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Interaction of Povidone with Aromatic Compounds I: Evaluation of Complex Formation by Factorial Analysis

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Abstract □ In a study of complex formation between macromolecules and small ligands such as drugs, it appeared that the association constants must be calculated with more care (*i.e.*, after a thorough investigation of the influencing parameters such as buffer composition, ionic strength, and temperature) to allow meaningful interpretations of the phenomena. For this purpose, factorial analysis seems to be the method of choice; it offers the advantage of evaluating the influence of several variables and their interactions at the same time with a minimum of experiments. The method was applied to the association of povidone with two ligands, salicylic acid and benzoic acid. Parameters such as buffer composition and ionic strength, which affect binding, could be distinguished. Especially at pH 7.00, a great positive influence of buffer ions (phosphate buffer) and a relative positive interaction between temperature and ionic strength were noted. Knowledge of the influences of these parameters allowed comparison of the effects of the functional groups attached to the ligand molecules, as well as their degree of dissociation, on adsorption to permit more meaningful interpretation of thermodynamic constants.

Keyphrases □ Salicylic acid—binding to povidone, effects of temperature, buffer composition, and ionic strength □ Benzoic acid—binding to povidone, effects of temperature, buffer composition, and ionic strength □ Povidone—complex formation with salicylic acid and benzoic acid, effects of temperature, buffer composition, and ionic strength □ Complexation—povidone with salicylic acid and benzoic acid, effects of temperature, buffer composition, and ionic strength

The binding of various drugs with macromolecules in aqueous solution has been reviewed (1–3). Vallner (3) showed the discrepancies among the results obtained by several investigators and cited the need for the calculation of the association constants in a more meaningful manner. These discrepancies often are due to the uncontrolled influence of variables such as buffer composition, pH, ionic strength, and temperature. There have been few reports concerning the influence of buffer ions on the binding onto macromolecules (4, 5). The parameters usually are studied in the classical manner, *i.e.*, by successively changing one parameter at a time. However, as demonstrated previously (6), an accurate interpretation of the individual effects of the variables or their interactions is not possible with that approach.

Factorial analysis offers the possibility of evaluating the influence of individual variables and their interactions at the same time with a minimum of experiments. This method seems to be a first step for a thorough study of complex formation. Once the influence of these parameters is known, comparison of the effects of the functional groups attached to the ligands and their degree of dissociation on adsorption is possible, and the association constants can be interpreted more meaningfully. The application of factorial analysis to the binding of two simple ligands, benzoic acid and salicylic acid, onto povidone demonstrates the advantages and possibilities of this method in the study of complex formation.

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. Salicylic acid[2] (p.a.) and benzoic acid[3] (p.a.) were used without further purification.

Buffer solutions were used at pH 7.00 (phosphate buffer) and at pH values equal to the pKa value of the two cosolutes (McIllvaine buffers) plus and minus 0.80; the solutions were brought to a determined ionic strength with sodium chloride. 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 of the normal capacity are indicated as 0.5. The pH of the solutions at 25 and 50° was controlled with a potentiometric pH measurement[4] and adjusted if necessary.

Methods—The solutions containing the ligand and povidone were prepared in the respective buffers and allowed to stand overnight to attain equilibrium. Ultrafiltration[5] was performed, using a membrane of regenerated cellulose with a claimed cutoff value of ~10,000 mol. wt. units. Compressed nitrogen (4 kg/cm^2) was used, and the filtration was performed under continuous stirring of the sample solution to avoid accumulation of the macromolecule at the membrane–solution interface (7).

The concentration of unbound ligand in the filtrate was assayed spectrophotometrically. Corrections were made for membrane adsorption effects, and the concentration of bound ligand was calculated. The spectrophotometric measurements were performed with a double-beam spectrophotometer[6] at 296 nm for salicylic acid and at 224 nm for benzoic acid.

Calculations—Complex formation can be studied as a function of either macromolecule concentration or ligand concentration. In the first method, the relative tendencies of several ligands to form complexes are expressed as the ratio of the total ligand concentration, T, to the con-

[1] Polyvinylpyrrolidone (Kollidon K90), BASF, Brussels, Belgium.
[2] Merck.
[3] U.C.B.
[4] Radiometer, Copenhagen, Denmark.
[5] Amicon model 52.
[6] Perkin-Elmer model 124.

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Table I—Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 2.20

Ionic Strength	Buffer	Temperature	Percentage of Ligand Bound		Difference
		Influence of Temperature[a]	25°	50°	
Low	Low	—	56.9	54.0	−2.9
Low	High	—	57.1	55.0	−2.1
High	Low	—	62.9	62.2	−0.7
High	High	—	62.8	62.4	−0.4
		Influence of Ionic Strength[b]	μ = 0.15	μ = 1.00	
—	Low	Low	56.9	62.9	+6.0
—	Low	High	54.0	62.2	+8.2
—	High	Low	57.1	62.8	+5.7
—	High	High	55.0	62.4	+7.4
		Influence of Buffer Ions[c]	0.5 Buffer	1 Buffer	
Low	—	Low	56.9	57.1	+0.2
Low	—	High	54.0	55.0	+1.0
High	—	Low	62.9	62.8	−0.1
High	—	High	62.2	62.4	+0.2

[a] Factor A = temperature; low level = 25° and high level = 50°. [b] Factor B = ionic strength (μ); low level = 0.15 and high level = 1.00. [c] Factor C = buffer ions; low level = 0.5 and high level = 1.

centration of the free form, *F*, as a function of the percentage of povidone (8–10):

$$Q = \frac{T}{F} = \frac{r}{F}[\text{PVP}] + 1 \qquad \text{(Eq. 1)}$$

where *r* is the number of moles of ligand bound per mole of macromolecule and [PVP] is the molar concentration of povidone in the solution. If *r*/*F* is constant (*i.e.*, if the same type of binding is taking place with increasing povidone concentration), a linear relationship is obtained (11) between *T*/*F* and [PVP].

The second method permits the calculation of equilibrium constants and the maximum number of binding sites on the macromolecule. The basic theory of the calculation of ligand–macromolecule equilibria was described previously (12–15). If no interaction phenomena take place, then reversible binding can be described by:

$$r = \frac{nkF}{1 + kF} \qquad \text{(Eq. 2)}$$

where *n* represents the total number of sites available and *k* is the intrinsic binding constant. The relationship between the intrinsic binding constant, *k*, and the constants for the individual equilibrium reactions, k_i, is given by (16):

$$k_i = \left[\frac{n - (i-1)}{i}\right]k \qquad \text{(Eq. 3)}$$

where *n* is the total number of available sites and *i* is the number of possible combinations of *n* binding sites taken exactly *i* at a time.

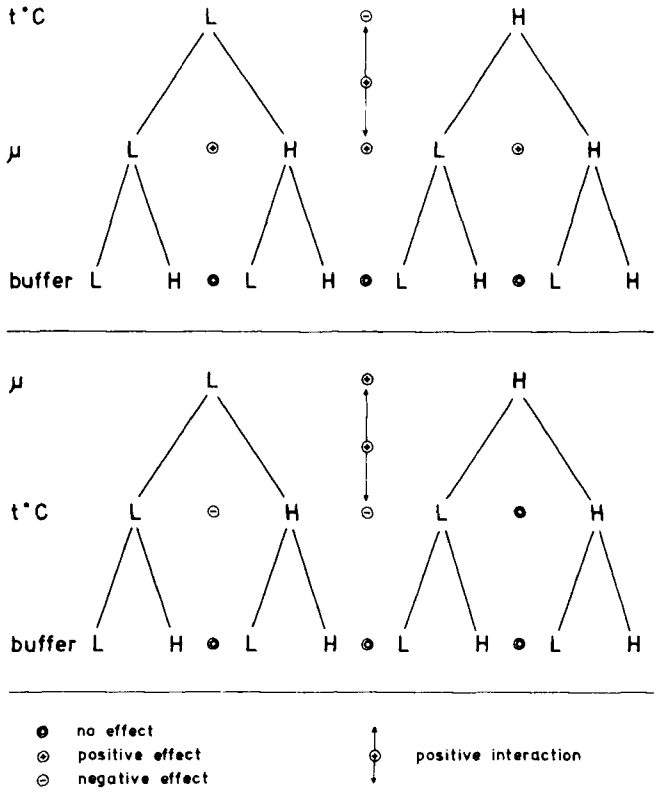

Figure 1—*Influence of parameters on the complexing of salicylic acid (1.00×10^{-2} M) with povidone (6.00%) at pH 2.20.*

The hyperbolic graph of *r versus F* (Eq. 2) can be converted into linear form. The approach of Klotz *et al.* (12) usually is used:

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nkF} \qquad \text{(Eq. 4)}$$

The Scatchard equation (15) is:

$$\frac{r}{F} = kn - kr \qquad \text{(Eq. 5)}$$

Consequently, graphical representations of $1/r$ *versus* $1/F$ or r/F *versus* *r* are used to obtain *n* (the number of binding sites) and *k* (the intrinsic binding constant). Curvature of such plots usually indicates the existence of more than one class of sites (17).

Factorial Analysis—The overall objective of factorial analysis is to obtain a general picture of how the results are affected by changing the parameters or factors. The theory and practice of factorial analysis were given by Philippe (6) and Chatfield (18). In complex formation, the principal parameters or factors to be considered are ionic strength, buffer

Table II—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 2.20

Column 1: Source of Variation	Column 2: Percentage of Ligand Bound Exp. 1	Exp. 2	Column 3: Sum	Column 4	Column 5	Column 6	Column 7: Degrees of Freedom	Column 8: Sum of Squares (Column 6)^2 / 2 × 8	Column 9: Variance	Column 10: *F*	Column 11: Significant Level $F_8^{1\ 5\%\ 5.32}_{1\%\ 11.26}$
1	57.0	56.8	113.8	221.8	471.9	946.4					
A[a]	53.7	54.3	108.0	250.1	474.5	−12.2	1	9.303	9.303	33.83	Temperature: $-0.001 < p$
B[b]	63.2	62.6	125.8	224.2	−7.3	54.4	1	184.960	184.960	672.58	Ionic strength: $+0.001 < p$
AB	62.5	61.8	124.3	250.3	−4.9	7.4	1	3.423	3.423	12.45	Temperature and ionic strength: $+0.01 < p < 0.001$
C[c]	56.9	57.2	114.1	−5.8	28.3	2.6	1	0.423	0.423	1.54	Buffer ions: 0
AC	55.7	54.4	110.1	−1.5	26.1	2.4	1	0.360	0.360	1.31	
BC	62.6	63.0	125.6	−4.0	4.3	−2.2	1	0.303	0.303	1.10	
ABC	62.9	61.8	124.7	−0.9	3.1	−1.2	1	0.090	0.090	0.33	
Experimental error							8		4.40/2 × 8		

[a,b,c] See Table I footnotes for explanation.

Table III—Analysis of Variance at the Different Levels of Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 2.20

Factor	Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1\ 5\%\ 7.71}_{4\ 1\%\ 21.20}$
			Low Temperature (25°)		
1					
B	1	69.031	69.031	849.62	Ionic strength: $+0.001 < p$
C	1	0.001	0.001	0.02	Buffer ions: 0
BC	1	0.031	0.031	0.39	
Experimental error	4		0.65/2 × 4		
			High Temperature (50°)		
A					
AB	1	119.351	119.351	254.62	Ionic strength: $+0.001 < p$
AC	1	0.781	0.781	1.67	Buffer ions: 0
ABC	1	0.361	0.361	0.77	
Experimental error	4		3.75/2 × 4		
			Low Ionic Strength ($\mu = 0.15$)		
1					
A	1	12.005	12.005	44.06	Temperature: $-0.001 < p$
C	1	0.720	0.720	2.64	Buffer ions: 0
AC	1	0.405	0.405	1.49	
Experimental error	4		2.18/2 × 4		
			High Ionic Strength ($\mu = 1.00$)		
B					
AB	1	0.720	0.720	2.60	Temperature: 0
BC	1	0.005	0.005	0.02	Buffer ions: 0
ABC	1	0.045	0.045	0.16	
Experimental error	4		2.22/2 × 4		

ions, and temperature. In the present experiment, all of these factors were investigated at two levels. For simplicity, a letter was written when the corresponding factor was in the high level and not written when it was in the low level. When all of the factors were in the low level, a number one was written. Generally, with n factors at two levels, 2^n treatment combinations were possible. To obtain an estimation of the experimental error, each factor was determined in duplicate. The sum of the duplicates was analyzed using the Yates method (19), which is a systematic method for estimating the individual effects and performing the analysis of variance.

RESULTS AND DISCUSSION

To determine the influence of the dissociation of the ligand molecules on their binding to povidone, each ligand was investigated at two pH values: at pH = pKa − 0.80 and at pKa + 0.80. At these pH values, the drugs are 86.3% in the undissociated and dissociated state, respectively. Salicylic acid also was investigated at pH 7.00, where the drug is fully dissociated.

Salicylic Acid (pKa 3.0)—Salicylic acid was investigated at pH 2.20 and 3.80. Table I summarizes the factors and their respective levels at pH 2.20. To demonstrate the difficulties in interpreting the results, they are represented in Table I by changing one parameter from the low to the high level, with the other variables being either at a low or high level. For instance, by observing the ionic strength at the low and the high levels, the positive influence of this variable can be seen since the results change from ~56 to 62% of the ligand bound. However, the results obtained by changing the temperature from 25 to 50° are not so easy to interpret. The influence seems to be negative and is greater at low than at high ionic strength, probably due to an interaction of the temperature and the ionic strength. An accurate interpretation is impossible.

With factorial analysis, the parameter effects and interaction phenomena can be interpreted easily and accurately. In Table II, the results given in Table I are analyzed according to Yates (19). In the first column, all of the treatment combinations are listed in a systematic way; the results are determined in duplicate for each factor and are expressed as the percentage of ligand bound (column 2); their sum is listed in column 3.

According to Yates, with three factors, three columns have to be calculated (columns 4–6). Each column is generated from the preceding column in the same way. The first 2^{n-1} values in a column are the sums of successive pairs of numbers in the preceding column. The next 2^{n-1} values are the differences of the successive pairs. In this way, the final column gives the total effect corresponding to the particular treatment combinations. With these estimates, an analysis of variance is performed. This analysis consists of splitting a total sum of squares into sums of squares for the factors considered. The total effect of each factor is squared (column 8) and divided by the number of observations ($2^3 \times 2$), with each factor possessing one degree of freedom. The estimate of the residual variation, *i.e.*, the variance of the experimental error, is calculated by the sum of squares of the variations between the duplicates, divided by 2×8.

With an F test, the significance of the observed effects is tested (column 10). Table II shows that the ionic strength has an overall positive influence; however, increasing the temperature reduces the adsorption, and buffer ions have no influence at all. Moreover, a relative positive interaction is noted between ionic strength and temperature (factor AB).

By splitting up the results at low and high temperatures, more infor-

Table IV—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of the Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 2.20

Source of Variation	Percentage of Ligand Bound		Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1\ 5\%\ 5.32}_{8\ 1\%\ 11.26}$
	Exp. 1	Exp. 2					
1	57.0	56.8					
A[a]	53.7	54.3	1	38.131	38.131	217.11	Temperature: $-0.001 < p$
B[b]	56.8	57.5	1	0.051	0.051	0.29	Ionic strength: 0
AB	53.2	54.2	1	0.076	0.076	0.43	
C[c]	56.9	57.2	1	0.001	0.001	0.00	Buffer ions: 0
AC	53.7	54.4	1	0.031	0.031	0.17	
BC	56.7	57.0	1	0.031	0.031	0.17	
ABC	53.6	54.1	1	0.076	0.076	0.43	
Experimental error			8		2.81/2 × 8		

[a] Factor A = temperature; low level = 25° and high level = 50°. [b] Factor B = ionic strength (μ); low level = 0.15 and high level = 0.40. [c] Factor C = buffer ions; low level = 0.5 and high level = 1.

Table V—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of the Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 3.80

Source of Variation	Percentage of Ligand Bound Exp. 1	Exp. 2	Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1}_{8}{}^{5\%}_{1\%}{}^{5.32}_{11.26}$
1	52.4	53.1					
A[a]	46.6	45.5	1	147.623	147.623	524.88	Temperature: $0.001 < p$
B[b]	52.0	52.4	1	0.063	0.063	0.22	Ionic strength: 0
AB	45.9	46.8	1	1.103	1.103	3.92	
C[c]	52.2	53.1	1	0.000	0.000	0.00	Buffer ions: 0
AC	45.8	46.5	1	0.160	0.160	0.57	
BC	51.8	52.0	1	0.000	0.000	0.00	
ABC	46.3	47.0	1	0.040	0.040	0.14	
Experimental error			8		4.50/2 × 8		

[a,b,c] See Table I footnotes for explanation.

Table VI—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of the Binding of Salicylic Acid (1.00×10^{-2} *M*) to Povidone (5.00%) at pH 7.00

Source of Variation	Percentage of Ligand Bound Exp. 1	Exp. 2	Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1}_{8}{}^{5\%}_{1\%}{}^{5.32}_{11.26}$
1	49.0	50.2					
A[a]	42.8	41.6	1	268.141	268.141	1059.32	Temperature: $-0.001 < p$
B[b]	49.3	50.0	1	0.001	0.001	0.00	Ionic strength: 0
AB	42.0	42.5	1	0.006	0.006	0.02	
C[c]	56.6	56.4	1	102.516	102.516	405.00	Buffer ions: $+0.001 < p$
AC	47.6	47.6	1	2.481	2.481	9.80	Temperature and ionic strength: $-0.05 < p < 0.01$
BC	56.4	56.7	1	0.006	0.006	0.02	
ABC	47.8	47.5	1	0.006	0.006	0.02	
Experimental error			8		4.05/2 × 8		

[a] Factor A = temperature; low level = 25 and high level = 50°. [b] Factor B = ionic strength (μ); low level = 0.25 and high level = 0.50. [c] Factor C = buffer ions; low level = 0.5 and high level = 1.

Table VII—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of the Binding of Benzoic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 3.40

Source of Variation	Percentage of Ligand Bound Exp. 1	Exp. 2	Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1}_{8}{}^{5\%}_{1\%}{}^{5.32}_{11.26}$
1	39.0	39.2					
A[a]	41.0	40.9	1	14.251	14.251	281.49	Temperature: $+0.001 < p$
B[b]	39.4	38.9	1	0.016	0.016	0.31	Ionic strength: 0
AB	41.2	40.9	1	0.001	0.001	0.01	
C[c]	39.1	39.0	1	0.001	0.001	0.01	Buffer ions: 0
AC	41.3	40.7	1	0.001	0.001	0.01	
BC	39.1	39.2	1	0.001	0.001	0.01	
ABC	41.1	40.9	1	0.006	0.006	0.11	
Experimental error			8		0.81/2 × 8		

[a,b,c] See Table VI footnotes for explanation.

Table VIII—Experimental Conditions of the Treatment Combinations with the Resulting Percentage of Ligand Bound and the Analysis of Variance of the Binding of Benzoic Acid (1.00×10^{-2} *M*) to Povidone (6.00%) at pH 5.00

Source of Variation	Percentage of Ligand Bound Exp. 1	Exp. 2	Degrees of Freedom	Sum of Squares	Variance	*F*	Significant Level $F^{1}_{8}{}^{5\%}_{1\%}{}^{5.32}_{11.26}$
1	16.7	16.8					
A[a]	14.3	14.8	1	17.640	17.640	109.40	Temperature: $-0.001 < p$
B[b]	16.9	16.6	1	0.040	0.040	0.25	Ionic strength: 0
AB	14.4	14.6	1	0.090	0.090	0.56	
C[c]	17.0	16.3	1	0.023	0.023	0.14	Buffer ions: 0
AC	15.2	14.7	1	0.063	0.063	0.39	
BC	17.2	16.3	1	0.023	0.023	0.14	
ABC	14.1	14.9	1	0.063	0.063	0.39	
Experimental error			8		2.58/2 × 8		

[a,b,c] See Table VI footnotes for explanation.

mation about the influence of the ionic strength can be obtained; by considering the results at low and high ionic strengths, the influence of temperature can be analyzed more specifically. The results obtained by splitting at the different levels together with their analysis of variance are given in Table III. Considering the results at the different levels (Table III), the overall positive influence of ionic strength is maintained for the two temperatures, while the negative influence of temperature is observed at low ionic strength.

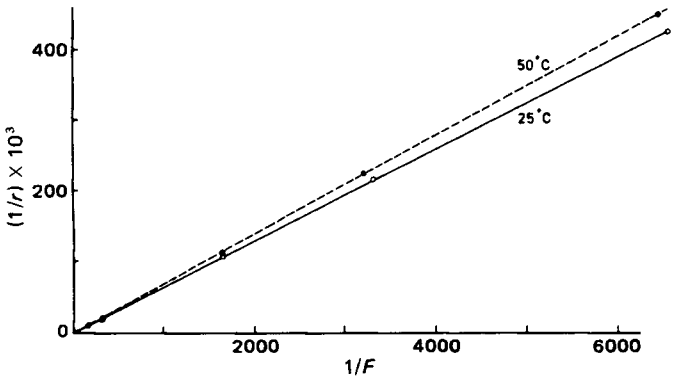

Figure 2—*Influence of temperature on the complexing of salicylic acid with povidone at pH 2.20. The salicylic acid concentration was 5.00 × 10^{-4}–1.00 × 10^{-2} M, the povidone concentration was 6.00% (8.57 × 10^{-5} M), the ionic strength was 0.15, and the buffer ions were 1.*

In Fig. 1, the influence of the different variables investigated before and after splitting the results at the different levels is represented. This figure also offers a good idea of how much information can be obtained from factorial analysis. In the middle of the graph, the overall effects are indicated; on the side, the results after splitting the results are represented.

By lowering the high level of the ionic strength from 1.0 to 0.40 and maintaining the other factors at their original levels, the percentage of ligand bound is determined by ultrafiltration, and a factorial analysis is carried out again. From these results (Table IV), it is noted that ionic strength no longer has an effect on the binding at these levels whereas temperature and buffer ions behave as indicated in Table I.

For salicylic acid at pH 3.80, where the drug is mainly dissociated, the same factors as are given in Table I were investigated. The levels used and the corresponding results are summarized in Table V. In contrast with the phenomena observed at pH 2.20, ionic strength no longer influenced the binding of salicylic acid to povidone while increasing the temperature also diminished the adsorption. The observations remained after splitting the results at the different levels of ionic strength and temperature.

The adsorption of salicylic acid in the dissociated state onto povidone was investigated at pH 7.00 using a phosphate buffer. Factorial analysis was carried out with three factors at two levels (Table VI). Increasing the temperature and the ionic strength had a negative and no influence, respectively, on the binding of salicylic acid. At pH 7.00, in contrast to pH 2.20 and 3.80, the buffer ions enhanced complex formation (from 49.6 to 56.9% of the ligand was bound). At the two lower pH values, the buffer ions were mainly monovalent phosphate and citrate ions. At pH 7.00, the phosphate ions were present as dibasic phosphate ions at 38%, indicating the great influence of these ions on complex formation. However, dibasic phosphate ions do not always enhance complex formation. Thus, no influence was found for the binding of methyl orange to povidone at pH 7.00 (20). On the other hand, dibasic phosphate ions increased binding for chlorpromazine with carboxymethylcellulose sodium (21), as well as citrate ions, by complex formation of local anesthetics and polyvinyl alcohol (22).

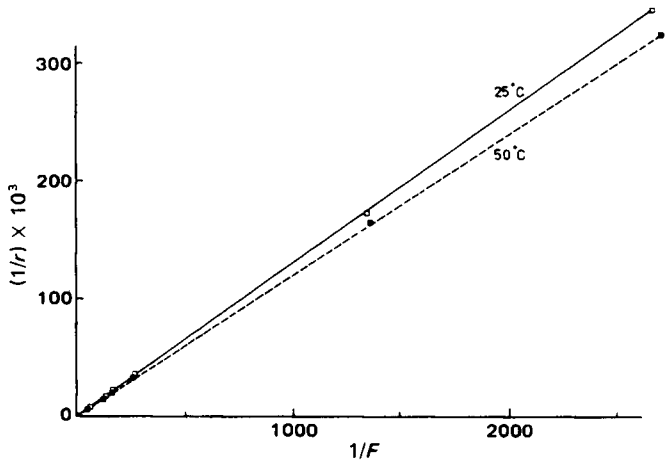

Figure 4—*Influence of temperature on the complexing of benzoic acid with povidone at pH 3.40. The benzoic acid concentration was 1.00 × 10^{-4}–1.00 × 10^{-2} M, the povidone concentration was 6.00% (8.57 × 10^{-5} M), the ionic strength was 0.25, and the buffer ions were 1.*

It is concluded that binding depends not only on the kind of buffer ions present but also on the structure of the macromolecule and the ligand. A second important conclusion is evident; without knowledge of the influence of buffer ions, the binding tendency of the undissociated and dissociated forms of salicylic acid cannot be compared.

Benzoic Acid (pKa 4.2)—For benzoic acid, the same factors and levels were investigated as for salicylic acid. However, the buffers used were McIllvaine buffers at pH 3.40 and 5.00. The ionic strength was maintained at 0.25 and 0.50. At pH 3.40 and 5.00, the ionic strength and buffer ions had no influence on the binding of benzoic acid to povidone (Tables VII and VIII). At pH 3.40, where benzoic acid is mainly undissociated, the adsorption was enhanced by increasing the temperature; at pH 5.00, the binding was diminished.

Isotherms and Thermodynamic Calculations—Isotherms were obtained at 25 and 50°; they are represented graphically using the Klotz isotherm (Eq. 4) in Figs. 2 and 3 for salicylic acid and in Figs. 4 and 5 for benzoic acid.

For the two derivatives, a linear relationship was observed, with the two lines nearly going through the origin. From Eq. 2, it is seen that this finding implies that $kF \ll 1$; from Eq. 4, it is shown that $1/n$ equals zero or n is infinite, indicating a nearly infinitely large number of adsorption sites. The fraction of the ligand bound to the macromolecule does not vary significantly with the ligand concentration, and the binding process ap-

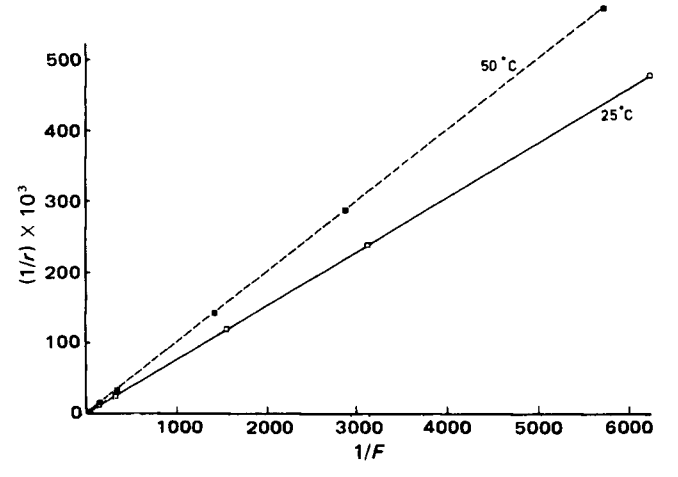

Figure 3—*Influence of temperature on the complexing of salicylic acid with povidone at pH 3.80. The salicylic acid concentration was 5.00 × 10^{-4}–1.00 × 10^{-2} M, the povidone concentration was 6.00% (8.57 × 10^{-5} M), the ionic strength was 0.15, and the buffer ions were 1.*

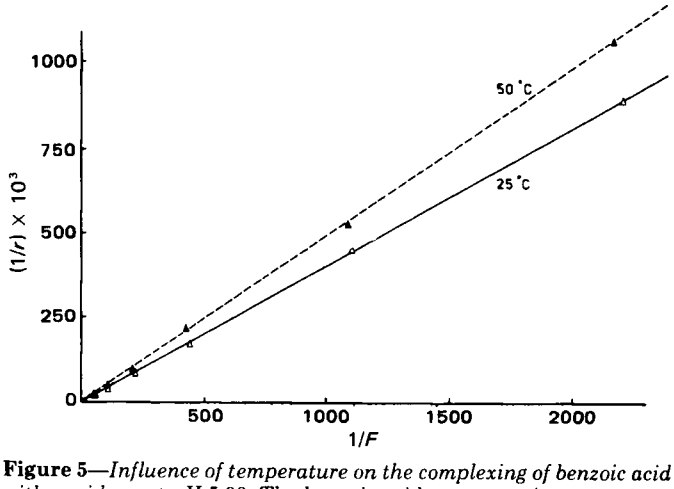

Figure 5—*Influence of temperature on the complexing of benzoic acid with povidone at pH 5.00. The benzoic acid concentration was 1.00 × 10^{-4}–1.00 × 10^{-2} M, the povidone concentration was 6.00% (8.57 × 10^{-5} M), the ionic strength was 0.25, and the buffer ions were 1.*

Table IX—Thermodynamic Data for the Binding of Salicylic Acid and Benzoic Acid to Povidone

Cosolute	pH	nk ($\times 10^{-3}$) at 298 °K, liters/mole	nk ($\times 10^{-3}$) at 323 °K, liters/mole	$\Delta F°$ (323 °K), kcal/mole	$\Delta H°$, kcal/mole	$\Delta S°$, kcal/mole degree
Salicylic acid	2.20	16.2	15.4	−6.2	−0.4	+18.0
	3.80	13.1	9.3	−5.9	−2.1	+11.8
Benzoic acid	3.40	7.8	8.5	−5.8	+0.7	+20.1
	5.00	2.5	2.0	−4.9	−1.5	+10.4

pears to be unsaturable. The same finding was made by Higuchi and Kuramoto (23) in a study of complex formation between benzoic acid and povidone[7] in aqueous solution. Values for nk are deduced from the slopes. According to Scholtan (20), the value of n can be determined only with much uncertainty, so it has little physical meaning. In this case, it is better to use the value of $nk = k_1$, which is a measure for the strength of the binding, while n is indicative of the binding capacity.

The thermodynamic functions calculated from k_1 do not necessarily refer to a single site on the macromolecule. However, the values are useful in considering the nature of the binding, in comparing the binding of different ligands with the same macromolecule, and in analyzing the effects of parameters such as pH and ionic strength (14). The standard free energy, $\Delta F°$, for complex formation is calculated from:

$$\Delta F° = -RT \ln nk \qquad \text{(Eq. 6)}$$

With the assumption that there is no significant temperature dependence of the enthalpy change within the temperature range used, the standard enthalpy change, $\Delta H°$, for the association of 1 mole of ligand with 1 mole of macromolecule is given by:

$$\ln \frac{nk_{25\,°C}}{nk_{50\,°C}} = -\frac{\Delta H°}{R}\left(\frac{1}{298} - \frac{1}{323}\right) \qquad \text{(Eq. 7)}$$

and the entropy change, $\Delta S°$, is obtained by substituting $\Delta H°$ and $\Delta F°$ in the Gibbs–Helmholtz equation:

$$\left(\frac{\delta \Delta F°}{\delta T}\right)_p = -\Delta S° = \frac{\Delta F° - \Delta H°}{T} \qquad \text{(Eq. 8)}$$

Thermodynamic data for the association reactions are reported in Table IX. The negative sign for $\Delta F°$ means that the binding process occurs spontaneously. The high positive entropy and small negative enthalpy values are characteristic for hydrophobic binding (24–26). The positive entropies are associated with many reactions involving the binding of ligands to macromolecules as well as proteins (27–30) and to macromolecules such as polymethacrylic acid (31), polyvinyl alcohol (32), methylcellulose (33), hydroxyethyl cellulose (33), and povidone (34–36). With the knowledge that the buffer composition and ionic strength have no influence on the adsorbing tendency of the two derivatives studied, the thermodynamic constants calculated from the isotherms are due entirely to the binding of the ligand to the macromolecule. Thus, comparison of the thermodynamic constants and the effect of the substituted groups as well as the effect of their degree of dissociation on the binding tendency is possible now.

For salicylic acid (Table IX), the binding strength was lowered by increasing temperature, both for the undissociated and dissociated forms; at the two temperatures, binding was highest for the undissociated form. The same findings can be noted for benzoic acid, except for the undissociated form, where the binding strength was highest at 323 °K. Substitution of a hydroxyl function in the *ortho*-position of the carboxyl function increased the binding strength at 298 °K by a factor of 2.1 for the undissociated form and by a factor of 5.3 for the dissociated form; at 323 °K, it increased by factors of 1.8 and 4.6, respectively. The observations indicate that hydrogen binding also may play an important role in the binding. The application of factorial analysis is under investigation in the study of complex formation between povidone and a series of benzene and pyridine derivatives.

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[7] Kollidon K-30.