.1 | 39.1 | 0 | |
| 5-Nitrosalicylic acid | 25° | 1.30[a] | 25.5 | 25.5 | 0 | |
| | 25° | 3.30[b] | 38.1 | 38.2 | 0 | |
| | 25° | 7.00[c] | 46 6 | 53.9 | + | 15.7 |
| | 50° | 7.00[c] | 41.9 | 49.3 | + | 17.7 |
| Methylsalicylate | 25° | | 88.1[f] | 87.3[c] | 0 | |
| Ethylsalicylate | 25° | | 95.5[f] | 91.1[c] | ?[g] | |

[a] Hydrochloric acid–potassium chloride buffer. [b] MacIlvaine buffer. [c] Phosphate buffer. [d] Boric acid–disodium tetraborate buffer. [e] Boric acid–sodium hydroxide buffer. [f] Water. [g] Since a little difference in free ligand concentration exerts a great influence on the percentage of bound ligand, the difference for the two levels is not significant.

**Theory of Multiple Equilibrium**—The principles and concepts of complex formation with macromolecules were delineated previously (11, 23–30).

**Ionic Strength**—Table I gives the results for the influence of ionic strength studied at two levels, at a temperature of 25° and a buffer concentration of half the normal buffer capacity.

With the experimental conditions used, ionic strength had no significant effect on complex formation of the cosolutes. However, for salicylic acid at pH 2.20, a significant positive effect was observed when the ionic strength was changed from 0.15 to 1.00 ($p > 0.001$). At this pH, a relative positive interaction was also noted between ionic strength and temperature ($0.01 < p < 0.001$), although this last factor exerted a negative effect when considered individually (see effect of temperature). This effect of ionic strength is represented in detail in Fig. 1.

Complex formation as a function of ionic strength is represented in three ways: the ratio of bound to free ligand concentration ($B/F$), the percentage of bound ligand ($B\%$), and the logarithm of bound ligand (log $B\%$).

Ionic strength has a great positive influence since the amount of bound ligand rose with a factor of 1.8 from $\mu = 0.15$ to 4.00. At higher ionic strength (>4.00) the macromolecule precipitated.

It was previously reported (31–38) that neutral salts enhance complex formation with nonionic macromolecules. This activity was attributed to the dehydrating effect of the salt making the water molecules less available for hydrogen bonding with the macromolecules, thereby de-

**Table III—Complex Formation of Various Cosolutes with Povidone (4.0%) as a Function of Temperature Using Low Ionic Strength and 0.5 Buffer**

| Cosolute | pH | Ionic Strength | Bound Ligand, % 25° | Bound Ligand, % 50° | Significant Difference |
|---|---|---|---|---|---|
| Benzoic acid | 3.40[a] | 0.25 | 30.0 | 31.7 | + |
| | 5.00[a] | 0.25 | 11.9 | 10.2 | − |
| Nicotinic acid | 5.60[a] | 0.32 | 6.3 | 4.2 | − |
| Isonicotinic acid | 5.60[a] | 0.32 | 8.3 | 6.3 | − |
| Salicylic acid | 2.20[a] | 0.15 | 46.8 | 43.9 | − |
| | 3.80[a] | 0.15 | 42.7 | 36.3 | − |
| | 7.00[b] | 0.25 | 44.0 | 36.9 | − |
| Salicylamide | 5.00[a] | 0.25 | 34.6 | 32.5 | − |
| | 7.20[c] | 0.15 | 32.7 | 27.3 | − |
| | 9.20[d] | 0.15 | 10.7 | 7.5 | − |
| Salicylic acid hydrazide | 7.00[b] | 0.25 | 33.1 | 30.6 | − |
| 4-Hydroxysalicylic acid | 2.22[a] | 0.25 | 71.4 | 61.6 | − |
| | 4.22[a] | 0.25 | 61.9 | 51.4 | − |
| | 7.00[b] | 0.25 | 64.5 | 49.7 | − |
| 5-Hydroxysalicylic acid | 1.93[a] | 0.25 | 58.2 | 52.3 | − |
| | 3.93[b] | 0.25 | 51.3 | 39.1 | − |
| 5-Nitrosalicylic acid | 7.00[c] | 0.25 | 46.6 | 41.9 | − |
| Methyl salicylate | Water | | 88.1 | 88.1 | 0 |
| Ethyl salicylate | Water | | 95.5 | 94.6 | 0 |

[a] MacIlvaine buffer. [b] Phosphate buffer. [c] Boric acid–disodium tetraborate buffer. [d] Boric acid–sodium hydroxide buffer.

creasing the aqueous solubility of the polymer and favoring the competing ligand polymer interaction. The precipitation of povidone observed at higher ionic strength (>4.00) indicated that this parameter effectively exerted a dehydrating effect on the macromolecule.

From the results represented in Fig. 1, the following relationships could be deduced:

$$\log B\% = a\mu + b \qquad \text{(Eq. 1)}$$

or:

$$B/F = \frac{10^{(a\mu + b)}}{100 - 10^{(a\mu + b)}} \qquad \text{(Eq. 2)}$$

The values $a$ and $b$, determined from the slope and intercept of Fig. 1*c* were 0.068 and 1.655, respectively.

Together with the macromolecule dehydration, which is a logarithmic function of the ionic strength (39), the percentage of bound ligand increased as an exponential function of ionic strength until precipitation. From the log $B\%$ graph as a function of the ionic strength, it was possible to eliminate the effect of this parameter by extrapolation to the ordinate. An increasing complexing tendency at higher ionic strength was observed with hydrophobic binding and Van der Waals forces.

**Buffer Concentration**—The influence of buffer concentration, studied at two levels, is reported in Table II.

No significant effect was noted with hydrochloric acid–potassium chloride buffer (pH 1.0), boric acid–disodium-tetraborate buffer (pH 7.20), boric acid–sodium hydroxide buffer (pH 9.20), or the MacIlvaine buffer (pH 2.20–5.60).

For the compounds investigated at pH 7.00 in phosphate buffer, a large positive effect of the buffer concentration was observed. Doubling the concentration of the buffer ions considerably enhanced complex formation: 16.4% for salicylic acid, 6.4% for 4-hydroxysalicylic acid, 15.7% for 5-nitrosalicylic acid, and 2.7% for salicylic acid hydrazide at 25°.

At the investigated pH values, the MacIlvaine buffer was composed mainly of monovalent phosphate and citrate ions. The phosphate buffer (pH 7.00) consisted of monobasic (61.9%), and dibasic ions (30.1%). Thus, the dibasic phosphate ions presumably affected the complexing tendency.

This enhancement in complex formation with bivalent ions can perhaps be explained in terms of macromolecule dehydration. This presumption is supported by the fact that the salting-out effect is a function of size and the hydration state of the ions (11). The pronounced dehydrating effect of bivalent phosphate ions was emphasized in an earlier experiment (see effect of temperature).

The effect of phosphate buffer was also investigated for three ligand molecules (Fig. 2). The logarithm of the percentage of bound ligand as a function of the buffer concentration showed a linear relationship. The intercept, obtained by extrapolation to the ordinate, represented the log percentage of bound ligand at infinite dilution, thereby eliminating the

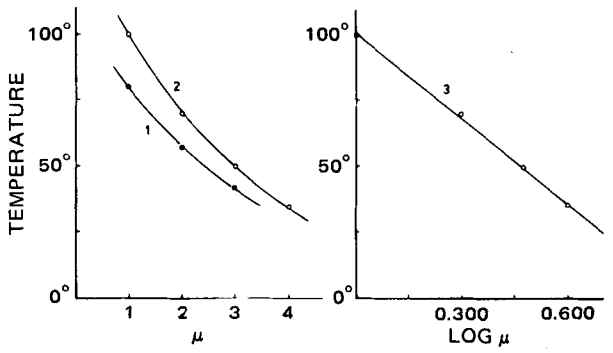


**Figure 3**—*Cloud point of the povidone (4.0%)–salicylic acid ($1.00 \times 10^{-2}$ M) complex as a function of temperature at pH 2.20. Key: 1, cloud point; 2, precipitation; and 3, cloud point.*

effect of the buffer. These values represented 38.8% of bound ligand for salicylic acid; 60.0 and 47.9%, respectively, at 25 and 50° for 4-hydroxysalicylic acid; and 40.6% for 5-nitrosalicylic acid. Comparison of these results with those of Table II shows that the complexing tendency of salicylic acid and 4-hydroxysalicylic acid was lowest at pH 7.00 where the two ligand molecules were fully dissociated. However, 5-nitrosalicylic acid seemed to have a higher complexing tendency onto povidone at higher pH values.

Ionic strength (Table I), had no effect on complex formation, and the effects of buffer concentration and ionic strength were compared. For both parameters, a linear relationship was found between the logarithm of the percentage of bound ligand and the ionic strength and buffer capacity.

**Temperature**—The effect of temperature was evaluated by factorial analysis carried out at 25 and 50°. Binding diminished significantly at the higher temperature (Table III). However, an exception was noted for benzoic acid at pH 3.40. The same effect was found for this compound in the nonionic state with povidone[11] (5). Methyl and ethyl salicylate also did not influence complexation.

Thermal energy would be expected to exert two antagonistic effects on complex formation. By thermal desolvation of the polymer chain, a more suitable environment for the interaction with the ligand molecules would be expected. However, thermal agitation might also decrease the association of the ligand molecules with the polymer by weakening the attractive forces between them (31, 40). It is the authors' opinion that another effect must be considered; temperature also enhances the solubility of the ligand molecules, probably decreasing the complexing tendency.

The results of the desolvating effect of temperature on povidone are given in Table V. No cloud point could be obtained with povidone solutions in water, ethanol, and 20% (v/v) ethanol when the solutions were heated to 100°. However, the solutions containing salts became clouded by temperature elevation, suggesting dehydration of povidone by salt. This effect, most pronounced with bivalent phosphate ions, could explain the positive effect of these ions on complex formation.

The same experiment was performed on the solutions represented in Fig. 1, and the results are shown in Fig. 3.

The cloud point and precipitation occurred at lower ionic strength as the temperature increased. This finding is in accordance with the relative positive interaction noted between ionic strength and temperature. When representing the logarithm of the ionic strength as a function of temperature where the solutions are clouded, a linear relationship is observed (Fig. 3). These results were also indicative of the dehydrating effect of temperature. When the clouded solutions were cooled, turbidity disappeared with the exception of the solution heated to 100° ($\mu = 1$).

**Effect of pH and Substituents**—The results obtained from factorial analysis (Tables I and II) indicated that ionic strength and buffer concentration had no influence on complex formation. Therefore, it is possible to discuss in a more meaningful way the influence of pH and dissociation of ligand molecules on complex formation. An exception was observed at pH 7.00, where the buffer effect was considerable (Table II). At this pH, the extrapolated values from Fig. 2 were used, representing the percentage of bound ligand at infinite buffer dilution and excluding the effect of the buffer.

The results for the different ligand molecules as a function of their pKa values and degree of dissociation are summarized in Table IV. The

[11] Kollidon K30, BASF, Brussels, Belgium.

**Table IV—Complex Formation of Various Cosolutes in Percentage of Bound Ligand with Povidone (4.0%) as a Function of pKa and pH at 25° Using Low Ionic Strength and 0.5 Buffer**

| Cosolute | pKa Cosolute | pH 1.0 | pKa Cosolute −1.00 | pKa Cosolute −0.80 | pKa Cosolute +0.80 | pKa Cosolute +1.00 | pH 7.00 |
|---|---|---|---|---|---|---|---|
| Benzoic acid | 4.2[a] | 34.3 | — | 30.0 | 11.9 | — | — |
| Nicotinic acid | 4.83[a] | 0.0 | — | 1.0 | 6.3 | — | — |
| Isonicotinic acid | 4.84[a] | 0.0 | — | 0.8 | 8.3 | — | — |
| Salicylic acid | 2.97[a] | 47.7 | — | 46.8 | 42.7 | — | 38.7[b] |
| 4-Hydroxysalicylic acid | 3.22[a] | 72.2 | 71.4 | — | — | 61.9 | 60.3[b] |
| 5-Hydroxysalicylic acid | 2.93[a] | 59.0 | 58.2 | — | — | 51.3 | — |
| 5-Nitrosalicylic acid | 2.30[a] | — | 25.5 | — | — | 38.1 | 40.2[b] |
| Methyl salicylate | — | — | — | — | — | — | 87.3 |
| Ethyl salicylate | — | — | — | — | — | — | 91.1 |
| Nicotinamide | 3.35[c] | — | 0.0 | — | 0.0 | — | 0.0 |
| Salicylamide | 8.20[d] | 34.6[e] | 32.7 | — | — | 10.7 | — |
| Benzoic acid hydrazide | 3.27[f] | — | — | 6.2 | — | — | 11.6 |
| Nicotinic acid hydrazide | 3.63[c] | 0.0 | 0.0 | — | — | — | 0.0 |
| Isonicotinic acid hydrazide | 3.81[c] | 0.0 | 0.0 | — | — | — | 0.0 |
| Iproniazid phosphate | — | 0.0 | — | — | — | — | 0.0 |
| Salicylic acid hydrazide | — | 0.0 | — | — | — | — | 33.1 |

[a] pKa = carboxyl function. [b] Extrapolated values for 4.0% povidone. [c] pKa = nitrogen of pyridine ring. [d] pKa = hydroxyl function. [e] pH 5.00. [f] pKa = hydrazide function.

**Table V—Cloud Point of Povidone Solutions as a Function of Temperature**

| Grams of Povidone + 25 ml of Solvent | Water | 20% (v/v) Ethanol | Ethanol | 3 *M* NaCl in Water | 3 *M* NaCl in 20% (v/v) Ethanol | 3 *M* NaCl + 0.12 *M* $H_2PO_4^-$ in Water | 3 *M* NaCl + 0.12 *M* $HPO_4^{2-}$ in Water |
|---|---|---|---|---|---|---|---|
| 1.0 | no | no | no | 83° | 90° | 78° | 73° |
| 2.0 | no | no | no | 83° | 90° | 79° | 74° |
| 3.0 | no | no | no | 83° | 90° | 79° | 75° |
| 5.0 | no | no | no | 84° | 94° | 80° | 76° |
| 6.0 | no | no | no | 85° | 98° | 83° | 76° |
| 7.5 | no | no | no | — | — | — | — |

compounds generally interacted to a lesser extent when dissociated than when undissociated. Exceptions were noted for nicotinic acid, isonicotinic acid, and 5-nitrosalicylic acid.

The low interaction tendency of the dissociated compounds could be attributed to their more hydrophilic nature. For the carboxylic acid derivatives, the complexing tendency followed the series: salicylic acid > benzoic acid > nicotinic acid ≃ isonicotinic acid. The same order was observed for the amide derivatives (salicylamide > nicotinamide) and the hydrazide derivatives (salicylic acid hydrazide > benzoic acid hydrazide > nicotinic acid hydrazide = isonicotinic acid hydrazide = iproniazide phosphate = no interaction).

A large negative influence of the pyridine ring with regard to the benzene ring was striking. Where benzoic acid, in the undissociated state (pH 1.0), showed complex formation of 34.3% and benzoic acid hydrazide (pH 7.00) showed complex formation of 11.6%, no interaction was noted for the pyridine derivatives. The introduction in the molecular structure of a hydroxyl function enhanced the complexing tendency; *e.g.*, compare salicylic acid with 47.7% of bound ligand against benzoic acid with 34.3%, and salicylic acid hydrazide with 33.1% of bound ligand against benzoic acid hydrazide with 11.6%. A second hydroxyl function enhanced complex formation considerably; *e.g.*, compare 4-hydroxysalicylic acid and 5-hydroxysalicylic acid with 72.2 and 59.0%, respectively, of bound ligand against salicylic acid with 47.7%. However, the introduction of a nitro function decreased the interaction (5-nitrosalicylic acid < 5-hydroxysalicylic acid).

The position of the substituents on the benzene ring also played an important role; *i.e.*, 4-hydroxysalicylic acid showed a higher complexing tendency than the 5-hydroxy derivative. Esterification of the carboxyl function with a methyl or ethyl function enhanced the interaction considerably. The alkyl chain length seemed to have a positive influence [ethyl salicylate (91.1 *B*%) > methylsalicylate (87.3 *B*%).] The negative effect of a nitro group as compared with a hydroxyl function was also observed by Jürgensen Eide and Speiser (5). In the case of the bulky nitro group, steric hindrance should play a role. The difference in interaction between 5-hydroxysalicylic acid and 4-hydroxysalicylic acid could be due to the higher solubility of the 5-hydroxy derivative in contrast to the 4-hydroxy derivative, accompanied by a decreased complexing tendency. However, a correlation between solubility and complex formation had exceptions. The solubility of 5-nitrosalicylic acid was much lower than that of the 5-hydroxy derivative. While the latter showed a higher complexing tendency, the same statement could be made for 5-hydroxysalicylic acid and salicylic acid, with the former showing a greater solubility and a more pronounced complexing tendency. The increasing complexing tendency of compounds in the dissociated state containing hydroxyl groups indicates that hydrogen bonding must play an important role in the interaction.

Moreover, the difference in complex formation between undissociated and respectively dissociated ligand molecules was not important; *e.g.*, for salicylic acid, the complexing tendency only decreased from 47.7 to 38.7 *B*%. Also, for 4-hydroxysalicylic acid and 5-hydroxysalicylic acid, the decrease in the percentage of ligand bound when going from the undissociated (pH 1.0) to the dissociated state (pH 7.00) was not very important. The correlation between degree of complexing tendency and pH and pKa of the drug and partition coefficients is being investigated.

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# Polymorphism of Spray-Dried Microencapsulated Sulfamethoxazole with Cellulose Acetate Phthalate and Colloidal Silica, Montmorillonite, or Talc

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**Abstract** □ Sulfamethoxazole was microencapsulated with cellulose acetate phthalate and talc, colloidal silica, or montmorillonite clay by a spray-drying technique. The surface topography of the products varied with the type of excipient used and the pH of the suspending medium. The products without the excipient were coated with flake-like crusts, while the products containing the excipient tended to become well-rounded spheres. In addition, the crystalline form of sulfamethoxazole converted from Form I to an amorphism and Form II during the spray-drying process. This polymorphic transformation was attributed to the interaction of cellulose acetate phthalate with sulfamethoxazole. Increasing the concentration of cellulose acetate phthalate in the formulation increased the attainment of amorphism. Form II was also obtained by freeze and vacuum drying. Talc was the only excipient that contributed to polymorphism, which occurred in the alkaline suspension medium. Montmorillonite products prepared from the acidic medium exhibited an exothermic differential scanning calorimetry thermogram, which might be interpreted in terms of adsorption of the fused sulfamethoxazole with the internal surface of montmorillonite.

**Keyphrases** □ Microencapsulation—sulfamethoxazole, spray drying with cellulose acetate phthalate and colloidal silica, montmorillonite, or talc, polymorphism □ Polymorphism—microencapsulation of sulfamethoxazole, spray drying with cellulose acetate phthalate and colloidal silica, montmorillonite, or talc □ Sulfamethoxazole—microencapsulated, polymorphism, spray drying with cellulose acetate phthalate and colloidal silica, montmorillonite, or talc

The appropriate selection of the most suitable polymorphic form of medicaments with high thermodynamic activities frequently is the key to improving bioavailability or preventing caking in an aqueous vehicle.

The polymorphic form of a compound depends on the nature of crystallization, *i.e.*, the type of solvent used, the temperature of crystallization, *etc.* It has been documented that polymorphism occurs during spray drying. Fell and Newton (1) produced spray-dried lactose that was a mixture of $\alpha$-monohydrate, $\alpha$-anhydrous, and $\beta$-lactose forms. Kawashima *et al.* (2) reported that spray-dried sodium salicylate was polymorphic, composed of normal crystals and amorphisms. Another study (3) showed that the crystalline form of spray-dried sulfamethoxazole with cellulose acetate phthalate converted from Form I to Form II and an amorphism.

The present study examined the effects of cellulose acetate phthalate and excipients such as colloidal silica, talc, and montmorillonite clay on polymorphism. Tests were performed by freeze drying and vacuum drying at 70° using the same formulations as for spray drying. Another microcapsule of sulfamethoxazole with cellulose acetate phthalate was prepared by the coacervation method of Merkle and Speiser (4) as a reference to test polymorphism.

## EXPERIMENTAL

**Preparations of Microcapsules and Agglomerates**—Sulfamethoxazole[1] (50 g) and cellulose acetate phthalate[2] (10–50 g) were dis-

[1] Shionogi Pharmaceutical Co., Japan.
[2] Kishida Chemical Co., Japan.



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