Interaction of Bishydroxycoumarin with Polyvinylpyrrolidone

M. J. CHO*, A. G. MITCHELL, and M. PERNAROWSKI†

Abstract □ Bishydroxycoumarin forms a highly soluble complex with polyvinylpyrrolidone in aqueous solution. A 1.0% solution of polyvinylpyrrolidone at pH 7.4 increased the solubility of bishydroxycoumarin, the increase being approximately 21.5 times that observed for the drug in the absence of the macromolecule. Equilibrium dialysis, viscometric, and spectrophotometric methods were used to study binding mechanisms. A polyvinylpyrrolidone molecule with a molecular weight of 40,000 provides approximately 50 identical sites for the bishydroxycoumarin molecule. This implies that one binding site consists of seven or eight monomer units of the polymer. The intrinsic association constant is in the order of 3×10^3 l./mole at 20° and decreases with an increase in temperature. Thermodynamic data indicate that hydrophobic bonds form at low temperature. UV spectral changes of the drug in the presence of the macromolecule suggest that the α,β-unsaturated lactone structure of bishydroxycoumarin is involved in the complexation. Intrinsic viscosity data indicate that coiling occurs during the binding process.

Keyphrases □ Bishydroxycoumarin, polyvinylpyrrolidone—interaction □ Complex formation—bishydroxycoumarin, polyvinylpyrrolidone □ Dialysis, equilibrium—bishydroxycoumarin, polyvinylpyrrolidone complex □ Viscometry—bishydroxycoumarin, polyvinylpyrrolidone complex □ UV spectrophotometry—analysis

Polyvinylpyrrolidone has several properties of pharmaceutical interest, including its ability to complex with drugs, dyes, toxins, and other substances (1). The results reported herein indicate that a highly soluble complex is formed when the polymer and bishydroxycoumarin are combined at a pH of 7.4. Studies were carried out to evaluate the mechanism of this interaction.

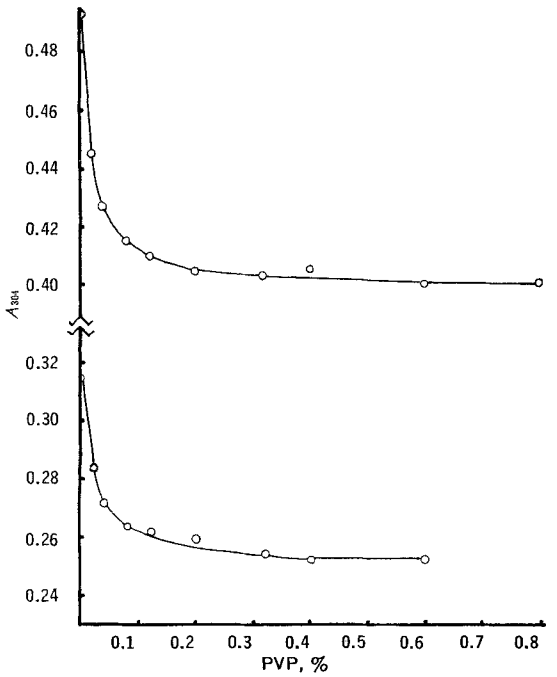

Figure 1—*Effect of polyvinylpyrrolidone on the* A_{304} *value for bishydroxycoumarin (upper curve: 26.5* $\times$ 10^{-6} *mole/l. bishydroxycoumarin; lower curve: 16.9* $\times$ 10^{-6} *mole/l.).*

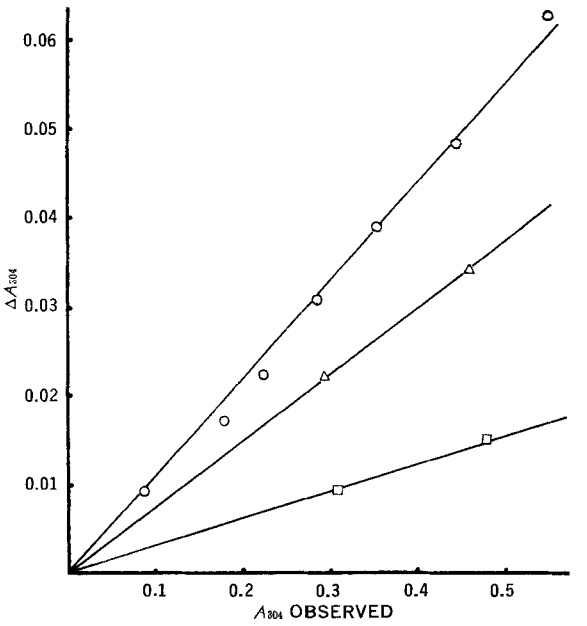

Figure 2—*Calibration curves for the analysis of bishydroxycoumarin in the presence of polyvinylpyrrolidone. Polyvinylpyrrolidone concentrations: 0.004%, □; 0.01%, △; and 0.02%, ○.*

EXPERIMENTAL

Materials—Specifications for bishydroxycoumarin, tris(hydroxymethyl)aminoethane, and dialyzer tubing were given in a previous paper (2). Polyvinylpyrrolidone[1] was extracted with anhydrous ether in a continuous-extraction apparatus for 24 hr. to remove any subfractions or impurities and then dried in a vacuum oven at 25–40°. The average water content, based on loss of weight at 110–115° for 24 hr., was 3.8%. A buffer system of pH 7.4 and ionic strength 0.15 [0.18 mole/l. tris(hydroxymethyl)aminoethane and 0.15 *N* HCl] was used in this investigation.

Spectrophotometric Analysis—Bishydroxycoumarin absorbs a maximum of radiant energy at 304 nm. The A_{304} value decreases as polyvinylpyrrolidone concentration increases. A series of solutions containing a constant quantity of bishydroxycoumarin and varying quantities of polyvinylpyrrolidone in tris(hydroxymethyl)aminoethane buffer was prepared, and A_{304} values were measured[2]. Tris(hydroxymethyl)aminoethane buffer was used as a reference solution. Results for two concentrations of bishydroxycoumarin in the presence of varying quantities of polyvinylpyrrolidone are shown in Fig. 1.

Calibration curves for the analysis of bishydroxycoumarin in the presence of polyvinylpyrrolidone are shown in Fig. 2. Three series of solutions containing varying amounts of bishydroxycoumarin and 0.004, 0.01, and 0.02% polyvinylpyrrolidone in tris(hydroxymethyl)aminoethane buffer were prepared. Polyvinylpyrrolidone solutions containing no bishydroxycoumarin were used as reference solutions. The A_{304} values were measured. A second set of solutions

[1] Plasdon C, General Aniline Corp., New York, N. Y.; average molecular weight, 40,000.

[2] A Beckman DU-2 spectrophotometer equipped with a thermospacer and maintained at 20° was used to measure A_s values.

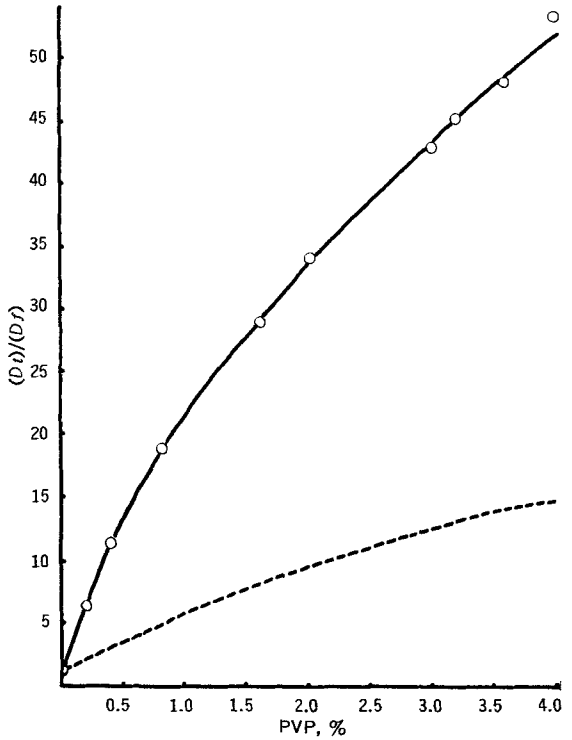

Figure 3—*Effect of polyvinylpyrrolidone on the apparent solubility of bishydroxycoumarin at 20°. Dotted line (see* Reference 2) *shows the effect of human serum albumin.*

containing identical quantities of bishydroxycoumarin but no polyvinylpyrrolidone was prepared. Tris(hydroxymethyl)aminoethane buffer was used as the reference solution. The A_{304} values were measured. For each pair of solutions containing identical quantities of bishydroxycoumarin, the former A_{304} value was subtracted from the latter A_{304} value. This yielded the ΔA_{304} value, which was then plotted against the A_{304} value for solutions containing polyvinylpyrrolidone. Results are shown in Fig. 2. For example, if a bishydroxycoumarin solution in 0.02% polyvinylpyrrolidone had an A_{304} value of 0.400, the corrected value of 0.443 was used to calculate bishydroxycoumarin concentrations.

Solubility Analysis and Equilibrium Dialysis Studies—Solubility and dialysis procedures were described in a previous paper (2). Dialysis experiments were carried out at 10, 20, 30, and 40° using 0.1 and 0.4% polyvinylpyrrolidone. A correction factor of 4% of the total bishydroxycoumarin was used to correct for losses due to membrane binding (2). It was reported (3) that some polyvinylpyrrolidone passes through the membrane even if the average molecular weight is over 40,000. A colorimetric method of analysis for polyvinylpyrrolidone (4) showed that approximately 0.25% of the total amount of polyvinylpyrrolidone present passed through the membrane. No correction, therefore, was made for polyvinylpyrrolidone losses through the membrane.

Viscometric Analysis—Using capillary viscometers[3], the intrinsic viscosity of polyvinylpyrrolidone in tris(hydroxymethyl)aminoethane buffer was determined in the presence and absence of bishydroxycoumarin at 10, 20, 30, and 40°. Temperatures were controlled to ± 0.05°. Polyvinylpyrrolidone concentrations ranged from 0.5 to 4.0%. The bishydroxycoumarin concentration was 1.85×10^{-4} mole/l. Procedures for the determination of intrinsic viscosity are given in the literature (6). The relative viscosities of bishydroxycoumarin solutions and tris(hydroxymethyl)aminoethane buffer were considered to be unity in the calculation of relative viscosity. Densities were measured using a calibrated Westphal balance[4] maintained at a constant temperature.

[3] Cannon Fenske routine viscometer (size 50), Cannon Instrument Co., State College, Pa. The instrument was calibrated with freshly distilled water. The kinetic energy correction term (5) was neglected.

[4] E. Machlett & Son Scientific Apparatus, New York, N. Y. The reproducibility was ±0.0001 density unit.

RESULTS AND DISCUSSION

Spectrophotometric Analysis—Polyvinylpyrrolidone alters the spectral characteristics of bishydroxycoumarin. A similar phenomenon was observed for the human serum albumin–bishydroxycoumarin interaction (2). It was suggested in that paper (2) that the α,β-unsaturated lactone structure present in bishydroxycoumarin was involved in the interaction. Figure 1 shows that the A_{304} value does not decrease beyond a certain polyvinylpyrrolidone concentration. This implies that, beyond that point, the bishydroxycoumarin molecules are in the bound form. On the basis of these data and those obtained from bishydroxycoumarin solutions containing no polyvinylpyrrolidone, the ϵ values for free (1.87×10^4) and bound (1.5×10^4) bishydroxycoumarin can be calculated. The concentration of free drug (D_f) and the r value can be estimated from the ϵ values for free and bound drug.

Solubility Analysis—The effect of polyvinylpyrrolidone on the apparent solubility of bishydroxycoumarin is illustrated in Fig. 3 which, for comparison, includes the effect of human serum albumin (2). The solubility of bishydroxycoumarin in tris(hydroxymethyl)-aminoethane buffer of pH 7.4 is 1.90×10^{-4} mole/l. The solubility curve is linear to about 0.4% polyvinylpyrrolidone.

The molar ratio of total drug to free drug, $(D_t)/(D_f)$, at a given macromolecule concentration has been widely used as a measure of binding. Literature values for approximately 40 compounds in 1.0% polyvinylpyrrolidone show that the ratio rarely exceeds 1.2. Bishydroxycoumarin has the unusually high value of 21.5. The molar ratio of bound drug to total macromolecule, $(D_b)/(M_t) = r$, is also frequently used as a measure of binding. The r value, calculated from the initial slope of Fig. 3, is 20.

Equilibrium Dialysis—Results for the dialysis experiments are shown in a Langmuir-type plot (Fig. 4). The binding curve is expressed by Eq. 1, which is derived from the law of mass action (7, 8):

$$r = \frac{nk(D_f)}{1 + k(D_f)} \qquad \text{(Eq. 1)}$$

where n is the maximum number of independent binding sites on the polymer, and k is the intrinsic association constant. The Langmuir-type plot (Fig. 4) shows that saturation of the binding sites was not achieved by the dialysis method. However, an upper limit to the binding curve was obtained from the solubility data, *i.e.*, $r = 20 = n$.

Equation 1 can be rearranged in a number of ways for convenient plotting to yield values of k and n by extrapolation, *e.g.*:

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nk(D_f)} \qquad \text{(Eq. 2)}$$

i.e., a plot of $1/r$ *versus* $1/(D_f)$ is a straight line with a slope of $1/nk$ and an intercept of $1/n$. The curves pass through approximately the same intercept, the reciprocal of which (*i.e.*, 50) was taken as the maximum number of binding sites on the polymer. Intrinsic association constants were calculated from the slopes at different temperatures and are listed in Table I.

Thermodynamic Analysis and Mechanism of Interaction—A van't Hoff-type plot is shown in Fig. 6. The standard enthalpy change, $\Delta H°$, at a given temperature was estimated from the tangent to the curve. Other thermodynamic functions were calculated in the usual way, including the "unitary" entropy change, ΔSu (9), and are reported in Table I. The unitary function depends only on factors that involve the interaction of bishydroxycoumarin molecules and the binding sites with the solvent and with each other. Contributions due to randomness of mixing with the solvent are excluded.

Table I—Thermodynamic Data for Interaction of Bishydroxycoumarin with Polyvinylpyrrolidone

Temperature	$k \times 10^{-3}$, l./mole	$\Delta H°$, kcal./mole	$\Delta G°$, kcal./mole	$\Delta S°$, e.u.	ΔSu, e.u.
10°	3.64	−2.74	−4.61	+6.63	+14.61
20°	3.05	−2.96	−4.67	+5.86	+13.84
30°	2.48	−4.69	−4.71	+0.05	+8.03
40°	1.72	−8.57	−4.64	−12.56	−4.58

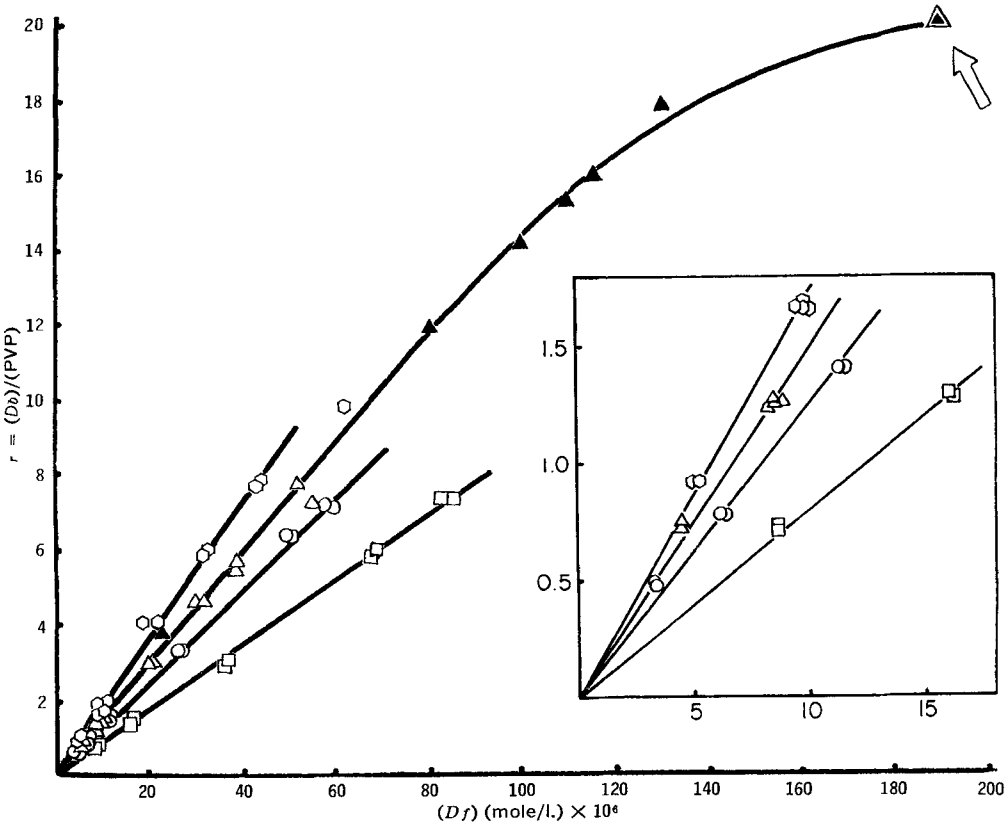

Figure 4—*Langmuir-type plot for the polyvinylpyrrolidone–bishydroxycoumarin interaction at 10°, ○; 20°, △; 30°, ○; and 40°, □. Polyvinylpyrrolidone concentrations are 0.4% (open symbols) and 0.1% (closed symbols). Solubility data are indicated by an arrow.*

The thermodynamic parameters obtained were interpreted on the basis of the "iceberg" concept of water structure (10). The number of hydrogen bonds surrounding the polyvinylpyrrolidone–bishydroxycoumarin complex is less than the number around the two unbound entities. Hence, it might be expected that complexation should be accompanied by a positive enthalpy change. Table I, however, shows that binding is exothermic from 10 to 40°. In studies in the interaction of polyvinylpyrrolidone with a number of substances, Molyneux and Frank (11) calculated that the net enthalpy change associated with: (*a*) the heat needed to overcome specific interaction between water and the polymer and water and the small molecule (*i.e.*, "dehydration"); (*b*) exothermic binding between the "dehydrated" entities to form a complex; and (*c*) exothermic interaction between the complex and neighboring water (*i.e.*, "rehydration") is essentially a constant value of −5 kcal./mole (ΔHb in *Reference 11*). If this value is subtracted from $\Delta H°$ in Table I, the net enthalpy changes associated with the melting of the icebergs around the two separate entities and the reformation of hydrogen bonds in icebergs around the complex become +2.3, +2.0, +0.3, and −3.6 kcal./mole at 10, 20, 30, and 40°, respectively. The decrease in enthalpy with increasing temperature may be explained by the decrease in "icelikeness" around hydrocarbons with increasing temperatures (Table IX in *Reference 12*).

Positive entropy changes on the interaction of polyvinylpyrrolidone with a variety of small molecules or ions have been attributed to the formation of hydrophobic bonds (11, 13, 14). It is postulated that the polyvinylpyrrolidone bishydroxycoumarin complex is accompanied either by a less ordered iceberg or by an

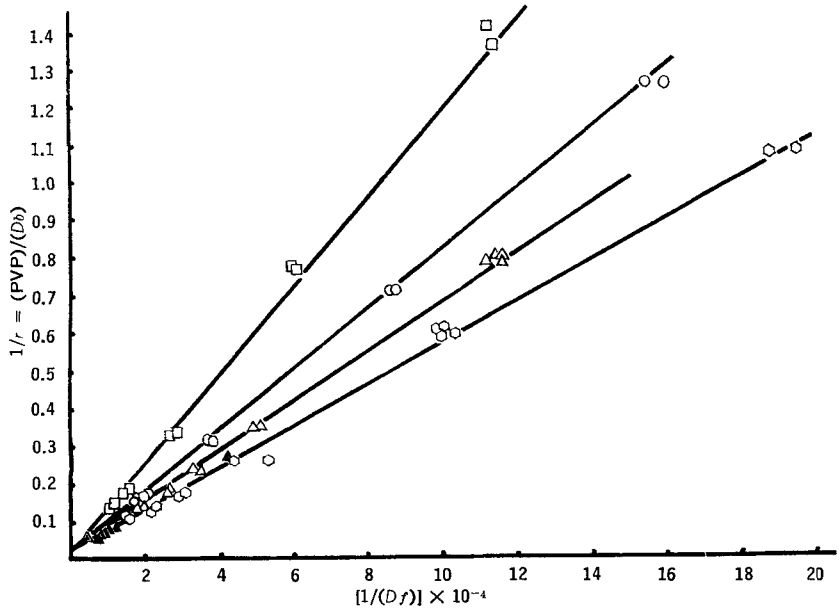

Figure 5—*Double reciprocal plot for the polyvinylpyrrolidone–bishydroxycoumarin interaction (symbols as in Fig. 4).*

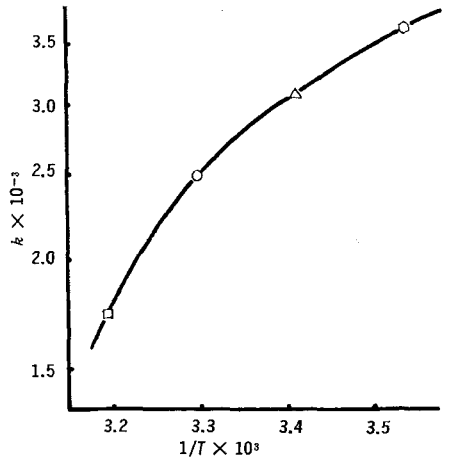

Figure 6—*Van't Hoff plot (log* k versus *1/*T*) for the polyvinylpyrrolidone–bishydroxycoumarin interaction.*

iceberg containing a smaller number of water molecules than the icebergs of the two separate entites. The release of water molecules from the ordered structure should produce a proportional gain in positive entropy (increase in randomness). Thus, the positive entropy values observed between 10 and 30° (Table I) is attributed to the formation of hydrophobic bonds. The contribution of the entropy term to free energy is 41 and 37% at 10 and 20°, respectively. At higher temperatures the binding process is increasingly exothermic; the role of hydrophobic bonding, which is essentially endothermic (9, 15), becomes less important.

At pH 7.4, bishydroxycoumarin exists mainly as the anion (2). However, polyvinylpyrrolidone has no ionizable groups (16) and it is unlikely that electrostatic interactions will be involved in complex formation. The dependence of binding strength on temperature also rules out this possibility (17). However, Frank *et al.*

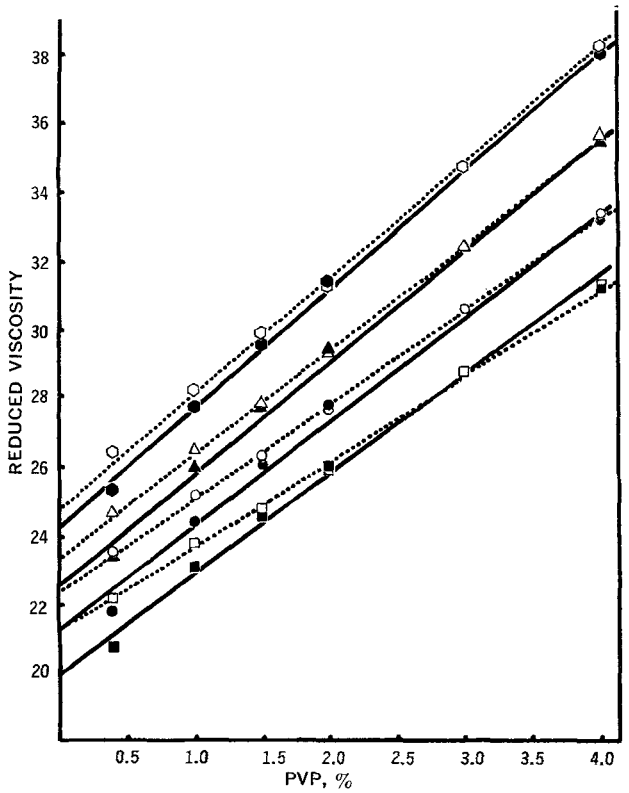

Figure 7—*Reduced viscosity of polyvinylpyrrolidone as a function of polyvinylpyrrolidone concentration at various temperatures (symbols as in Fig. 4). Solid and dotted lines represent the viscosities in the presence and absence of bishydroxycoumarin (185 × 10⁻⁶ mole/l.), respectively.*

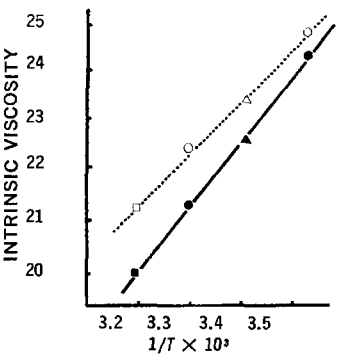

Figure 8—*Effect of temperature on the intrinsic viscosity of polyvinylpyrrolidone in the presence of bishydroxycoumarin (symbols as in Fig. 4).*

(18) reported that the lactam bond in the pyrrolidone ring is likely to undergo ion-dipole interaction with an anion, and van der Waals' forces will stabilize the complex.

A polyvinylpyrrolidone molecule with a molecular weight of 40,000 has approximately 360 monomer units. The binding data shown in Fig. 5 indicate that the average number of binding sites on a polyvinylpyrrolidone molecule is approximately 50, irrespective of temperature; *i.e.*, on the average, 7.2 repeating units provide a binding site for one bishydroxycoumarin molecule. Molecular models show that a bishydroxycoumarin molecule fits on approximately eight pyrrolidone rings. The polyvinylpyrrolidone model shows that the pyrrolidone rings appear to provide a channel-type cavity in both sides of the paraffin backbone. The possibility of another bishydroxycoumarin anion binding on the other side of the backbone is unlikely because of repulsive forces between the bound anion and an oncoming anion.

Viscometric Analysis—The relationship between changes in intrinsic viscosity and configurational changes in macromolecules is discussed in *References 19* and *20*. Viscometry has been widely used for complexation studies of polyvinylpyrrolidone with a variety of substances (18, 21–23).

In Fig. 7, the reduced viscosity is plotted *versus* polyvinylpyrrolidone concentration. The correlation coefficient between these two variables is in the range of 0.980–1.048. The intercept (*i.e.*, intrinsic viscosity) was determined by means of the method of least squares. No attempts were made to interpret changes in the Huggins constant (24) associated with the interaction since they are, in general, too irregular to be correlated with any definite molecular effect (22).

In the absence of bishydroxycoumarin, the intrinsic viscosity of polyvinylpyrrolidone decreases with increase in temperature. This finding was explained in terms of a progressive coiling of the polymer with temperature (25). A monoionized bishydroxycoumarin molecule is expected to have few rotational degrees of freedom around the methylene bridge because of the one remaining intramolecular hydrogen bond (Scheme 1b in *Reference 2*). A model of the complex indicates that a better fit is achieved by a slight bending or folding of the polymer toward the bishydroxycoumarin anion. This postulate is supported by the decrease in intrinsic viscosity on binding which suggests coiling of the polymer chain.

The variation of intrinsic viscosity with temperature is shown in an Arrhenius-type plot in Fig. 8. From the slope, a term analogous to activation energy was estimated to be 0.92 and 1.13 kcal./mole in the presence and absence of bishydroxycoumarin, respectively. The difference in slopes indicates a reduction in the extent of coiling on complexation as the temperature is decreased. Theoretically, at approximately −5°, no configurational changes will occur.

In contrast to these results, Molyneux and Frank (22) found an increase in intrinsic viscosity of polyvinylpyrrolidone in the presence of many organic anions. This was explained in terms of expansion of the polymer due to coulombic repulsion between the bound anions. However, in tris(hydroxymethyl)aminoethane buffer of ionic strength 0.15, repulsion between bound anions is reduced by the "screening" effect of the free counterions (RNH_3^+ form) and coiling of the polymer becomes possible.

Changes in intrinsic viscosity are not, in general, a linear function of the concentration of the cosolute (21). Due to the low solubility of bishydroxycoumarin, viscosity determinations were impractical, except at a concentration near the solubility limit. Hence, the changes measured serve only to ascertain the existence of an effect at that concentration and the general nature of this effect.

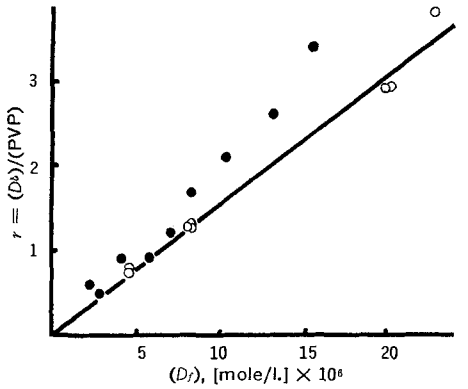

Figure 9—*Comparison of binding data obtained from spectrophotometric analysis* (●) *with data obtained from equilibrium dialysis* (○) *for the polyvinylpyrrolidone–bishydroxycoumarin interaction at 20°.*

Summary and Comparison of Methods Used to Evaluate Binding—Four methods were used to evaluate the binding mechanism of bishydroxycoumarin and polyvinylpyrrolidone: spectrophotometric, equilibrium dialysis, solubility, and viscometric. The first three methods are based on the analysis of changes in the properties of bishydroxycoumarin due to complex formation. Viscometry measures changes in rheological properties occurring in polyvinylpyrrolidone.

Figure 9 compares spectrophotometric data with the results obtained from dialysis experiments. At low (D_f), data from both methods agree. The agreement may be due to the fact that polyvinylpyrrolidone has only one type of binding site for bishydroxycoumarin. However, the precision of the spectrophotometric method falls off rapidly at higher (D_f).

The spectrophotometric method is important since information on the binding process can be obtained with accuracy at very low (D_f) and it is unnecessary to separate free and bound drug as in the dialysis technique. Binding data obtained in this way must be complemented by other methods because drug concentrations are limited by Beer's law.

Solubility analysis has been used widely to study molecular interaction in solution (26). If there is a sharp break in the solubility curve, the overall step stability constant, K_{ov}, can be estimated[5]. However, polyvinylpyrrolidone has a high aqueous solubility and formed a soluble complex with bishydroxycoumarin. The solubility curve was nonlinear and failed to show a break. The unusually high solubility of the complex deserves further investigation. Coprecipitates of bishydroxycoumarin and polyvinylpyrrolidone would be expected to produce a significant increase in dissolution rate.

The value of n obtained by the solubility method was approximately 20, compared with a value of 50 obtained from dialysis experiments. The molecular model of the complex supports the latter value. It is suggested that all binding sites are not accessible to bishydroxycoumarin molecules and that uptake of bishydroxycoumarin anion interferes with the interaction at other sites.

Viscometry does not provide quantitative information; in the present study, its applicability was limited by the low solubility of bishydroxycoumarin at pH 7.4. However, a configurational change in polyvinylpyrrolidone was detected from measurements made at one bishydroxycoumarin concentration. Coiling of the polymer, as a result of binding, is a possible explanation of the inaccessibility of all binding sites to bishydroxycoumarin molecules.

[5] The value of K_{ov} is defined by $(MD_n)/(M_f)(D_f)^n$ for the reaction $nD + M = MD_n$. The relationship of this constant with the intrinsic association constant, k, is (27):

$$K_{ov} = k^n \left[n \left(\frac{n-1}{2}\right)\left(\frac{n-2}{3}\right)\left(\frac{n-3}{4}\right) \cdots \left(\frac{1}{n}\right)\right]$$

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