

The actual nature of the hydrodynamic conditions at the tablet surface can be determined from the relationship between the dissolution rate and the angular velocity of the rough pellet surface. The relationship between  $R_n$  and angular velocity ( $\omega$ ) can be written in the form (10):

$$R_n \sim \omega^p \quad (\text{Eq. 8})$$

At smooth rotating disk surfaces,  $p = 0.5$  under laminar conditions and  $p = 0.9$  in a completely turbulent flow pattern. At a rough rotating disk surface, consisting of a regular, geometric pattern of pyramids, the following results were obtained (11-14): with a pyramid height of  $>1$  mm the mass flux varied with  $\omega^{0.67}$ . But when the height of the roughness elements diminished, the exponent of  $\omega$  increased from 0.67 to  $>0.9$  (14). The experimental data collected in Fig. 3 were used to investigate the effect of the presence of pores on the hydrodynamics at the tablet surface. Since the dissolution rates can be compared only for equal values of  $n$  at the different rotation speeds, interpolated values of  $R_n$  were calculated by Eq. 7 from the best fit of the experimental data for various numbers of pores at each rotation speed (Table II). A linear regression analysis of the plot of  $\log R_n$  versus  $\log \omega$  for any number of pores was performed on the data collected in Table II. The slopes of these plots correspond to  $p$  in Eq. 8. Exponent  $p$  increases from  $\sim 0.5$  at  $n = 0$  (smooth surface) to 0.8-0.9 for surfaces with  $>250$  pores. This confirms earlier observations that, due to the presence of pores, the flow regimen in the boundary layer near the surface shows a transition from laminar to turbulent, resulting in a changed dissolution mechanism of the surface.

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## Interaction of Povidone with Aromatic Compounds V: Relationship of Binding Tendency in a Macromolecular Solution Treated as a Pseudo Two Phase and a Monophase

J. A. PLAIZIER-VERCAMMEN

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**Abstract** □ The pseudo-two-phase model is proposed to correlate complex formation of ligand molecules with povidone with partition coefficients ( $\log P$  or  $\Pi$  constants). The conditions which permit the use of the pseudo-two-phase model for binding of ligand onto macromolecules are determined. This model seems to be a more rational choice than the frequently used complex formation model (monophase). This is demonstrated theoretically and confirmed experimentally. The advantages of the use of such a model are also discussed.

**Keyphrases** □ Povidone—interaction with aromatic compounds, pseudo-two-phase and monophase systems, salicylic acid, complex formation □ Pseudo-two-phase model—compared with monophase, complex formation aromatic compounds with macromolecules

The important role of lipophilicity of ligand molecules and hydrophobic bonding in complex formation of a series of ligand molecules with povidone has been previously investigated; ligand molecules in the nonionic state showed a higher complexing tendency than those in the ionic state, (1-3). Complex formation of ligand molecules with povidone was explained in terms of hydrophobic bonding (1, 4-8). In earlier work, we studied the complexing tendency of ligand molecules in the nonionic and ionic state, and the importance of the hydrophobic bonding especially for nondissociated molecules was confirmed (9). The correlation observed between the solubility of the ligand molecule in solvent mixtures and its binding to povidone (10) also provided support for the occurrence of complex formation by hydrophobic interactions.

Hansch *et al.* (11) showed that complex formation by hydrophobic bonding of neutral organic molecules with macromolecules, such as serum albumin, can often be correlated with partition coefficients ( $\log P$  or  $\pi$  constants) (12, 13) between 1-octanol and water. This correlation was found for phenols and serum albumin, barbiturates and homogenized rabbit brain (14), penicillin and serum albumin (15), aniline derivatives and nylon and rayon (16), and phenols and mitochondrial protein (17).

These results, correlated with octanol-water partition coefficients ( $\log P$ ), are expressed either as  $1/C$  (11) (where  $C$  represents the molar concentration of ligand to produce a one-to-one complex of ligand and macromolecule), as  $\log B\%$  [percent bound ligand (14)], or as  $\log B/F$  (15), where  $B$  refers to bound and  $F$  to free ligand concentrations, respectively. The methods for expressing complex formation are not comparable. As indicated by Bird and Marshall (15),  $B/F$  is a more rational choice than  $B\%$  for correlating the results with  $\log P$ , because  $B/F$  is analogous to an organic solvent-water partition coefficient. Moreover, as opposed to the other expressions,  $B$  is not a linear function of the association constants.

However, the use of the  $B/F$  expression has a disadvantage; one must work at a constant macromolecular concentration in order to compare the  $B/F$  expressions for the individual ligand molecules with the  $\log P$  or  $\pi$  values. This implies that one must expect errors in the results obtained for ligand mol-

ecules with a low or a high tendency for complex formation (18). The  $1/C$  expression offers the advantage over the  $B/F$  expression in that it is directly related to the association constant and is independent of the macromolecular concentration. However, for the determination of  $1/C$ , a series of ligand concentrations must be investigated to determine the concentration of ligand ( $F$ ) where  $r = 1$  [ $r$  is defined as mole of bound ligand/mole of macromolecule (11)]. Moreover, expressions used to correlate complex formation to  $\log P$  values suffer from the fact that notations such as  $T$  (total ligand),  $F$  (free ligand), and  $B$  (bound ligand) calculated from a monophasic point of view (the theory of complex formation) are correlated to the same notations, calculated from a biphasic point of view (partition theory), but have different significance.

The purpose of this report was to investigate the possibilities of expressing complex formation of ligand molecules with macromolecules from a pseudo-two-phase point of view. For this purpose, we have regarded the macromolecular solution both as a true solution (monophase) and as a pseudo two phase comparable with a biphasic. We have theoretically investigated the expression of results calculated according to the theory of complex formation in partition terms, and the relationship of the two systems. The theory is experimentally verified with two different experiments and the possibilities and limitations of the two systems are discussed.

## EXPERIMENTAL SECTION

**Materials and Methods**—Povidone<sup>1</sup> was used as the macromolecule and salicylic acid<sup>2</sup> was used as the ligand molecule. The buffer employed was a phosphate buffer (pH 7.00) (19) containing dibasic sodium phosphate and monobasic potassium phosphate. Ultrafiltration was used to investigate the ligand-macromolecule interactions. Previously described equipment was employed (20). The concentration of unbound ligand was determined spectrophotometrically in the ultrafiltrate. The spectrophotometric measurements were performed at 296 nm with a double-beam spectrophotometer<sup>3</sup> after appropriate dilution.

**Partial Specific Volume**—The partial specific volume ( $\bar{V}_2^0$ ) (21, 22) of povidone in water and phosphate buffer (pH 7.00) is calculated from the density increment with all other components at constant chemical potential using the equation:

$$\bar{V}_2^0 = \frac{1}{\rho'} \left[ 1 - \left( \frac{\Delta\rho}{\Delta C_2} \right)' \right] \quad (\text{Eq. 1})$$

where  $\bar{V}_2^0$  is the partial specific volume;  $C_2$  is the povidone concentration;  $\Delta\rho/\Delta C_2$  is the density increment with respect to component 2; and  $\rho'$  is the buffer density. The densities of the macromolecular solution and the solvent were measured with a digital precision density meter<sup>4</sup>, calibrated with air and solvent, at 25°C. In a second method, the partial specific volume was determined using a pycnometer.

**Determination of the Binding Tendency**—A solution containing salicylic acid and 2.50% povidone in a phosphate buffer (pH 7.00) was prepared. Ultrafiltration was carried out at 25°C, several ultrafiltrate fractions (~0.5–1.0 mL each) were collected, and the free ligand concentration in each sample was determined spectrophotometrically. The volume of the sample was calculated by weighing the sample and determining its density.

## RESULTS AND DISCUSSION

**Treatment of Povidone Solutions as Pseudo Two Phase**—The question of homogeneity or heterogeneity of macromolecules in solution is still a point of discussion. Some authors (23, 24) consider that macromolecular dispersions are monophasic (homogeneous), while other authors (25, 26) believe they are clearly biphasic (heterogeneous). In fact, the problem of phases is a problem of scale. A phase dispersed in another phase ceases to exist, as such, when its

degree of division reaches the molecular level. At any point along the chain, a macromolecule has a molecular size in two of the spatial dimensions and a macromolecular size in the third.

The "pseudo-two-phase" model (27, 28) was used by a number of investigators (29–33) to describe aqueous micellar solutions in general and solubilization in particular (28, 34). Such a model leads one to think of the solubilized dispersion as being distributed or partitioned between the micelles ("micellar phase") and the external "aqueous phase."

The elementary requirement that these "phases" be mechanically separable is satisfied, since such a separation can be effected with dialysis or ultrafiltration. Moreover, these micellar solutions are consistent with the Gibbs phase rule for two-phase systems; the intensive properties of the aqueous phase are nearly constant with overall surfactant concentration above the critical micellar concentration at constant temperature and pressure. The pseudo-two-phase treatment of micelles has gained strong support from the ultrafiltration and solubilization studies of Hutchinson *et al.* (30), which showed the number of free detergent molecules to be nearly constant above the micelle point.

The pseudo-two-phase model is useful if the micelles are both large and do not interact; these requirements, if they are fulfilled, should be reflected in the distribution coefficient, being independent of the surfactant concentration for a given micellar composition. All these requirements and limitations, valid for the micellar solutions, are also fulfilled in the case of macromolecular solutions. Moreover, macromolecular solutions are seen as gel particles (35) and occupy a definite volume in solution, indicated as their partial specific volume (21, 22).

Povidone, like other nonionic macromolecules, contains separate hydrophobic and polar groups, making it comparable to nonionic tensides. A molecular model of the povidone molecules showed that the polar pyrrolidone ring and the hydrophobic paraffin backbone were both accessible for the ligand molecules (36). Taking into account these considerations, we thought the pseudo-two-phase model, describing micellar solutions, could also be used for describing macromolecular solutions.

**Relationship Between Binding Results, Expressed in a Monophasic (Complex Formation) and a Pseudo-Two-Phase System (Partition)**—According to the theory of complex formation (monophase) (18, 23, 37–39) the following equation is valid:

$$T = B + F \quad (\text{Eq. 2})$$

where  $T$ ,  $B$ , and  $F$  represent the molarities of total, bound, and free ligand and are calculated on the total volume ( $V_T$ ) of the solution.

Complex formation can be studied as a function of either macromolecule concentration or ligand concentration. In the first method, the ratio of  $T$  to  $F$  can be expressed as a function of the amount of povidone (40–42):

$$\frac{T}{F} = a g_{\text{PVP}} + 1 \quad (\text{Eq. 3})$$

where  $g_{\text{PVP}}$  is grams of povidone and  $a$  is a constant.

The second method permits the calculation of intrinsic binding constants ( $k$ ) and the number of binding sites ( $n$ ) on the macromolecule:

$$r = \frac{B}{g_{\text{PVP}}} = \frac{nkF}{1 + kF} \quad (\text{Eq. 4})$$

For isotherms of several ligand molecules with povidone, a straight line was obtained by plotting  $r$  as a function of  $F$  (3, 9, 43, 44). This finding implies that at  $kF \ll 1$ ,  $1/n$  equals zero or  $n$  is infinite, indicating a nearly infinitely large number of adsorption sites (9, 20). So Eq. 4 reduces to:

$$r = \frac{B}{g_{\text{PVP}}} = nkF \quad (\text{Eq. 5})$$

If both equations are valid, the constant  $a$  of Eq. 3 equals  $nk$  of Eq. 5.

According to the pseudo-two-phase model (partition), the general equation for partition between two phases is valid:

$$Kd = \left( \frac{T'V_T - F \cdot V_F}{F} \right) \cdot \left( \frac{1}{V_B} \right) \quad (\text{Eq. 6})$$

where:

$$B' = T' - F, \quad (\text{Eq. 7})$$

$Kd$  is the partition coefficient between "the pseudo or macromolecule phase" and the aqueous or "outer" phase;  $T'$  is the concentration (in molarity) of the aqueous phase before partition;  $V_T$  is the volume of the aqueous phase before partition;  $F$  is the concentration (in molarity) of the aqueous phase after equilibrium (*i.e.*, the free ligand concentration);  $V_F$  is the volume of the "outer" or aqueous phase after equilibrium;  $B'$  is the concentration of ligand in the pseudo or macromolecule phase (*i.e.*, the bound ligand concentration);  $V_B$  is the volume of the "pseudophase," in our case, the volume occupied by

<sup>1</sup> Polyvinylpyrrolidone (Kollidon K 90); BASF, Brussels, Belgium.

<sup>2</sup> E. Merck, Darmstadt, F.R.G.

<sup>3</sup> Model 124; Perkin-Elmer.

<sup>4</sup> Digital density meter DMA 40; Anton Parr.

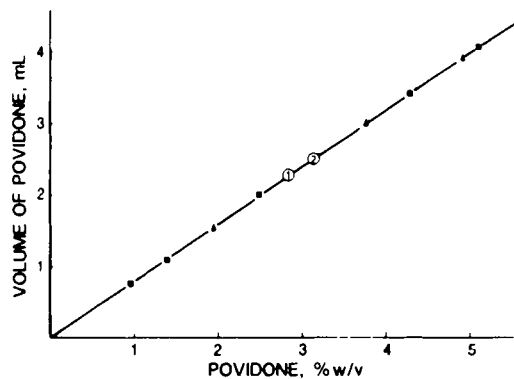


Figure 1—Volume increment of povidone in water (1) and phosphate buffer (2) solution as a function of its weight.

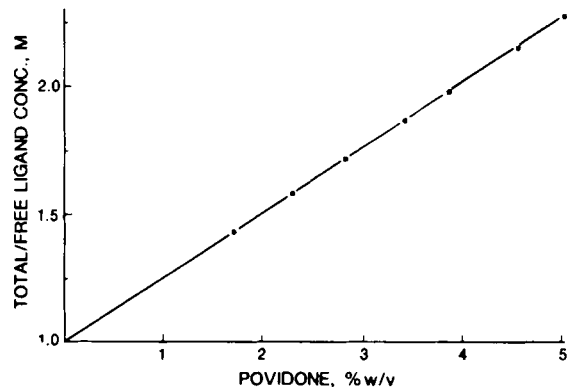


Figure 2—Complexing tendency of salicylic acid onto povidone. Salicylic acid,  $7.60 \times 10^{-3}$  M; pH 7.00; ionic strength, 0.25;  $25^\circ\text{C}$ .

povidone in solution. The partial specific volume of povidone was taken. As  $V'_T = V_F$ , Eq. 6 can be written:

$$Kd = \left( \frac{T' - F}{F} \right) \frac{V_F}{V_B} \quad (\text{Eq. 8})$$

As in the pseudo-two-phase system,  $T'$  represents the total concentration of the "outer" or aqueous phase and the relationship between total concentration in the monophasic and pseudo-two-phase system is given by:

$$T' = T \left( \frac{V_T}{V_T - V_B} \right) \quad (\text{Eq. 9})$$

By comparing Eqs. 2, 7, and 9, it is noted that  $B'$  expressed in a pseudo-two-phasic system is larger than  $B$  expressed in a monophasic system. Further, we can write:

$$V_B = b \cdot g_{PVP} \quad (\text{Eq. 10})$$

where  $g$  is grams of povidone used in the experiment and  $b$  is the volume occupied by one gram of povidone in solution.

Substitution of Eqs. 9 and 10 in Eq. 8 gives:

$$Kd = \left( \frac{T \cdot V_T}{V_T - b \cdot g_{PVP}} - F \right) \cdot \left( \frac{V_T - b \cdot g_{PVP}}{b \cdot n \cdot g_{PVP}} \right) \quad (\text{Eq. 11})$$

resulting in:

$$\frac{T}{F} = \left( \frac{b \cdot g_{PVP}}{V_T} \right) \cdot (Kd - 1) + 1 \quad (\text{Eq. 12})$$

or:

$$\frac{B}{F} = \left( \frac{b \cdot g_{PVP}}{V_T} \right) \cdot (Kd - 1) \quad (\text{Eq. 13})$$

Therefore, a plot of  $T/F$  as a function of  $g_{PVP}$  results in a straight line with slope,  $b/V_T (Kd - 1)$  and an intercept of 1. The slope is equal to the slope  $a$  of Eq. 3 or  $nk$  of Eq. 5.

The relationship between  $Kd$  and  $a$ , the slope of Eq. 3, or  $nk$  of Eq. 5 can be calculated from Eqs. 3, 5, and 12 and results in:

$$Kd = a \frac{V_T}{b} + 1 = nk \frac{V_T}{b} + 1 \quad (\text{Eq. 14})$$

From Eq. 14, it can be concluded that the linear relationship between  $T/F$  and  $g_{PVP}$ , which is found using the theory of complex formation (Eqs. 3 and 5), can be directly correlated with the pseudo-two-phase theory. This holds true for all povidone concentrations.

It has only been assumed that  $V_B = V_{PVP}$  is a linear function of  $g_{PVP}$ . The linearity has been investigated using a pycnometer and is graphically represented in Fig. 1. The volume occupied by povidone is indeed a linear function of  $g_{PVP}$ . From the slope of the line, the  $b$  value was calculated and equals 0.80 mL/g (i.e., every gram of povidone occupies a volume of 0.80 mL). This holds true for both water and phosphate buffer. The partial specific volume, calculated from density increments of povidone solutions using Eq. 1, agreed with the results obtained with a pycnometer.

**Comparison of Results in a Macromolecular Solution Treated as Monophasic and Pseudo Two Phase**—To verify the results experimentally, 100-mL solutions containing a constant concentration of salicylic acid as ligand ( $7.60 \times 10^{-3}$  M) and different amounts of povidone were prepared in a phosphate buffer (pH 7.00). From the monophasic point of view, and according to Eq. 2,  $T = 7.60 \times 10^{-3}$  M and  $V_T = 100$  mL.

Ultrafiltration was carried out and the free ligand concentration ( $F$ ) was determined. The results calculated from the mono- and pseudo-two-phasic points of view (Eqs. 2, 3, and 11) are presented in Table I and Fig. 2.

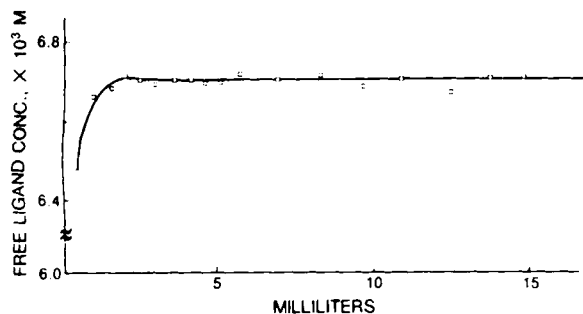
The slope of the straight lines,  $T/F$ , as a function of the povidone concentration was 0.260. The corresponding  $Kd$  value, according to Eq. 13 where  $V_T = 100$  mL and  $b = 0.80$ , was 33.50. The mean of the  $Kd$  values from the data of Table I, calculated according to Eq. 11, was 33.11. The  $Kd$  values agree quite well.

The results of binding onto povidone can be interpreted from the monophasic as well as from the pseudo-two-phasic point of view. The results calculated in one system can easily and correctly be expressed in the other system by use of a simple equation. The calculated  $Kd$  values are independent of the macromolecular concentration, as can be expected with partition between two phases. The  $Kd$  values offer the advantage above the  $B/F$  and  $1/C$  determinations, to correlate with log  $P$  constants (11, 14, 15) so that the desired information can be obtained from one experiment and the macromolecular concentration can be varied from one ligand to another to minimize the error in the results obtained for ligand molecules with a low or high complexing tendency (18).

Table I—Complexing Tendency of Salicylic Acid Onto Povidone<sup>a</sup>

Povidone, %	Monophasic				Pseudo Two Phase					
	$T^b$	$F$	$B$	$T/F$	$T'$	$F$	$B'$	$V_{PVP}$	$V_{H_2O}$	$Kd$
1.70	0.00760	0.00530	0.00230	1.43	0.00770	0.00530	0.00240	1.36	98.64	32.91
2.27	0.00760	0.00478	0.00282	1.59	0.00774	0.00478	0.00296	1.82	98.18	33.49
2.81	0.00760	0.00444	0.00316	1.71	0.00777	0.00444	0.00333	2.25	97.75	32.66
3.39	0.00760	0.00404	0.00356	1.88	0.00781	0.00404	0.00377	2.71	97.29	33.49
3.84	0.00760	0.00384	0.00376	1.98	0.00784	0.00384	0.00400	3.07	96.93	32.87
4.53	0.00760	0.00352	0.00408	2.16	0.00789	0.00352	0.00437	3.62	96.38	32.98
5.01	0.00760	0.00331	0.00429	2.30	0.00792	0.00331	0.00461	4.01	95.99	33.34

<sup>a</sup> Calculations were made using Eqs. 2, 3, and 11. The total concentration of salicylic acid was  $7.60 \times 10^{-3}$  M; the pH was 7.00; the ionic strength was 0.25; the temperature was  $25^\circ\text{C}$ . <sup>b</sup>  $T$ ,  $B$ , and  $F$  are molarities of total, bound, and free ligands, respectively;  $T'$ ,  $B'$ , and  $Kd$  are molar concentrations of the aqueous phase before partition, the bound ligand concentration, and the partition coefficient between the "pseudo" and aqueous phase, respectively.



**Figure 3**—Determination of the free ligand concentration of salicylic acid in the successive ultrafiltrate samples. Salicylic acid,  $1.00 \times 10^{-2}$  M; povidone, 2.50%; pH 7.00; 25°C.

**Determination of the Complexing Tendency in Successive Ultrafiltrate Samples**—The free ligand concentrations of successive fractions were determined from a solution containing salicylic acid ( $1.00 \times 10^{-2}$  M) and 2.50% povidone dissolved in a buffer (pH 7.00) (Fig. 3). The free ligand concentrations in the successive aliquots remain constant, although the volume of the solution is more than halved while the povidone concentration is more than doubled.

The constancy of the free ligand concentration in the successive aliquots was tested in terms of the mono- and pseudo-two-phase models. The results are consistent with a pseudo-two-phase model. According to the partition theory (and Eq. 6),  $F$  corresponds to the concentration of the "outer" or the aqueous phase, in opposition to the theory of complex formation. Since we are dealing with concentrations and not with amounts, the free ligand concentration ( $F$ ) at equilibrium is not changed by eliminating a portion of the "outer" or aqueous phase.

On the other hand, with complex formation, all results are expressed as the total volume of the solution. Therefore, the concentration of the macromolecule will increase due to solvent elimination during ultrafiltration. However, the change in total volume must simultaneously also affect the other factors. This change in the concentration of total ligand, total povidone concentration, bound ligand, and especially free ligand was theoretically investigated. These effects are considered first for a 1:1 complex:

$$M_F + F \rightleftharpoons ML \quad (\text{Eq. 15})$$

$$k = \frac{(ML)}{(M_F) \cdot (F)} \quad (\text{Eq. 16})$$

where  $ML$  is the concentration of bound ligand or bound macromolecule,  $M_F$  is the free concentration of macromolecule, and  $F$  is the free ligand concentration. If  $T$  and  $M_T$  represent the total concentration of ligand and macromolecule, respectively, we may write:

$$M_F = M_T - T + F \quad (\text{Eq. 17})$$

so:

$$k = \frac{T - F}{(M_T - T + F) \cdot (F)} \quad (\text{Eq. 18})$$

Expressing Eq. 18 in terms of concentration (w/v), with the volume before ultrafiltration as  $V_0$ , then Eq. 18 can be written:

$$k = \frac{\frac{m_T}{V_0} - \frac{m_F}{V_0}}{\left(\frac{m_{M_T}}{V_0} - \frac{m_T}{V_0} + \frac{m_F}{V_0}\right) \left(\frac{m_F}{V_0}\right)} \quad (\text{Eq. 19})$$

where  $m_T$ ,  $m_F$ , and  $m_{M_T}$  are the mass of total ligand, free ligand, and mac-

**Table II**—Changes in Povidone Concentration of Total, Free, and Bound Ligand as a Function of Successive Eliminated Ultrafiltrate Fractions

	$dv^a$	$V_0 - \sum dv^b$	Povidone, $M \times 10^7$ $c$	$T$ , $M \times 10^4$ $d$	$F$ , $M \times 10^4$ $e$	$B$ , $M \times 10^4$ $f$	$r$ $g$	Amount $F$ Eliminated, $g \times 10^5$	$\Sigma F$ Eliminated, $g \times 10^5$
Before	—	25	357	100.0	—	—	—	—	—
After	0.51	—	357	100.0	64.7	35.3	98.88	45.6	45.6
Before	—	24.49	365	100.7	—	—	—	—	—
After	0.50	—	365	100.7	66.6	34.1	93.42	46.0	91.6
Before	—	23.99	372	101.4	—	—	—	—	—
After	0.47	—	372	101.4	66.8	34.6	93.01	43.3	135
Before	—	23.52	380	102.1	—	—	—	—	—
After	0.53	—	380	102.1	67.1	35.0	92.11	49.1	184
Before	—	22.99	388	102.9	—	—	—	—	—
After	0.48	—	388	102.9	67.0	35.9	92.53	44.4	228
Before	—	22.51	397	103.7	—	—	—	—	—
After	0.51	—	397	103.7	66.9	36.8	92.70	47.1	275
Before	—	22.00	406	104.6	—	—	—	—	—
After	0.58	—	406	104.6	67.0	37.6	92.61	53.7	329
Before	—	21.42	417	105.6	—	—	—	—	—
After	0.59	—	417	105.6	67.0	38.6	92.57	54.6	384
Before	—	20.83	429	106.7	—	—	—	—	—
After	0.51	—	429	106.7	66.9	39.8	92.71	47.1	431
Before	—	20.32	439	107.7	—	—	—	—	—
After	0.48	—	439	107.7	66.9	40.8	92.87	44.4	475
Before	—	19.84	450	108.7	—	—	—	—	—
After	0.60	—	450	108.7	67.1	41.6	92.36	55.6	531
Before	—	19.24	464	110.0	—	—	—	—	—
After	1.21	—	464	110.0	67.0	43.0	92.61	112.0	643
Before	—	18.03	495	112.8	—	—	—	—	—
After	1.38	—	495	112.8	67.1	45.7	92.40	127.9	771
Before	—	16.65	536	116.6	—	—	—	—	—
After	1.35	—	536	116.6	66.8	49.8	92.96	124.6	896
Before	—	15.30	584	121.0	—	—	—	—	—
After	1.25	—	584	121.0	67.0	54.0	92.46	115.7	1012
Before	—	14.05	635	125.8	—	—	—	—	—
After	1.54	—	635	125.8	66.6	59.2	93.23	141.7	1154
Before	—	12.51	714	133.1	—	—	—	—	—
After	1.27	—	714	133.1	67.0	66.1	92.58	117.5	1272
Before	—	11.24	794	140.5	—	—	—	—	—
After	1.08	—	794	140.5	67.0	73.5	92.59	99.9	1372
Before	—	10.16	879	148.3	—	—	—	—	—

<sup>a</sup> Volume of the eliminated ultrafiltrate fraction. <sup>b</sup>  $V_0 - \sum dv$  = volume remaining in the ultrafiltrate cell. <sup>c</sup> Povidone concentration in the ultrafiltrate cell, calculated according to Eq. 22. <sup>d</sup> Total ligand concentration in the ultrafiltrate cell, calculated according to Eq. 21. <sup>e</sup>  $F$  = free ligand concentration, determined in the ultrafiltrate fraction. <sup>f</sup>  $B = T - F$  = bound ligand concentration. <sup>g</sup>  $r$  = moles of ligand bound/mole of macromolecule.

romolecule, respectively, or:

$$k = \frac{m_T - m_F}{(m_{M_T} - m_T + m_F) \left( \frac{m_F}{V_0} \right)} \quad (\text{Eq. 20})$$

After eliminating a volume fraction  $dv$ , with mass  $dm$ , the total ligand concentration is also changed and equals:

$$T = \frac{m_T - dm}{V_0 - dv} \quad (\text{Eq. 21})$$

and the total macromolecular concentration:

$$M_T = \frac{m_{M_T}}{V_0 - dv} \quad (\text{Eq. 22})$$

the free ligand concentration is changed to:

$$F = x_F / (V_0 - dv) \quad (\text{Eq. 23})$$

where  $x_F$ , the mass of free ligand, *a priori* is not known. The new equilibrium is then:

$$k = \frac{\frac{m_T}{V_0 - dv} - \frac{dm}{V_0 - dv} - \frac{x_F}{V_0 - dv}}{\left( \frac{m_{M_T}}{V_0 - dv} - \frac{m_T}{V_0 - dv} + \frac{dm}{V_0 - dv} + \frac{x_F}{V_0 - dv} \right) \cdot \left( \frac{x_F}{V_0 - dv} \right)} \quad (\text{Eq. 24})$$

or:

$$k = \frac{m_T - dm - x_F}{(m_{M_T} - m_T + dm + x_F) \left( \frac{x_F}{V_0 - dv} \right)} \quad (\text{Eq. 25})$$

The intrinsic binding constants before and after eliminating free ligand concentration must be equal. This is the case if:

$$\frac{m_F}{V_0} = \frac{x_F}{V_0 - dv} = \frac{dm}{dv} \quad (\text{Eq. 26})$$

*i.e.*, the free ligand concentrations before and after ultrafiltration are identical. This is also true for complex formation in general. In the equation,  $r = nkF/(1 + kF)$  (18, 23, 37, 38),  $nk$  and  $F$  are constants, then  $r$ , the amount of bound ligand per mole of macromolecule, should also be constant.

The results from Fig. 3, were calculated according to Eqs. 21 and 22 (Table II). The results are given before and after the removal of a volume fraction. The molarity of povidone (Col. 4), as well as the total (Col. 5), and bound (Col. 7) ligand concentrations increase according to Eqs. 21 and 22, while the free ligand concentration (Col. 6) and  $r$ , the mole bound ligand/macromolecule ratio remain constant (Col. 8). Consequently, the results correspond to Eq. 26.

Using somewhat different calculations, the same conclusions were found in a study of the binding of manganese to concanavalin A (45). In the first aliquots (to ~2 mL), the free ligand concentrations are somewhat lower than in the following aliquots, resulting in an overestimation of  $B$  (Fig. 3 and Table II). This effect was also noted in experiments with other ligand molecules. This observation must be attributed to a dilution effect of the membrane, which is immersed in a buffer solution before use. Therefore, the first sample must always be eliminated to obtain correct values of  $B$  and  $F$ . We can conclude that the experiments are in accordance with the treatment of a macromolecular solution as a pseudo two phase as well as a monophasic model.

Another important conclusion can be made. Some authors consider ultrafiltration as suspect due to changes in the macromolecular concentration during the experiment which disturb the binding equilibrium (46). The use of small aliquots of filtrate should overcome this objection (47, 48). Indeed, binding equilibrium is disturbed by removing small aliquots of filtrate, but the intrinsic binding constant must be a constant. From Eq. 26 it is seen that this is the case if free ligand concentrations are identical before and after ultrafiltration, whatever the volume fraction of filtrate taken; therefore, it is not necessary to take small aliquots of filtrate.

Indeed, since the free ligand concentration in the filtrate was constant, regardless of the volume fraction of filtrate taken, the concentration of bound ligand is also unchanged since it is calculated by subtracting the free from the total ligand concentration. The treatment of macromolecular solutions as a pseudo two phase is also generally applicable if Eqs. 3 and 5 are fulfilled.

It is expected that the treatment of a macromolecule solution as a pseudo two phase and calculation of partition coefficient ( $K_d$ ) will have advantages over the theory of complex formation and will be useful to study and interpret

complex formation between macromolecules and ligand molecules as a function of the  $pK_a$  and dissociation of the ligand and the pH of the solvent. This investigation will be reported in a following paper.

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# Improved Competitive Indicator Methods for the Study of $\alpha$ -Cyclodextrin Complexes

DAVID D. PENDERGAST\* and KENNETH A. CONNORS\*

Received January 6, 1984, from the *School of Pharmacy, University of Wisconsin, Madison, WI 53706*. Accepted for publication March 9, 1984. \* Present address: The Upjohn Company, Kalamazoo, MI 49001.

**Abstract** □ The competitive indicator method for studying molecular complexes is extended to systems forming 1:1 (SL) and 1:2 (SL<sub>2</sub>) complexes of substrate (S) and ligand (L). A modification is described for slightly soluble substrates, in which the presence of solid substrate establishes a constant concentration of uncomplexed substrate. These methods are applied to complexes of  $\alpha$ -cyclodextrin with some aromatic substrates, with methyl orange as the indicator in acid solution; nitrazine yellow is introduced as an indicator for these studies in basic solution.

**Keyphrases** □  $\alpha$ -Cyclodextrin—competitive indicator methods, methyl orange, nitrazine yellow □ Competitive indicator methods— $\alpha$ -cyclodextrin, methyl orange, nitrazine yellow complexes

The cyclodextrins are naturally occurring cyclic oligosaccharides possessing a central cavity into which smaller molecules may "partition," forming noncovalently bonded inclusion complexes. The physical and chemical properties of the included molecule (the substrate or guest) may be altered by complexation with the cyclodextrin (the ligand or host). This phenomenon has attracted much recent attention, in part because of its potential applications in drug dosage forms (1-3). Among the properties of a drug that can be affected by cyclodextrin complexation are its solubility, dissolution rate, chemical stability, and bioavailability.

Systematic studies in this laboratory have been designed to provide information on the stoichiometry and thermodynamic stability of cyclodextrin complexes, with the goal of understanding structure-stability relationships and thus developing predictive capability. It has been necessary, in these studies, to make use of several experimental techniques for the measurement of complex stability constants and in some instances to develop new methods. One technique that has been found useful is the so-called "competitive indicator" method. In this technique, an equilibrium is established between the ligand (cyclodextrin) and an indicator whose absorption spectrum differs significantly in its complexed and uncomplexed forms. This equilibrium is then perturbed by the addition of a substrate, which competes with the indicator for the ligand. By measuring the spectral change produced by this perturbation, the stability constant for the substrate-ligand complex can be deduced. The methyl orange- $\alpha$ -cyclodextrin equilibrium was exploited in this way by Broser and co-workers (4) to study the adrenalin- $\alpha$ -cyclodextrin complex and by Casu and Ravà (5)

to study a series of substituted benzoic acids. The method was recently improved in this laboratory and was applied to the study of the  $\alpha$ -cyclodextrin complexes of some phenols (6). All of these applications have made use of methyl orange as the indicator in acidic solution and have been limited to the study of 1:1 complexes between substrate and ligand.

Our most recent work has required three new capabilities of the competitive indicator method: (a) that it be applicable to systems containing both 1:1 (SL) and 1:2 (SL<sub>2</sub>) complexes, where S and L represent substrate and ligand respectively; (b) that it be applicable to slightly soluble substrates; (c) that it be applicable in alkaline medium. The present paper describes extensions to the theory and practice of the method that permit its application in these circumstances.

## THEORETICAL SECTION

**Standard Competitive Indicator Method**—Attention is restricted to systems containing a 1:1 complex 1L of indicator I with ligand plus the 1:1 (SL) and 1:2 (SL<sub>2</sub>) complexes of substrate with ligand. The average number of ligand molecules bound per substrate molecule ( $\bar{n}$ ) is:

$$\bar{n} = \frac{\Sigma(\text{L bound to S})}{\Sigma(\text{all S})} \quad (\text{Eq. 1})$$

The mass balance equations for S and L are:

$$S_t = [S] + [SL] + [SL_2] \quad (\text{Eq. 2})$$

$$L_t = [L] + [SL] + 2[SL_2] + [IL] \quad (\text{Eq. 3})$$

where S<sub>t</sub> and L<sub>t</sub> are the (known) total concentrations of substrate and ligand. Further define the quantity P as  $\Sigma$  (L bound to S) can be defined by:

$$P = [SL] + 2[SL_2] = L_t - [L] - [IL] \quad (\text{Eq. 4})$$

Combining Eqs. 1, 3, and 4:

$$\bar{n} = \frac{P}{S_t} \quad (\text{Eq. 5})$$

The equilibrium constants in this system are given by:

$$K_{11} = \frac{[SL]}{[S][L]} \quad (\text{Eq. 6})$$

$$K_{12} = \frac{[SL_2]}{[SL][L]} \quad (\text{Eq. 7})$$

$$K_1 = \frac{[IL]}{[I][L]} \quad (\text{Eq. 8})$$

Combining Eqs. 5, 6, and 7 yields: