A LIGHT-SCATTERING INVESTIGATION
OF THE INTERACTION OF
POLY-4-VINYL-N, n-BUTYL-PYRIDINIUM BROMIDE
AND BOVINE SERUM ALBUMIN

DISSERTATION

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I. INTRODUCTION

In the last few years the study of polyelectrolytes and their interactions with both small ions and other polyelectrolytes has become an interesting phase of physical chemistry, mainly because of the improved methods of measurement of the molecular weight, size, and shape of the complexes found. The method of angular light scattering has previously been used to good advantage in this and other laboratories since this method enables one to unambiguously determine the molecular weight of high molecular weight materials without first assuming a shape. This method, implemented to a small degree by ultracentrifugation and electrophoresis, has been used to study the mutual interaction of bovine serum albumin (hereafter called BSA) and poly-4-vinyl-N,n-butyl-pyridinium bromide (hereafter called PVP). The purpose of this work was to study polyelectrolyte interactions as a whole by the investigation of the interaction of two specific, oppositely-charged polyelectrolytes of known structure. Any information obtainable on this system and the methods of study developed could perhaps be used eventually in defining some natural systems such as nucleoproteins. It was also the purpose of this investigation to develop methods for the characterization of multiple equilibria in polyelectrolyte systems.
II. LITERATURE REVIEW OF POLYELECTROLYTE INTERACTIONS

Until recently most of the polyelectrolyte interaction work was confined to proteins. This work may be divided into several parts; the first studied were protein-small ion, protein-dye, protein-detergent, and protein-protein interactions. Klotz (1) and Putnam (2) surveyed the first two major divisions quite thoroughly. Fuo, Ottewill, and Farreira (3) used the methods of viscosity and equilibrium dialysis to study the interaction of bovine plasma albumin and dodecyltrimethyl-ammonium bromide. Saito also did work on this type of interaction (4).

Oster (5) and Cohly (6) have recently investigated protein-dye binding, as have Kusunoki and Kimura (7). H. Heilweil (8) investigated the interaction of desoxyribonucleic acid with acriflavine by means of partition analysis and fluorescence techniques. Protein-small ion interactions were studied by Rosenberg, et al. (9), Fredricq (10), and Gregor, et al. (11).

Studies of protein-protein interactions were carried out by Longsworth, et al. (12), which studies were among the first in a series of investigations of polyelectrolyte-polyelectrolyte interactions. This type of investigation was extended to antibody-antigen (13) and protein enzyme systems (14,15); also investigated were the complexes formed by the interaction of proteins with naturally
occurring polyelectrolytes (16,17,18) and with uncharged molecules (19,20,21).

Most of the above investigations used analytical methods and studies of the viscosities of the resulting solutions. However, recently, steps have been taken to incorporate other methods, such as osmotic pressure (22), x-rays (23), diffusion (24), and streaming birefringence (25), into the general pattern for study of polyelectrolyte interactions.

Light scattering has been used as a method for studying interacting systems for the last decade. Doty, et al. (26) and Nord, et al. (27) used the method of light scattering to study the aggregation of polyvinyl acetates in various solvents. The method was particularly useful in aggregation studies and was carried over to aggregation studies on proteins (28,29) and recently to rubber solutions (30). However, Geiduschek and Doty (31) were among the first to use angular light scattering to investigate the interaction between two different polyelectrolytes. They studied the interaction of bovine serum albumin and sodium desoxyribonucleate and found that the complexes formed dissociated at low total concentrations. Their report was followed by that of Yasnoff and Bull (32) on ovalbumin-pepsin interaction and by Steiner's papers (33, 34, 35) on reversible association between globular proteins. In 1954 a thorough investigation of the interaction of
ovalbumin-PVP was completed in these laboratories by I. Heilweil (36,37). Heilweil studied the effects of changing pH, ionic strength, and weight mixing ratio of BSA to PVP on the complexes formed, by using the angular light scattering method. He found very strong complexing, even at very low total polymer concentrations. Thus, it was impractical to try to determine association constants for the complexes.

The BSA-PVP system was chosen for this series of investigations with the hope that the complexes formed on interaction would exhibit measurable dissociation under suitable conditions. The tendency of BSA to form weak complexes had been demonstrated previously by other experimenters (3,31). If weak complexing were found, an attempt would be made to characterize the equilibria involved by a mathematical treatment, perhaps similar to that given by Klotz (1) for small ion binding by proteins.
III. THEORY OF LIGHT SCATTERING

The theory of light scattering has been excellently and completely reviewed in the literature of the last few years (38,39,40). The subject has also been discussed in Doctoral Dissertations from these laboratories (36,41,42). However, a brief review here is deemed to be necessary for the sake of completeness.

A. Fundamental Relationships for Light Scattering by Ideal Isotropic, Monodisperse Particles

Ideal, isotropic, monodisperse particles, whose dimensions are less than one-twentieth of the wavelength of light used follow Rayleigh's Law:

\[
R_{eu} \propto \frac{i_o r^2}{I_o} = \frac{8\pi^2 a^2 \gamma}{\lambda^4} (1 + \cos^2 \theta) \tag{1}
\]

where \( R_{eu} \) = Rayleigh's ratio for unpolarized incident light, measured at the angle \( \Theta \); known, also, as "reduced scattered intensity."

\( i_o \) = scattered intensity at the angle \( \Theta \), for small particles

\( I_o \) = intensity of unpolarized incident beam

\( \gamma \) = number of particles per unit volume

\( a \) = excess polarizability of the particles over the surrounding medium

\( \lambda \) = wavelength of light used, in vacuum

After a number of substitutions which introduce the average square excess optical dielectric constant and the
refractive index increment, the following expression is obtained:

\[ R_{eu} = \frac{2\pi^2 V}{\lambda^4} \left( n_0 \frac{dn}{dc} \right)^2 \left( \frac{\Delta c}{c} \right)^2 \]  

(2)

where \( V \) = a volume element

\[ \frac{\Delta c}{c} = \text{average square concentration fluctuation} \]

\[ \frac{dn}{dc} = \text{refractive index increment for the solute} \]

\[ n_0 = \text{refractive index of the solvent} \]

In this expression the \( \frac{\Delta c}{c}^2 \) is evaluated in terms of the change of osmotic pressure with concentration, leading to the following relationship:

\[ R_{eu} = \frac{2\pi^2 n_0^2}{\lambda^4 N} \left( \frac{dn}{dc} \right)^2 \frac{\Delta \Pi}{\Delta c} \left( 1 + \cos^2 \theta \right) \]  

(3)

where \( \frac{\Delta \Pi}{\Delta c} = \text{change of osmotic pressure with concentration.} \)

Let \( K = \frac{2 n_0^2}{\lambda^4 N} \)

Then \( R_{eu} = \frac{K \Delta \Pi}{\frac{\Delta \Pi}{\Delta c}} \left( 1 + \cos^2 \theta \right) \)  

(4)

Since \( \frac{\Delta \Pi}{\Delta c} = RT \left( \frac{1}{M} + Bc + Cc^2 + \ldots \right) \)

\[ \frac{\Delta \Pi}{\Delta c} = RT \left( \frac{1}{M} + 2Bc + 3Cc^2 + \ldots \right) \]

then

\[ \frac{Kc}{R_{eu}} = \frac{1}{M} + 2Bc + 3Cc^2 + \ldots \]  

(5)

where \( M \) is the molecular weight of the material and \( B \) and \( C \) are interaction constants.
If \( R_e = \frac{R_{eu}}{1 + \cos^2 \theta} \)

then

\[
\frac{K_o}{R_e} = \frac{1}{M} + 2Bc + 3c^2 + \ldots...
\]

(6)

which is equivalent to an expression suggested by Flory:

\[
\frac{K_o}{R_e} = \frac{1}{M} (1 + 2c + 3c^2 + \ldots)\]

(7)

Therefore, \( \left( \frac{K_o}{R_e} \right)_{c=0} = \frac{1}{M} \)

**B. Relationships for Large Isotropic, Monodisperse Particles**

In the case when particle dimensions are larger than one-twentieth of the wavelength of the light used, corrections to the above equations must be made. This is caused by an intraparticle interference effect brought about when oscillating dipoles in different parts of the particles are no longer in phase. This interference is a function of the size of the particle, as well as the viewing angle, and decreases the intensity measured. At the angle \( \theta = 0^\circ \) no interference effects are noticed. So, the light scattering relationship is modified in the following manner:

\[
\left( \frac{K_o}{R_e} \right)_{c=0} = \frac{1}{M P(\theta)} = \frac{1}{M F(\theta)}, \quad \text{and} \quad \left( \frac{K_o}{R_e} \right)_{\theta=0} = \frac{1}{M}
\]

(8)

where \( P(\theta) = \int_0^\infty \rho(r) \frac{\sin \theta r}{\mu r} \, dr \)

and \( \rho(r) \) = distribution function for the occurrence of
the distance \( r \) between pairs of scattering elements in the same solute molecule

\[
\mu = \frac{4\pi n}{\lambda} \sin \frac{\theta}{2}
\]

\( n = \) index of refraction

Several methods have been developed for treating light scattering data, including the dissymmetry method, the absorption method, the transmission method, and Zimm's double extrapolation method. These methods have been discussed by Doty and Steiner (43) and others. The dissymmetry method is the one normally used, since it involves measurements at only four angles - \( \theta = 0, 45, 90, \) and \( 135. \) The dissymmetry is defined as \( Z = \frac{I_{135}}{I_{45}}. \) This dissymmetry may be used in calculating a correction factor for the apparent molecular weight if, first, a shape is assumed for the molecule. The absorption, or turbidity extrapolation method also enables one to correct the molecular weight for interference effects, but when using this method, one measures the turbidity at various wavelengths of incident light in a spectrophotometer. The transmission method is complementary to the dissymmetry method.

The method used in this treatment is the Zimm double extrapolation method (44). Since \( P(\theta) \) can normally be expressed, for most differently shaped molecules, as a
power series in  
\[ \sin^2 \frac{\theta}{2}, \text{ i.e., } P_{\text{obs}}^{-1} = 1 + A \sin^2 \frac{\theta}{2} + A_1 \sin^4 \frac{\theta}{2} + \ldots \] (9)

it seems logical to plot \( \frac{Kc}{R_{\theta}} \) as a function of \( \sin^2 \frac{\theta}{2} \), and extrapolate to \( \theta = 0^\circ \) to overcome any interference effects in the measurement of the molecular weight. Zimm has suggested plotting \( \frac{Kc}{R_{\theta}} \) versus \( \sin^2 \frac{\theta}{2} \) + kc, where k is an arbitrary constant. This plot is normally a grid, in which the \( c = 0 \) and \( \theta = 0^\circ \) lines intersect at \( \left( \frac{Kc}{R_{\theta}} \right)_{c=0} = \frac{1}{M} \).

This method enables one to find a molecular weight unambiguously, without first assuming a shape for the molecule. At low concentrations \( \frac{Kc}{R_{\theta}} = \frac{1}{M} P_{\text{obs}}^{-1} + 2Bc \), if one assumes that terms in higher powers of c will be insignificant. Substituting for \( P_{\text{obs}}^{-1} \) from Equation (9) we obtain  
\[ \frac{Kc}{R_{\theta}} = \frac{1}{M} (1 + A \sin^2 \frac{\theta}{2}) + 2Bc \] at low angles. (10)

In this expression, \( A = f (\lambda, n, [D]) = \text{constant} \)
where \( [D] = \text{characteristic dimension for a given shape} \).

Thus, by taking limiting slopes, one can obtain both of the constants, A and B, since

\[ A = \frac{1}{\left( \frac{Kc}{R_{\theta}} \right)_{c=0}} \left[ \frac{d\left( \frac{Kc}{R_{\theta}} \right)_{c=0}}{d \sin^2 \frac{\theta}{2}} \right]_{\theta=0} \] (11)
and \( B = \frac{1}{2} \left( \frac{d \left( \frac{Kc}{R_{	heta}} \right)}{dc} \right)_{c=0} = \frac{k}{2} \left( \frac{d \left( \frac{Kc}{R_{	heta}} \right)}{dc} \right)_{c=0} \) \( (12) \)

\( B \) is the osmotic pressure virial coefficient and is a function of molecular interaction or repulsion, and \( A \) determines the dimensions of the particle whose shape must now be assumed. Likewise, \( P_{\theta_{\text{exp.}}} = \left( \frac{KcM}{R_{	heta}} \right)^{-1} \) \( (13) \)

So, \( P_{\theta_{\text{exp.}}} \) may be measured experimentally and compared with theoretical expression derived for different shapes of molecules, so as to enable one to decide on the true or closest shape of the molecule.

Several authors have calculated closed expressions for the most common shapes. Expressions for monodisperse spheres, rods, Gaussian coils (39), non-Gaussian coils (45), and cylindrical disks (46), have been developed in closed form, while power series expressions are available for ellipsoids, cylindrical disks (39), non-Gaussian chains (47), and randomly-oriented cylindrical rods (48). Zimm has derived an expression for polydisperse coils (44), while Goldstein developed an expression for polydisperse rods (49). Values of \( P_{\theta} \) for spheres, rods, monodisperse coils, and polydisperse coils are tabulated by Doty and Steiner (43).

Even if no expression has been developed for a certain shape, a quantity called the "radius of gyration" can be
obtained unambiguously from the experimentally determined value $A$.

$$R_G = \frac{\sqrt{2} \lambda}{4 \pi n_0 \left[ \frac{d F(\theta)^{-1}}{d \sin^2 \theta/2} \right]_{\theta=0}} = \frac{\sqrt{2} \lambda}{4 \pi n_0 A}$$

The characteristic dimensions for certain simple shapes are related to $R_G$ thus:

- For a coil:
  $$R_G = (6)^{-1/2} \frac{1}{\langle r^2 \rangle^{1/2}}$$

- For a sphere:
  $$R_G = \frac{20}{3}^{-1/2} D$$

- For a rod:
  $$R_G = (12)^{-1/2} L$$

- For a thin disk:
  $$R_G = (8)^{-1/2} D^1$$

where $\langle r^2 \rangle^{1/2}$ = root mean square distance between ends of random coil

$D$ = diameter of sphere
$L$ = length of rod
$D^1$ = large diameter of thin disk

C. Other Corrections

Sadron (40) has shown that for anisotropic particles, the treatment becomes much more involved. However, for high molecular weight coils, the anisotropy correction becomes insignificant. Since we will show in a later section that the molecules and complexes under consideration are probably large coils, the anisotropy correction will not be considered.
D. Polydispersity

Debye (50) has shown that, for a monodisperse chain,

\[ P_\theta = \frac{2}{N^2 u^2} \left[ Nu - 1 + \exp(-Nu) \right] \tag{16} \]

where \( u = \frac{\mu b^2}{6} \), \( \mu = \frac{4 \pi N \eta \lambda}{\alpha} \sin \frac{\theta}{2} \)

\( N = \) degree of polymerization
and \( b = \) the length of a statistical element of the chain; if one inverts \( P_\theta \) and expands the exponential, a series results (51). This series, at high \( u \), reduces to

\[ P_\theta = \frac{1}{2} + \frac{Nu}{2} \]

which is a linear portion of the curve.
This asymptotic portion of the curve has an intercept of \( \frac{1}{N} \).

For polydisperse systems, Zimm (44) has defined \( P_\theta \) as:

\[ P_\theta = \frac{1}{\int \! f(N) dN} \int \! f(N) P_N(\theta) dN \tag{17} \]

where \( f(N) \) is the distribution function for the degree of polymerization.
If \( \langle N_n \rangle = \) number-average degree of polymerization

\[ = \frac{1}{\int \! f(N) dN} \int \! \frac{1}{N} f(N) dN \]

\( \langle N_w \rangle = \) weight-average degree of polymerization

\[ = \int \! Nf(N) dN \]
and \( \langle N_z \rangle = z \)-average degree of polymerization

\[ = \frac{1}{\langle N_w \rangle} \int \! N^2 f(N) dN \]
for low values of \( u \), or \( \sin^2 \frac{\theta}{2} \), \( P_\theta^{-1} \) becomes

\[
P_\theta^{-1} = 1 + \langle N_z \rangle \frac{u}{3} + \ldots
\]

and thus

\[
\left( \frac{Kc}{R_\theta} \right)_{c=0} = \frac{1}{M_N} \left( 1 + \langle N_z \rangle \frac{u}{3} + \ldots \right)
\]

For high values of \( u \) there appears to be some confusion as to just what the value of \( P_\theta \) is, but Benoit, et al. (52), states that:

\[
\left( \frac{Kc}{R_\theta} \right)_{c=0} \theta=0 = \frac{1}{M_N} \left( \frac{1}{2} + \langle N_z \rangle \frac{u}{2} + \ldots \right)
\]

So, if \( \left( \frac{Kc}{R_\theta} \right)_{c=0} \) is plotted versus \( \sin^2 \frac{\theta}{2} \),

\[
M_w = \frac{1}{\left( \frac{Kc}{R_\theta} \right)_{c=0} \theta=0}
\]

\[
R^2_{G_z} = \frac{3 \lambda^2}{16 \pi^2 n_0^2} \left( \frac{Kc}{R_\theta} \right)_{c=0} \theta=0
\]

\[
M_N = \frac{1}{2} \text{ (intercept of asymptote)}
\]

\[
R^2_{G_N} = \frac{\lambda^2}{16 \pi^2 n_0^2} \left( \frac{\text{slope of asymptote}}{\text{intercept of asymptote}} \right)
\]

Thus, the weight- and number-average molecular weights, and the z- and number-average (radius of gyration)^2 may be found. Since \( \frac{\langle N_z \rangle}{3M_w} \) = initial slope, and \( \frac{\langle N_N \rangle}{2M_N} \) = asymptotic slope, \( \frac{\langle N_z \rangle}{\langle N_N \rangle} = \frac{(\text{initial slope})(3M_w)}{(\text{asymptotic slope})(2M_N)} = \frac{M_Z}{M_N} \)

So, it is possible to characterize the polydispersity of the system quite well if there is a definite curvature in
the \( \frac{K_0}{R_G} \) vs. \( \sin^2 \frac{\Theta}{2} \) plot. In the reference noted (52), good results were obtained on solutions of cellulose tri-nitrate. However, the curvature was noticed only from values of \( (\bar{r}^2) = 6R_G^2 = 800 \AA \) to \( (\bar{r}^2) = 3000 \AA \), for common distributions.

Billmeyer and de Than (53) have shown that the value of \( M_N \) obtained by this method is probably high, since the curvature begins at higher angles than supposed by Benoit. However, the asymptotic slope is probably correct, giving a good value for \( (R_G^2) \).
IV. THEORY OF INTERACTION IN MULTICOMPONENT SYSTEMS

The general theory of light scattering in multi-component systems was developed by Stockmayer (54) and others (55,56). This theory has been extended to interacting systems by Steiner (35) and Geiduschek and Doty (31). The latter treatment will be used herein and will be developed in detail in the following sections.

A. General Theory

For the system in which a reaction $A + B \rightarrow AB$ takes place, it is possible to determine by light scattering the weight of $B$ which combines with a unit weight of $A$. Thus, if the molecular weights of the starting materials are known, it becomes possible to determine the number of molecules of $B$ combining with one molecule of $A$, if all of the $A$ in the reaction mixture is complexed.

Thus, if $A = PVP$ and $B = BSA$, we will apply this theory to the reaction $PVP + nBSA \rightarrow PVP(BSA)_n$. Following the symbolism of Geiduschek and Doty, where we use the subscript 2 for PVP, the subscript 4 for BSA, and the subscript 6 for the complex formed, we obtain the following quantities:

$S = \text{grams BSA bound / gram of PVP}$

$c_2 = 0$

$c_4 = c_4^0 - c_2 = \text{concentration of free BSA}$
\[ c_6 = c_2^o (1 + \delta) \text{ concentration of complex} \]

\[ \psi_6 = \frac{\psi_2 + \delta \psi_4}{1 + \delta} = \text{specific refractive index increment of the complex} \]

where \( c_2, c_4 \) and \( c_6 \) are equilibrium concentrations and \( c_2^o \) and \( c_4^o \) are initial concentrations in g/ml.

Since, for a single component,

\[ (R_{\theta 1})_{c=0} = K^n \psi_1^2 \psi_1^M \psi_1(\theta) \]  \hspace{1cm} (25)

then, for the whole system, at \( \theta=0 \), where \( P_1(0) = 1 \)

\[ (R_{\theta t})_{c=0} = \sum(R_{\theta 1})_{c=0} \]  \hspace{1cm} (26)

So, using the general theory for systems of more than one component, we obtain:

\[ (R_{\theta t})_{c=0} = K^n M_4 \psi_4^2 (c_4^o - \delta c_2^o) + K^n M_2 (\psi_2 + \delta \psi_4)^2 c_2^o \]  \hspace{1cm} (27)

Thus,

\[ \left[ \frac{K^n (c_2^o + c_4^o)}{R_{\theta t}} \right]_{c=0 \theta=0} = K = \frac{c_2^o + c_4^o}{c_2^o M_2 (\psi_2 + \delta \psi_4)^2 + (c_4^o - \delta c_2^o) M_4 \psi_4^2} \]  \hspace{1cm} (28)

In the above relationship, \( K^n = \frac{2 \pi^2 n_i^2}{M \lambda^4} \). The left side of the equation is experimentally determinable. By determining this quantity, \( K \), we can determine \( \delta \).

If \( r = \text{mixing ratio} = \frac{c_2^o}{c_2^o} \), we can obtain, by dividing both numerator and denominator of the right side of Equation 28 by \( c_2^o \) and rearranging:

\[ \delta^2 + \left[ 2 \frac{\psi_2 - M_4}{M_2} \right] \delta + \left[ \left( \frac{\psi_2}{M_2} \right)^2 + \frac{r M_4}{M_2} - \frac{(r+1)}{M_2 \psi_4^2 K} \right] = 0 \]  \hspace{1cm} (29)

This quadratic equation can be solved for \( \delta \), and the
molecular weight of the complex, the relative number of molecules of BSA per molecule of PVP, and the average molecular weight can be found.

\( c \), of course is an average value, and was determined in a non-rigorous manner to be a z-average binding ratio, by Heilweil (36,37).

If plots are made of \( \frac{c}{\phi} \) vs. \( c \), interacting systems which form strong complexes having large equilibrium constants will show straight lines with positive slopes. However, if the complexes formed are of only moderate stability, a minimum will appear in the aforementioned curve. However, the extent of binding may still be obtained by extrapolating the high concentration portion of the curve to zero concentration. This type of interaction was shown in the system, DNA-BSA, by Geiduschek and Doty (31).

B. Multiple Equilibria

The calculation of equilibrium constants from light scattering data is possible, theoretically, if certain requirements are fulfilled. That is, if the equilibria follow the law of mass action and if the complexes formed are of the type \( AB_n \), and not \( A_m B_n \), the calculation of an intrinsic equilibrium constant should be possible. The following sections are concerned with the development of such a method of calculation.
1. Multiple Equilibria, in the Absence of Electrostatic Effects

Some of the original work on multiple equilibria was done by Lassettre (78). A review of the theory of multiple equilibria was recently made in the literature by Klotz (1). If the following equilibria are simultaneously attained:

\[ A + B \rightleftharpoons AB \quad K_1 = \frac{[AB]}{[A][B]} \]

\[ AB + B \rightleftharpoons AB_2 \quad K_2 = \frac{[AB_2]}{[AB][B]} = \frac{[AB_2]}{[A][B]^2 K_1} \]

\[ AB_{i-1} + B \rightleftharpoons AB_i \quad K_i = \frac{[AB_i]}{[AB_{i-1}][B]} = \frac{[AB_i]}{[A][B]^{i-1} K_1 \cdots K_{i-1}} \]

\[ AB_{n-1} + B \rightleftharpoons AB_n \quad K_n = \frac{[AB_n]}{[AB_{n-1}][B]} = \frac{[AB_n]}{[A][B]^{n-1} K_1 \cdots K_{n-1}} \]

the accompanying association constants result. According to Klotz, by considering the number of sites on A remaining available for the binding of the "i"th B, and the number of molecules of B available for dissociation, one obtains

\[ K_i = \frac{(n-i+1)}{i} K \]  \hspace{1cm} (31)

where \( K \) is an intrinsic association constant and \( n \) is the number of sites on A available for the binding of B.

If \( h = \frac{\text{moles of bound B}}{\text{total moles of A}} \)

\[ h = \frac{(AB) + 2(AB_2) + \cdots + i(AB_i) + \cdots + n(AB_n)}{(A) + (AB) + \cdots + (AB_i) + \cdots + (AB_n)} \]  \hspace{1cm} (32)
Substituting for the concentrations in terms of association constants from Equations 30, we obtain:

\[ h = \frac{K_1(A)(B) + 2K_2K_1(A)(B)^2 + \ldots + iK_1 \ldots K_i(A)(B)^i + \ldots}{(A) + K_1(A)(B) + \ldots + K_1 \ldots K_i(A)(B)^i + \ldots} \]

\[ = \frac{K_1(B) + 2K_1K_2(B)^2 + \ldots + iK_1 \ldots K_i(B)^i + \ldots + nK_1 \ldots K_n(B)^n}{1 + K_1(B) + \ldots + K_1 \ldots K_i(B)^i + \ldots + K_1 \ldots K_n(B)^n} \]

\[ = \left( \frac{\partial}{\partial h(B)} \right) \frac{(1 + K(B))^n}{[1 + K(B)]^n} = \frac{nK(B)}{1 + K(B)} \] (33)

so, \[ h(B) = Kn - Kh \] (34)

In any one run where a dissociation is shown to be present by a minimum in the \( \left( \frac{K_e}{R_\theta} \right) \) vs. c curve, points may be found on the part of the curve which shows the dissociation. From these points can be calculated apparent \( \delta \)'s. The method will be described in a later section. If we can express Equation 34 in terms of our experimentally determined quantity \( \delta \), calculations for \( K \), the intrinsic association constant, and \( n \), the maximum number of sites on the PVP molecule available for binding of BSA should become possible.

Since \( \delta = \frac{\text{grams (BSA) in complex}}{\text{grams (PVP) in complex}} = \frac{\text{moles BSA/liter} \cdot M_{\text{BSA}}}{\text{moles PVP/liter} \cdot M_{\text{PVP}}} \)

\[ = h \cdot \frac{M_{\text{BSA}}}{M_{\text{PVP}}} \]
and \( h = \delta \frac{M_{PVP}}{M_{BSA}} \)

then, \[ \delta \left( \frac{\frac{M_{PVP}}{M_{BSA}}}{(B)} \right) = Kn - K \delta \left( \frac{M_{PVP}}{M_{BSA}} \right) \] (35)

where \((B) = \text{concentration of free BSA in moles/liter.}\)

Since \( r = \frac{\text{total grams BSA}}{\text{total grams PVP}} = \frac{\text{total grams BSA/liter}}{\text{total grams PVP/liter}} \)

\( r - \delta = \frac{\text{total grams BSA/liter}}{\text{total grams PVP/liter}} - \frac{\text{bound grams BSA/liter}}{\text{total grams PVP/liter}} \)

\( = \frac{(B) \left( \frac{M_{BSA}}{M_{BSA}} \right)}{\text{total grams PVP/liter}} \)

\[ \delta \left( \frac{\frac{M_{PVP}}{M_{BSA}}}{(r-\delta)} \left( \frac{\text{total grams PVP/liter}}{M_{BSA}} \right) \right) = Kn - K \delta \left( \frac{M_{PVP}}{M_{BSA}} \right) \] (36)

So, by plotting \( \delta \frac{M_{BSA}}{c_{PVP}} \) vs. \( \delta \), the slope should be \( K \) and the intercept should be \( Kn \left( \frac{M_{BSA}}{M_{PVP}} \right) \).

2. Charge Effects

The above treatment should enable one to obtain a value for the intrinsic association constant if neither of the interactants are charged. However, if the materials are charged, the effects of these charges and the ionic
strength must be considered. The treatment of these charge effects is greatly hindered by the fact that the PVP molecules in this investigation are definitely not spherical, except, perhaps, in solutions of very high ionic strength. So, the simple Debye-Hückel and Born approach to the problem is not usable. If this method were usable, the expression below should apply:

\[
\left(\frac{K_n}{K_h}\right)^{2Wh} = K_n - K_h
\]  

where \( W = \frac{NZ^2\varepsilon^2}{2\Delta RT} \frac{1}{b} \frac{1}{1+K\alpha} \)

and \( Z^+ = \) charge on BSA

\( b = \) radius of sphere (PVP)

\( a = \) distance of closest approach of salt ions

\( K = \) Debye reciprocal radius.

The above process is further complicated by the fact that the \( Z^+ \) on BSA changes with the pH. Therefore, since the amount of experimental information needed to substantiate the above theory would be prohibitive, no further attempt will be made to coordinate the experimental data with a theoretical curve, corrected for charge effects.

C. Theory of Polyelectrolytes

The subject of the characteristic properties of polyelectrolytes as opposed to those of uncharged polymers has been reviewed quite completely by Katchalsky (57), and Strauss and Fuoss (58).
tion in the light scattering of polyelectrolytes is the effect of the ionic strength on the shape of the polyelectrolyte molecules. This effect stems from the fact that the charged molecules have a definite electrostatic potential which, in the case of a highly charged molecule like PVP, causes a repulsion between charged groups. However, this repulsion is decreased as the ionic strength of the solution is increased, causing the molecules to change from highly extended forms in low ionic strengths to less extended forms in high ionic strengths. In the case of a molecule which, under moderate conditions of ionic strength, exists as a random coil, a decrease of ionic strength will cause the molecule to gradually assume a rodlike shape, since the repulsion between charged groups on the chain has been increased greatly.

Another effect brought about by increasing the ionic strength is the more intense binding of counter ions by the polyelectrolyte ion. This change in the number of bound counter ions also changes the electrostatic potential of the polyions.

The investigations at hand were carried on in solutions of moderate ionic strength. In this region, where the polyion becomes a random coil, no drastic change in molecular shape should be noticed, except for a gradual decrease in molecular size as the salt concentration is increased. However, in very low or zero ionic strength,
ordering effects should be noticed. These effects cause the destructive interference of light, which, in turn, causes a leveling off of the reciprocal reduced intensity versus concentration curve at high concentrations, necessitating quite accurate low-concentration measurements to avoid the calculation of low molecular weights.
V. EXPERIMENTAL PROCEDURE

A. Preparation of Compounds

1. Preparation of Poly-4-vinyl-N,n-butyl-pyridine Bromide

The polymerization of 4-vinyl pyridine was adapted from Fuoss, et al. (60), and Goodman (42). Commercial grade PVP with inhibitor was obtained from Reilly Tar and Chemical Company. The monomer was distilled under vacuum (13 mm Hg) until 100 ml of water-clear monomer had been collected. This amount was dissolved in 457 ml of toluene to make a 20% by weight solution. Seven and one-half g of benzoyl peroxide was added as an initiator, and the solution was allowed to polymerize for one hour at 60°C., with stirring, and in a nitrogen atmosphere. The product, a buff colored lumpy solid, was washed in toluene, blended in a Waring blender, and filtered. The product was then dried in a vacuum oven at 42°C.

The products obtained from a number of runs were combined (total weight = 90 g), and dissolved in 3000 ml of tertiary butyl alcohol. To this solution was added 5360 ml of benzene, with stirring, until the cloud point was reached. Then, an additional volume of 50 ml of benzene was added, precipitating a fraction containing about 25 g of poly-4-vinyl pyridine. This entire process was repeated with 90 more grams of monomer. The products formed were dried,
combined, and refractionated. The middle fraction was taken from the above fractionation, and lyophilized, which process formed a white, spongy material. There were 34 g of product formed.

The poly-4-vinyl pyridine was then dissolved in 645 ml of nitromethane, and 500 ml of 1-bromobutane was added. This solution was allowed to stand at 60°±2° for one week. The solution became quite red, and a brown viscous solid formed. This solid was dried, broken up, and dissolved in 300 ml of grain alcohol. The resulting solution was poured into nine liters of dioxane. A white flocculent precipitate appeared, which was filtered, and dried at 40°C. The resulting white powder was stored in a desiccator at room temperature. Parr-bomb bromine analyses showed complete quaternization.

A stock solution of the quaternized salt was made up by dissolving the polymer in distilled water and centrifuging this solution at 50,000 rpm in a Spinco preparative ultracentrifuge for one hour. Some surface scum was removed from each centrifuge tube, and the solutions were combined and dialyzed for several days against distilled water. The resulting stock solution was slightly colored. This stock solution was tightly capped and allowed to stand at room temperature. The solution was neutral, as determined on a Beckman Model G pH Meter.
2. Purification of Bovine Serum Albumin

Twenty-five grams of crystalline bovine plasma albumin (Armour Lot P-67704) was dissolved in 250 ml of double-distilled water and dialyzed against double-distilled water at 1°C for one day. The resulting solution was centrifuged at 40,000 rpm in a Spinco preparative ultracentrifuge for one hour and divided into portions containing about 1 g of BSA. These small portions were frozen in a deep-freeze and stored there. When a series of runs was to be made, one of these portions was melted and diluted to make up a stock solution, which was stored at 0°C until needed. No solution was stored more than one week.

B. Titration of BSA with PVP — pH Measurements

A series of titrations was made on dilute BSA solutions of varying concentration, by adding dilute PVP solution, and measuring the change in H⁺ concentration with a Beckman Model G pH-meter, standardized with Beckman Standard pH = 5.0 buffer, since the titrations began at a pH of about 4.9. In no case did the pH drop below 4.5, so that the calibration of the instrument at pH = 5.0 was deemed sufficient. In all titrations, the initial volume of BSA solution was 20 ml. Solutions were stirred by manual swirling of the titration beaker in between additions of the PVP solution.
C. Light Scattering Measurements

1. Preparation of Solutions

All light scattering runs were made in sodium acetate-acetic acid buffers, of varying ionic strength and pH. Concentrated stock solutions of these buffers were made up, using a nomograph prepared by Boyd (61), and stored for further use. Solutions of the pure BSA for light scattering were made up by pipetting the required amount of stock buffer into a 125 ml glass-stoppered Erlenmeyer flask, and then adding the calculated amounts of double-distilled water and stock BSA, as determined by a concentration measurement on the stock BSA. Separate solutions were made up for each concentration desired for light scattering purposes. Care was taken during the pipetting of the BSA to keep the tip of the pipette below the surface of the solution, so as to avoid as much surface denaturation of the protein as possible.

Solutions for the molecular weight determinations on PVP were made up by pipetting the calculated amounts of buffer and PVP into 100 ml volumetric flasks and diluting to volume with double-distilled water. Again, separate solutions were made up for each concentration.

Solutions for the interaction runs were prepared by pipetting the calculated amount of stock buffer, PVP, double-distilled water, and BSA into 125 ml glass-stoppered Erlenmeyer flasks, in that order. Again, care had to
be taken to keep the tip of the pipette below the surface of the liquid during the addition of the BSA. The solutions were swirled and allowed to stand at 0°C, in a refrigerator until about two hours before use. Since some of the interactions involved equilibria which were temperature-dependent, care had to be taken to let the solutions come up completely to instrument temperature before actual measurements could be made.

2. Description of Difficulties

The measurement of the intensities of the light scattered angularly by solutions of large molecules is rather simple. The calculation of the average molecular weights and shape factors, too, is quite easy. However, experimentally, there are of course, some difficulties which arise. Low-angle light scattering is greatly increased by any dust included in the solutions. This difficulty necessitates the taking of great precautions in the transfer and filtration of solutions. The dust also causes great instability in the galvanometer deflections, if a galvanometer is used to measure light intensities.

Another difficulty arises in the measurement of concentrations, since the concentration of solutions used for light scattering purposes must be necessarily low. The determination of these low concentrations, accurately enough for calculation purposes, is quite difficult.
3. Filtration

Since all solutions used in the investigations were aqueous, the manner of filtration was dictated by the success of previous investigators who used Millipore filter disks, obtained from the Millipore Filter Corporation in Waterford, Connecticut. These disks are mountable on special Pyrex filter assemblies consisting of a funnel with a porous-sintered Pyrex supporting top to hold the disk, a solution vessel for containing the filterable solution, and a metal clamp, used to hold the solution vessel on top of the filter-holder and filter disk. This assemblage was inserted into the top of a bell-jar, which was evacuated by a water-pump aspirator. The light scattering cell was inserted into the bell-jar, directly under the filter assemblage, and solutions were filtered directly into the cell.

One very important part of the filtration procedure is the preconditioning of the filter assemblage. The first step in this procedure is the backwashing of the filter holder, or funnel, with distilled water for about four hours. Next, the filter assemblage is put together, with the Millipore disk in place, inserted in the bell-jar, and washed by pulling distilled water through the filter for about one hour. This step completes the conditioning process.

The actual filtering of solutions is carried out as
(1) A new Millipore disk is placed in the assembly, and the clean light scattering cell is inserted in the bell-jar. Water is filtered into the cell five or six times. The cell is inserted so that the filter-funnel touches the side, so as to obtain a steady flow with no dripping or splattering. The funnel stem has previously been bent at a gentle angle. Also, water is pulled through very slowly, so as to avoid splattering.

(2) A sample of water is taken for a light scattering determination of its dissymmetry. The cell is rewashed in a similar manner until a constant low dissymmetry is obtained. During this whole procedure, the filter must never be allowed to run dry, since such an accident would necessitate another conditioning of the filter holder.

(3) When the dissymmetry has been found to be sufficiently low, the remaining water in the filter may be poured out and replaced with the buffer-solvent. The light scattering cell is again washed several times with this buffer, until the dissymmetry of the buffer remains constant. Care must be taken in this and previous steps to avoid contamination with dust when the cell is being covered and transferred to the light scattering apparatus. When the dissymmetry becomes constant, a complete light scattering angular envelope is taken for the buffer.
(4) About one-half of the 100 ml sample of the most
dilute solution is then placed in the cell, after pouring
out the remaining buffer. The cell is again placed under
the bell-jar, and solution is slowly filtered into the
cell until the cell becomes approximately half full. This
filtered solution is then carefully poured out the back
side of the cell (keeping the cell covered as much as
possible with the bell-jar), into another container, and
is saved.

(5) The cell is reinserted in the bell-jar, and the
remainder of the solution is poured into the filter.
Solution is pulled into the cell to the proper amount
needed for light scattering (about 35 ml). The remaining
solution, plus that which had been saved from the washing,
is left in the filter while the light scattering measure­
ments are being made. The cell is again covered with its
thin glass cover. (The procedure of careful covering of
the cell must be followed throughout the filtering process,
since each exposure to the atmosphere results in the in­
corporation of dust into the cell).

(6) After the scattering measurements have been
made on the first solution, it is carefully poured into
another container and saved for concentration measure­
ments. However, while the cell is still covered, an
exchange of solutions must be made in the filter by the
process previously mentioned.
(7) The same procedure is repeated for each concentration. The method seems to be quite effective in removing extraneous particles and gives reproducible and steady low-angle light scattering readings.

(8) After all the light scattering measurements have been made, the filter assemblage is taken apart, rinsed in distilled water, and placed in boiling detergent solution for several hours. Each piece is then removed, and the filter is restored to its distilled water storage, while all other parts, including the bell-jar, are covered to keep out the dust.

4. Procedure of Light Scattering

The light scattering procedure may be divided into two parts. The first deals with the actual mechanical procedure for operation of the instrument and the precautions which must be taken to insure optimum sensitivity and stability. The second concerns the various constants involved in the calculation of the turbidity or Rayleigh ratio if the relative intensities of light at various angles are known. These subjects will be discussed in the following sections.

a. Description of the Light Scattering Photometer

The light scattering photometer used in these investigations is a modified B. S. Light Scattering Photometer, made by the Phoenix Precision Instrument Company. A schematic diagram of this instrument is shown in Figure 1.
LEGEND

A. Light source
B. Slit
C. Color filter
D. Diaphragm
E. Neutral density filters
F. Collimating lens
G. Collimating tube
H. Polarizer
I. Sleeve slit
J. Table slit
K. Light scattering cell
L. Table
M. Phototube compartment
N. Disc, marked in degrees
O. Phototube slit
P. Phototube
Q. Light trap
R. Working standard

Figure 1. Schematic Diagram of B.S. Light Scattering Photometer
The instrument consists of a mercury light source, A, in this case an A44 lamp, a slit, B, color filters, C, to pass light of a certain wavelength, neutral density filters, E, which enable one to reduce the light intensity, a diaphragm and shutter, D, and a collimating lens, F. Inside the instrument, the light passes through a collimating tube, G, and through two slits, H and I, into the light scattering cell, K. The cell is mounted on a fixed table, L, around which revolves the phototube compartment, M, fixed onto an arm, on the other end of which is mounted a working standard, W, consisting of a neutral filter and an opal glass diffusor. When the phototube compartment is at zero degrees (measured by a large ruled disk, W, revolving with the phototube arm), so that that incident light goes directly into the phototube compartment through a narrow slit, Q, the working standard is directly in the optical path, so as to keep the beam from falling on the phototube at full intensity.

The two slits on the incident beam side of the cell are fixed, one being on the end of the collimating and polarizing tube, and the other being mounted on the cell table, itself. When the cell being used is either the square or the semioctagonal cell, these two slits are large (1.2 cm). However, when the cylindrical cell is used for angular determinations, narrow slits are used. The slit leading into the phototube compartment remains the same.
in both cases.

When measurements requiring polarized light are to be made, it is possible to insert a polarizer $\mathcal{H}$ into the collimating tube, and another polarizer into the entrance to the phototube compartment $Q$. This analyzer is used in the determination of the constant, $b$, which will be mentioned in a later section.

The phototube current operates a sensitive galvanometer ($0.00064 \, \text{mA/mm}$). The phototubes used are selected RCA 931A tubes, with low dark current, high gain, and minimum sensitivity to a variation of the plane of polarization of the incident beam.

The input voltages of the light source transformer and the phototube power supply are regulated by two Sorensen voltage regulators, Model 1000S.

b. Actual Procedure for the Light Scattering Measurements

About one hour before measurements are to be taken, the Sorensen regulators are turned on and allowed to warm up. The leads to the light source and phototube power supply are connected to the regulators. At this time, the galvanometer light is also turned on. The phototube compartment should be kept as dry as possible at all times by the regular changing of the silica-gel in its desiccant compartment. This step should be made several hours before measurements are to be taken.
The light scattering cell should be inserted in the cell-table mount provided for it in the instrument, and the lid of the instrument closed. The phototube switch should be turned on next. With the shutter opened, and all four neutral density filters in place, the phototube sensitivity dial is slowly turned up until the galvanometer reaches almost a full-scale deflection. Then, the fine adjustment of the galvanometer is used to make the dark current exactly zero. At this point, angular readings may be taken. The method which is used is to go completely from 0° to 135°, taking readings at the angles selected, and then taking readings from 135° back to 0° at the same angles. Thus, an average reading for each angle is obtained. This method balances out any small changes in phototube voltage or light-source intensity during the series of readings.

c. Calculation of Rayleigh Ratio, $R(0)$, and $K$ from the Light Scattering Data

The relationship given (62) for the calculation of the turbidity, when a square or semioctagonal scattering cell is used is as follows:

$$
\Gamma_{\lambda, 90°} = \frac{16TDaN^2}{3(1.045)h} \frac{R_w n^2}{R_c} \frac{G_{90}}{G_0} \cdot \frac{F_90}{F_0} \tag{38}
$$

where $\Gamma_{\lambda, 90°} = \text{turbidity at wavelength } \lambda \text{ and } \theta = 90°$

$T = \text{apparent diffuse transmittance of the opal glass standard}$
D = light diffusion correction to adjust for the fact that the reference standard is not a perfect diffusor.

1.045 = reflection correction for the emergent beam.

h = width of the incident beam.

\( \left( \frac{R_M}{R_c} \right) \) = refraction correction dependent upon the refractive index of the solution.

n = refractive index of the solvent.

G = galvanometer deflection.

F = filter factor (transmittance) for neutral filters present in the beam.

\( \frac{a}{a_o} \) = transmittance of working standardtransmittance of opal standard.

In the above relationship, the constants T, D, 1.045, h, and all values of F_i are instrument constants and do not vary from run to run. Likewise, \( \frac{R_M}{R_c} \) and n are functions of the solvent involved and do not change with time. However, the value of \( \frac{a}{a_o} \) will change periodically, since this constant is affected by small geometrical changes in the alignment of the instrument, brought about by bringing the turntable against its stop, or any of several other factors, including the resetting of the lamp or changing of phototubes.

The above relationship is set up for the calculation of the turbidity. If the Rayleigh ratio is wanted for various angles, this relationship must be modified. The two quantities, \( \tau_{\lambda, 90^\circ} \) and \( R_{\lambda, 90^\circ} \) are related in the
following manner:

\[ \tau_{\lambda,90} = \frac{16\pi}{3} R_{\lambda,90} \]

so,

\[ R_{\lambda,90} = \frac{\pi D a}{1.045 n^2} \cdot \frac{R \gamma}{R_0} \cdot \frac{n^2}{G_0} \cdot \frac{F_0}{F_{90}} \]  \hspace{1cm} (39)

(1) Effect of Narrow Slits and Cylindrical Cell

on the Apparent Reduced Intensity

When making angular light scattering measurements, in order to obtain true relative values for each of the angles, it is necessary to use a conical or cylindrical cell, to avoid refraction effects. In this work a cylindrical cell was used. If a cylindrical cell is used, a much narrower slit must be used. These changes necessitate a change in the relationship involving the reduced scattering intensity and the galvanometer deflections. If one carefully filters a solution of Ludox (0.5%), which is colloidal silica obtained from E. I. du Pont de Nemours and Company, into both the semioctagonal cell, for which Equation 39 applies, and into the cylindrical cell, and measures \( \frac{G_{90} F_0}{G_0 F_{90}} \) semioct. and \( \frac{G_{90} F_0}{G_0 F_{90}} \) cyl.
as well as the corresponding values for the solvent in both cases, one obtains

\[ k' = \frac{\frac{G_{90} F_0}{G_0 F_{90}} \text{ semioct.}, \text{ net}}{\frac{G_{90} F_0}{G_0 F_{90}} \text{ cyl.}, \text{ net}} \]  \hspace{1cm} (40)
Thus, \( R_{90} = \frac{TD \pi (k') \cdot RW \cdot n^2 \left( \frac{G_{90} \cdot F_{0}}{G_0 \cdot F_{90}} \right)}{1.045 \ h} \) cyl., net. \ (41)

The value of \( k' \) appears to change slightly with time and with minor changes in the instrument. So, it must be re-measured periodically \((63)\). Normally, when \( a \) changes, \( k' \) changes also.

(2) Correction of the Rayleigh Ratio for Volume of Solution Viewed and Scattering Angle

Since the volume of the scattering solution viewed by the receiver is proportional to \( \frac{1}{\sin \theta} \), all \( R_\theta \) values must be multiplied by \( \sin \theta \) to obtain scattering intensities related to the same volume. Also, \( R_\theta = \frac{R_{\theta u}}{1 + \cos^2 \theta} \),

where \( R_{\theta u} = \) Rayleigh's ratio for unpolarized incident light, measured at the angle \( \theta \); this quantity is known also as the "reduced scattered intensity". Therefore to eliminate all dependence of the angle at which the scattering is viewed on the \( R_\theta \), we multiply all \( R_\theta \) values by \( \frac{1}{1 + \cos^2 \theta} \). Since the phototubes used were never entirely insensitive to the direction of polarization, one additional correction must be applied. This correction was determined by placing the standard opal glass diffuser at the center table and allowing unpolarized light to strike it. Then, the analyzer on the phototube housing was first set to pass just horizontal and then just vertical electric vector vibrations. The ratio of galvanometer deflections,
b = \frac{H_u}{V_u} is used as a correction factor in the 1+\cos^2\theta factor which corrects for the scattering angle, and we obtain a final correction factor of \frac{\sin \theta}{1 + b \cos^2 \theta}. Thus, the final relationship for R_\theta is:

\[
R_\theta = \frac{T_D a W k^2}{1.045 h} \cdot \frac{R_w}{R_c} \cdot n^2 \left( \frac{G_e}{G_o} \cdot F_o \right) \text{ cyl., net.} \cdot \frac{\sin \theta}{1 + b \cos^2 \theta}
\]

(3) Other corrections

In some systems, corrections must be made for other effects such as fluorescence of the solution, absorption of light by the system, back reflections, and depolarization. These corrections were found to be insignificant in the systems studied, and thus were not applied.

(4) Absolute Calibration of the Instrument

In the last few years, different methods have been developed for calibration of light scattering instruments (75). One of the most frequently used involves a measurement of the turbidity of very pure thiophene-free benzene. The value of R_{90, u} obtained in these laboratories for benzene was 4.91 \times 10^{-5} at 436 mu. This value compares favorably with a value of 4.94 \times 10^{-5} obtained as an average of values obtained by several authors and tabulated in the literature (64).

(5) Final Relationship for R_\theta

After incorporating all constants (given in Table I) into Equation 42, the final relationship for R_\theta becomes:
Rθ = 0.03724 \left( \frac{G_G \cdot F_G}{G_o \cdot G_e} \right) \text{ net, cyl.} \cdot \sin \theta \cdot \frac{1}{1+1.036 \cos^2 \theta} \quad (43)

Luckily, one phototube was used throughout all the light scattering runs, so b remained constant. However, a and k' were redetermined several times, and appropriate corrections were incorporated into the final expression for R₀.

**TABLE I**

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
<th>Filter</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.288</td>
<td>1</td>
<td>0.471</td>
</tr>
<tr>
<td>D</td>
<td>0.840</td>
<td>2</td>
<td>0.220</td>
</tr>
<tr>
<td>n</td>
<td>1.340 (for H₂O)</td>
<td>3</td>
<td>0.104</td>
</tr>
<tr>
<td>(R_m / R₀)</td>
<td>1.025 (for H₂O)</td>
<td>4</td>
<td>0.0277</td>
</tr>
<tr>
<td>h</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>0.2531</td>
<td></td>
<td></td>
</tr>
<tr>
<td>k'</td>
<td>1.3021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1.036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(6) Calculation of K

From the theory of light scattering, $K = \frac{2 \pi' n_o}{\lambda^2 N} \left( \frac{dn}{dc} \right)^2$

This expression contains several quantities, all of which are constant for any given solute and solvent under defined conditions. The specific refractive index increment $\left( \frac{dn}{dc} \right)$ was obtained for each of the starting materials in the interaction runs in water solution. It was assumed that the values obtained did not change appreciably when
the materials were dissolved in buffer solutions, at least over the region of buffer concentrations used in these measurements. The values of \( \frac{dn}{dc} \) used were 0.192 ml/g for BSA and 0.2036 ml/g for PVP. (36,62)

4. The Differential Refractometer, as Used in the Measurement of Concentrations for Light Scattering

The B.S. Differential Refractometer, made by the Phoenix Instrument Company, has been used to determine the concentrations of all solutions used in the investigations described. The use of this refractometer has been completely described by Brice and Halwer (65). The critical constants and variables are listed below:

\[ \Delta n = k_\lambda \Delta d \]

\( \Delta n \) = difference between refractive indices of a solution and its solvent

\( k_\lambda \) = apparatus constant for a given wavelength of light

\( \Delta d \) = a parameter which is experimentally determinable on the instrument

Since \( c = \Delta n \left( \frac{dn}{dc} \right)^{-1} \), it is thus possible to find the concentration of a solution if the specific refractive index increment is known.

The instrument was calibrated with 0.1992 M KCl. \( \left( \frac{dn}{dc} \right) \)'s used in the calibration were extrapolated from the data of Kruis (66). At 436 mp, the value of \( k_\lambda \) was 1.009 x 10\(^{-3}\) ml/g.
D. Electrophoresis and Ultracentrifugation

The methods of electrophoresis and ultracentrifugation were used in this investigation mainly to ascertain the purity of the starting materials and the polydispersities of the interaction complexes and starting materials.

1. Electrophoresis

The two electrophoresis runs that were made were run in a Tiselius-Klett electrophoresis apparatus. The cell used was a 1 ml Tiselius-type cell. This cell was filled in the usual manner (73) and was kept in a water bath at 1°C throughout the progress of the run. No mobilities were calculated, but the shapes and relative areas of the various peaks were investigated.

2. Ultracentrifugation

All centrifuge runs were carried out in a Spinco Model E ultracentrifuge at room temperature (25°C.). The cell was filled and put in place and the runs were carried out according to the procedure recommended in the Spinco instrument manual.
VI. EXPERIMENTAL RESULTS

The experimental work has been divided into several sections, including the pH titrations, the characterization of polymers, and the light scattering work. These topics will be covered in this chapter.

A. pH Titrations of BSA with PVP

The first experimental work attempted was the titration of varying concentrations of isionic BSA with a stock solution of PVP. The pH values resulting were measured and plotted versus the volume of PVP added. Characteristic curves were obtained, resembling the curves obtained by Hailweil (36) for ovalbumin versus PVP, but with three break-points in each concentration curve, instead of just two. These curves are shown in Figure 2. The curves showed some small variation in initial pH from concentration to concentration, so corrections were applied to bring all curves to a single starting pH of 4.92.

After the third break-point was reached in each of the titrations, the pH remained constant with increased addition of PVP. At the beginning of each titration, where BSA was in excess, no perceptible turbidity was noticed. However, in the two middle sections of the curve, a definite turbidity appeared. This turbidity disappeared when the third break-point was reached and no further
Figure 2. pH Titration of BSA by PVP
turbidity was noticed throughout the horizontal portion of the curve. The values of \( r \), the mixing ratio, at the break-points were calculated and are tabulated in Table II.

### Table II

<table>
<thead>
<tr>
<th>Initial ( c_{\text{BSA}} ) in g/ml ( \times 10^3 )</th>
<th>( r ) at 1st break</th>
<th>( r ) at 2nd break</th>
<th>( r ) at 3rd break</th>
</tr>
</thead>
<tbody>
<tr>
<td>.789</td>
<td>40.3</td>
<td>17.5</td>
<td>8.00</td>
</tr>
<tr>
<td>3.2</td>
<td>88.8</td>
<td>38.2</td>
<td>14.18</td>
</tr>
<tr>
<td>6.34</td>
<td>114</td>
<td>37.7</td>
<td>17.8</td>
</tr>
<tr>
<td>9.78</td>
<td>119.5</td>
<td>49.4</td>
<td>23.7</td>
</tr>
</tbody>
</table>

The values of \( r \) at the third break-point vary nearly linearly with \( c_{\text{BSA}} \), but approach a maximum at high values of \( c_{\text{BSA}} \), as do the curves for the second and first break-points. The significance of this will be discussed in a later section. Plots of these break-points can be seen in Figure 28. However, if mixing ratios are kept below the intercept of the third break-point curve with the ordinate axis, \( r = 6.2 \), no doubling or large aggregates will be formed, since the turbidity appears to be constant with respect to time in this region.

### B. Characterization of Pure Materials

Several different methods were used in the characterization of the starting materials, PVP and BSA, for the light scattering work. Light scattering measurements were
made on them to determine their molecular weights. The weight average molecular weight of PVP was determined to be 380,000, by the averaging of several different results obtained in different runs and the value obtained from the back-calculation of an interaction run in which complete interaction was known to have taken place. These values are shown in Table III. One of the light scattering runs is shown in Figure 3.

The molecular weight of BSA, as determined by light scattering, was found to be 82,200. This value, also, is an average value for two other runs, as shown in Table III.

**TABLE III**

Molecular Weights of BSA and PVP

<table>
<thead>
<tr>
<th>Conditions</th>
<th>BSA</th>
<th>PVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pH=4.5, =.01)</td>
<td>83,800</td>
<td>(pH=4.5, =.01)</td>
</tr>
<tr>
<td>(pH=4.5, =.01)</td>
<td>80,500</td>
<td>(pH=5.0, =.1)</td>
</tr>
<tr>
<td></td>
<td>Ave. 82,200</td>
<td>Interaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(pH=5.0, =.001, r=1.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ave. 380,000</td>
</tr>
</tbody>
</table>

Electrophoresis measurements were also made on the original polymers. Sample photographs from these runs are shown in Figure 4. The PVP moved as one peak. This indicated that PVP was composed of one main fraction with
Figure 3. Angular Light Scattering Data for PVP ($\text{pH} = 4.5, \frac{r}{2} = 0.01$)
FIGURE 4

**Electrophoresis Patterns for BSA and PVP**

A. BSA (pH = 8.5, $\frac{C}{2} = 0.1$, C = 2.0 %)
   - Ascending
   - Descending

B. PVP (pH = 8.5, $\frac{C}{2} = 0.1$, C = 0.5 %)
   - Ascending
   - Descending
all molecules having about the same charge density. No foreign material was shown to be present.

The electrophoresis run on BSA showed one large peak and a very small peak, with the area of the minor peak amounting to perhaps one per cent of the total area. The small peak moved more slowly than the peak representing the bulk of the BSA, indicating that the impurity has a smaller charge density than the BSA. The minor peak may possibly represent denatured BSA, or even some serum lipoprotein which had been carried down in the crystallizing procedure for the BSA. Either one of these impurities would increase the average molecular weights obtained for BSA. This explains why the molecular weights obtained for BSA by light scattering were larger than those usually found (68,70).

The previously mentioned impurity in BSA also showed up in an ultracentrifuge run, which was made, as a fast-moving component. Again, the area of this very minor peak was only a very small per cent of the total peak area. The aforementioned run is shown in Figure 5 along with several PVP runs. A measurement of the sedimentation constant of BSA at pH=5.0, $\frac{r}{2}=0.1$, gave $S_{20}=3.9$ Svedbergs, which value compares well with measurements by other experimenters (67,68,69,70,71).

A series of three centrifuge runs were made on different concentrations of PVP at pH=5.0, $\frac{r}{2}=0.1$. In these
FIGURE 5
ULTRACENTRIFUGE PATTERNS FOR BSA AND PVP

A. BSA (pH = 5.0, $\frac{C}{2} = 0.1$, C = 1.0 %)

B. PVP (pH = 5.0, $\frac{C}{2} = 0.1$, C = 0.235 %)

C. PVP (pH = 5.0, $\frac{C}{2} = 0.1$, C = 0.543 %)

D. PVP (pH = 5.0, $\frac{C}{2} = 0.1$, C = 0.775 %)
runs, PVP moved as a single peak, slightly sharpened on the solution side of the peak, as can be seen in Figure 5. A plot of $1/S_{20}$ versus the concentration gave $S_{20}^0=2.18$ Svedbergs. This slope of the $1/S_{20}$ line was nearly the same as that obtained by Maio (72) for a PVP run in NaCl solution at $\Gamma/2=0.1$. However, the value of $S_{20}$ obtained by Maio was much higher, since his PVP had a molecular weight of 620,000. The $1/S_{20}$ vs. $c_{\text{PVP}}$ curve may be seen in Figure 6.

C. Interaction Studies by Light Scattering

Three series of light scattering runs were made in the study of the interaction in solutions of PVP and BSA. There are three variable conditions in systems of this type. These conditions include the pH, the ionic strength, and the initial mixing ratio of the polymers. In each series of runs two of these conditions were held constant while the third was varied. Certain introductory runs were made to determine the proper ranges for each series of runs. A series of measurements at pH=4.5, $\Gamma=1.1$, and varying ionic strength determined that there was no interaction at this pH. So, higher pH's were indicated, since the BSA would become more negatively charged, as the isoelectric point was approached and passed.

1. Interaction Runs at Constant pH and Mixing Ratio

Light scattering runs were made on PVP-BSA inter-
Figure 6. Reciprocal Sedimentation Constant vs. Concentration of PVP (pH = 5.0, $\frac{c}{2} = 0.1$)
action mixtures at pH=5.0 and r=1.1. The ionic strength was varied (measurements were taken at \( \frac{\Gamma}{2} = 0.001, 0.005, 0.01, 0.025, 0.05, \) and \( 0.1 \)). The calculated values of \( \xi \) (where \( \xi = \text{weight ratio of BSA to PVP in the complex} \)), when plotted versus ionic strength (Figure 7), showed a behavior similar to ovalbumin-PVP (36,37). Complete interaction occurred at \( \frac{\Gamma}{2} = 0.001 \), and no interaction took place at \( \frac{\Gamma}{2} = 0.1 \). At values of ionic strength between these limits, as seen from Figure 7, the values of \( \xi \) calculated from the light scattering runs showed a rapid downward slope, gradually leveling out and approaching \( \xi = 0 \) as an asymptote at high values of ionic strength. It must be noted that \( \xi \), at low ionic strengths, approaches a maximum of 1.2 instead of the theoretical maximum of 1.1. This occurrence indicates that the value of \( M_{\text{PVP}} \) used in the calculation of \( \xi \) was slightly low, or that there has been some doubling up of PVP molecules on one BSA molecule, at least for a few of the BSA molecules. If a small percentage of the BSA was engaged in cross-linking in the manner described, the calculation of \( \xi \) in the previously determined way would lead to erroneously high results for \( \xi \). However, that little or none of this cross-linking was occurring was shown by an ultracentrifuge run on an interaction mixture at pH=5.0, \( \frac{\Gamma}{2} = 0.025 \), and \( r = 1.08 \) (Figure 8). Only two peaks were present, one being the free BSA, and the other (the faster-sedimenting peak) being the interaction complex.
Figure 7. Influence of Ionic Strength on Binding Ratio (pH = 5.0, r = 1.1, \( \frac{r}{2} \sim \))
FIGURE 8

Ultracentrifuge Pattern for PVP-BSA Interaction (pH = 5.0, $\Gamma = 0.025$, $r = 1.1$)
if any significant doubling had occurred, it would have shown up as a third, probably faster, peak. This run also verified the assumption made in the derivation of the equation defining $\mathcal{S}$, that all of the PVP was complexed.

Two representative runs in this series have been included (Figures 9, 10, 11). Figure 9, representing the interaction at $\Gamma=0.001$, is a normal Zimm plot. The only difference arises from the low-angle curvature of the $(c_T/\Re)_{\Theta=0}$ vs. $\sin^2 \Theta/2$ lines. This effect is particularly noticeable at high concentrations.

As the concentration of buffer ions was increased, minima appeared in the $(c_T/\Re)_{\Theta=0}$ vs. $c_T$ plots, necessitating the plotting of $(c_T/\Re)_{\Theta=0}$ vs. $\sin^2 \Theta/2$ to obtain values for $(c_T/\Re)_{\Theta=0}$ which were, in turn, plotted vs. total concentration. This phenomenon has occurred previously in the work of other investigators (31), and it normally is considered to be a sign of weak complexing. Thus, the weak complex begins to dissociate perceptibly at low total concentrations of interactants. Nevertheless, a value may still be obtained if one extrapolates the high-concentration points linearly to $c_T$=0. Figure 10 shows the $c_T/\Re$ vs. $\sin^2 \Theta/2$ curves for the interaction run at $\Gamma=0.025$, while Figure 11 contains the plot of $(c_T/\Re)_{\Theta=0}$ vs. $c_T$ for the same run. The relatively weak binding observed in Figure 11 becomes even weaker as
Figure 9. Angular Light Scattering Data for PVP-BSA Interaction (pH = 5.0, $r = 0.001$, $r = 11$)
Figure 10. Angular Light Scattering Data for PVP-BSA Interaction (pH = 5.0, $\frac{c}{2} = 0.025$, $r = 11$)
Figure II. Extrapolated Values of \( \frac{C_T}{R_0} \) at \( \Theta = 0 \) for PVP-BSA Interaction (pH = 5.0, \( \frac{m}{R} = .025 \), \( r = 1.1 \))
the ionic strength is increased.

Normally, no difficulties are encountered in obtaining values of $B$, the second virial coefficient, and $R_G$, the radius of gyration, from the experimental data, since

$$B = \frac{K}{2} \left[ \frac{d}{d \theta} \left( \frac{cT}{R_\theta^2} \right)_{\theta=0} \right]_{c=0}$$

and

$$R_G = \frac{\sqrt{3} \lambda}{4 \pi n_c} \frac{1}{\lambda},$$

where $\lambda = \frac{1}{\left( \frac{cT}{R_\theta^2} \right)_{\theta=0}}$.

In the light scattering plot for a dissociating system, $B$ is not constant, but can be considered to be effectively constant in the linear portion of the curve which is extrapolated to obtain $\mathcal{S}$. So, $B$ is determinable. However, in evaluating $R_G$, one must take the initial slope of a concentration line in the non-dissociating portion of the curve, instead of the slope at $cT=0$. Thus, the value obtained for $R_G$ is only a good approximation of the true value. If one might take a non-dissociating system such as shown in Figure 9 as a criterion, the values of $R_G$ obtainable from high-concentration data would be too large. So, probably in the case of a dissociating complex, the values obtainable for $R_G$ are slightly too large.

The values calculated for $\mathcal{S}$, $M_6$, $R_G$, and $B$ are compiled in Table IV. A plot of $R_G^2$ vs. $M_6$ is shown in Figure 12. The values of $R_G^2$ increase linearly with the
Figure 12. Radius of Gyration Squared versus Molecular Weight of PVP-BSA Interaction Complex for Varying Ionic Strength (pH = 5.0, r = 1.1, \( \theta \) ~ )
average molecular weight of the complex. A plot of $B$ vs. $\Gamma$ is shown in Figure 13. Values of $B$ decrease rapidly with ionic strength and then gradually slope off and approach $B=0$ at high ionic strengths. These effects are similar to those appearing in the interaction of PVP with ovalbumin (37).

### TABLE IV

Variation with Ionic Strength of $M_w$, $\bar{c}$, $B$, and $R_g$ for PVP-BSA Interaction (pH=5.0, $\frac{\Gamma}{2} = 0.025$, $r=1.1$)

<table>
<thead>
<tr>
<th>$\frac{\Gamma}{2}$</th>
<th>$\bar{c}$</th>
<th>$M_x 10^5$</th>
<th>$R_g(\bar{c})$</th>
<th>$B_x 10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>1.196</td>
<td>8.33</td>
<td>506</td>
<td>203</td>
</tr>
<tr>
<td>0.005</td>
<td>1.134</td>
<td>8.10</td>
<td>502</td>
<td>53.3</td>
</tr>
<tr>
<td>0.01</td>
<td>0.964</td>
<td>7.45</td>
<td>438</td>
<td>28.6</td>
</tr>
<tr>
<td>0.025</td>
<td>0.515</td>
<td>5.76</td>
<td>422</td>
<td>23.0</td>
</tr>
<tr>
<td>0.05</td>
<td>0.29</td>
<td>4.90</td>
<td>391</td>
<td>13.3</td>
</tr>
<tr>
<td>1</td>
<td>0.00</td>
<td>3.80</td>
<td>346</td>
<td>5.4</td>
</tr>
</tbody>
</table>

2. Interaction Runs at Constant pH and Ionic Strength

The next group of runs was made to check the changes in the characteristic constants of the polyelectrolyte complexes as the initial mixing ratio of BSA to PVP was changed. This series of runs crossed the preceding series, since all runs were made at pH=5.0 and $\frac{\Gamma}{2} = 0.025$, with mixing ratios of 0.5, 1.1, 1.8, 2.5, and 4.0. In this series, all runs showed that weak, dissociating complexes were being formed. The critical concentration below which the complexes dissociated varied from run to run. In general, as $r$ increased, the total concentration below which the complex dissociated also increased. Two plots showing
Figure 13. Variation of Second Virial Coefficient with Ionic Strength (pH = 5.0, r = 1.1, $\frac{r}{2} \sim$)
the interaction at pH=5.0, $\frac{\alpha}{2}=0.025$, $r=0.5$ are given in Figure 14 and 15. A plot of calculated $\delta$ vs. $\tau$ is shown in Figure 16. In the latter plot, $\delta$ varies linearly with $\tau$, with the experimental points being somewhat scattered at high values of $\tau$.

The calculation of $R_G$ for the complexes in this series of runs was again accompanied by the difficulties described in the previous section. However, values of this quantity were obtained; $R_G^2$ has again been plotted vs. the molecular weight of the interaction complex, which plot may be seen in Figure 17. The data appear to be somewhat scattered but show a distinct lowering of the radius of gyration as the molecular weight increases. The significance of this phenomenon will be discussed in a later section.

A plot of $B$ vs. $\omega$ for this series of runs is shown in Figure 18. $\omega$ is defined as the weight per cent of the PVP in the complex and is equal to $\frac{1}{1+\delta}$. This parameter was used, since $B$, the interaction constant, or second virial coefficient, will depend largely on the repulsion between the similarly charged groups on the PVP backbone, especially since the pH and ionic strength remained constant during this series of runs. As is shown in Figure 18, the values of $B$ vary almost linearly with $\omega$ at low values of $\omega$ (or high values of $\delta$) but curve upward at high values of $\omega$. Values of $M_6$, $\delta$, $R_G$, and $B$ are
Figure 14. Angular Light Scattering Data for PVP-BSA Interaction (pH = 5.0, \( \frac{r}{2} = 0.25, r = 0.5 \))
Figure 15. Extrapolated Values of \( \frac{c_t}{R_0} \) at \( \theta = 0 \) for PVP-BSA Interaction (pH = 5.0, \( \frac{r}{2} = 0.025 \), \( r = 0.5 \))
Figure 16. Change in Binding Ratio with Variation of Mixing Ratio (pH = 5.0, $r = 0.025 r$)
Figure 17. Radius of Gyration, Squared, versus Molecular Weight of PVP-BSA Interaction Complex for Various Mixing Ratio (pH = 5.0, $\frac{L}{L} = 0.025$)
Figure 18. Change in Second Virial Coefficient with Variation of $\omega$ (fraction PVP in complex) ($\text{pH} = 5.0$, $\frac{1}{2} = 0.25$).
given in Table V, for this series of runs.

TABLE V

Variation with Mixing Ratio of $M_y$, $\delta$, $B$, and $R_G$ for PVP-BSA Interaction (pH=5.0, $\frac{\gamma}{2}=.025$, $r \approx$)

<table>
<thead>
<tr>
<th>$r$</th>
<th>$\delta$</th>
<th>$M \times 10^5$</th>
<th>$R_G$ ($\AA$)</th>
<th>$B \times 10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>.5</td>
<td>.239</td>
<td>4.71</td>
<td>499</td>
<td>38.0</td>
</tr>
<tr>
<td>1.1</td>
<td>.515</td>
<td>5.76</td>
<td>422</td>
<td>23.0</td>
</tr>
<tr>
<td>1.8</td>
<td>.908</td>
<td>7.25</td>
<td>399</td>
<td>13.9</td>
</tr>
<tr>
<td>2.5</td>
<td>1.56</td>
<td>9.72</td>
<td>351</td>
<td>4.2</td>
</tr>
<tr>
<td>4.0</td>
<td>1.86</td>
<td>10.82</td>
<td>242</td>
<td>1.6</td>
</tr>
</tbody>
</table>

3. Interaction Runs at Constant Ionic Strength and Mixing Ratio

To round out the interaction work, a series of runs was made, holding the ionic strength constant at $\frac{\gamma}{2}=.025$, the mixing ratio constant at $r=1.1$, but varying the pH between 4.8 and 5.8. The calculated values of $\delta$ increased as the pH increased. The increase was nearly linear at low pH values, as can be seen in Figure 19. At high pH values, the curve approached a value of $\delta=1.1$ asymptotically, as it should, since the initial mixing ratio was $r=1.1$. Thus, no evidence has been shown for one molecule of BSA bridging between two molecules of PVP, since, in that case, the apparent $\delta$ would have increased above 1.1.

As in the case when the ionic strength was varied, a trend was seen in the relative strengths of the complexes formed at different values of the variable, in this series of runs, the pH. At low values of the pH, the complexes
Figure 19. Change of Binding Ratio with Variation of pH (pH-~ = .025, r = 1.1)
were relatively unstable, but at high values, the strength of complexing increased slightly. This trend is substantiated by theory, since the BSA molecules are more negatively charged at high values of pH. The runs at pH=4.8 (Figures 20 and 21) and pH=5.8 (Figure 22) are included here to illustrate the change in binding strength.

Values of $R_g$ were calculated for this series of runs and $R_g^2$ was plotted vs. $M_6$ in Figure 23. The plot is very interesting, since it shows that the radius of gyration increases abruptly at pH=4.8, which pH is on the acid side of the isoelectric point of BSA, where the net charge becomes positive. However, at all other values of pH, the radius of gyration remains nearly constant. This, also will be discussed later.

Figure 24 shows a plot of $B$ vs. pH at constant ionic strength and mixing ratio. This plot shows a slight increase in $B$ as the pH is decreased. As the isoelectric point of BSA is approached, the values of $B$ increase quite rapidly. A list of values of $M_6$, $\hat{c}$, $R_g$, and $B$ is given in Table VI.

D. Multiple Equilibrium Treatment of Data

In some of the interaction runs, a dissociation effect was noticed which caused minima in the plots of $\left( \frac{c_T}{R_g} \right)_{\theta=0}$ vs. total concentration. If it were possible to obtain values of $\hat{c}$ at points on the negatively sloped portions of
Figure 20. Angular Light Scattering Data for PVP-BSA Interaction (pH = 4.8, $\Gamma = 0.025$, $r = 1.1$)
Figure 21. Extrapolated Values of \( \frac{c_T}{R_{\Theta}} \) at \( \Theta = 0 \) for PVP-BSA Interaction 
(pH = 4.8, \( \frac{C}{2} = .025, r = 1.1 \))
Figure 22. Angular Light Scattering Data for PVP-BSA Interaction (pH = 5.8, $C = 0.025$, $r = 1.1$).
Figure 23. Radius of Gyration Squared vs. Molecular Weight of PVP–BSA Interaction Complex for Varying pH ($pH \sim, r \approx 1.1, \frac{R}{2} = .025$)
Figure 24. Change of Second Virial Coefficient with Variation of pH
(pH ~, $\frac{r}{a} = 0.025$, $r = 1.1$)
TABLE VI

Variation with pH of $M_H$, $\delta$, $B$, $R_G$ for PVP-BSA Interaction ($pH \approx r=1.1$, $\frac{v}{2}=0.025$)

<table>
<thead>
<tr>
<th>pH</th>
<th>$\delta$</th>
<th>$M \times 10^5$</th>
<th>$R_G(\AA)$</th>
<th>$B \times 10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>0.289</td>
<td>4.9</td>
<td>625</td>
<td>47.7</td>
</tr>
<tr>
<td>5.0</td>
<td>0.515</td>
<td>5.76</td>
<td>422</td>
<td>23.0</td>
</tr>
<tr>
<td>5.2</td>
<td>0.712</td>
<td>6.51</td>
<td>410</td>
<td>20.2</td>
</tr>
<tr>
<td>5.4</td>
<td>0.919</td>
<td>7.29</td>
<td>421</td>
<td>13.3</td>
</tr>
<tr>
<td>5.8</td>
<td>1.034</td>
<td>7.74</td>
<td>409</td>
<td>11.0</td>
</tr>
</tbody>
</table>

the curves, one should be able to obtain both the intrinsic association constant for the binding of BSA to PVP and the number of sites on the PVP available for binding, according to Equation 36. However, the process for obtaining values of $\delta$ from the curves was made difficult by the variation of $B$, the second virial coefficient, in the regions of the curves where the complexes were dissociating (31).

In the series of runs where only the mixing ratio, $\Gamma$, was varied, $B$ was plotted as a function of $\omega$, the fraction of PVP in the complex. However, $\omega$ was determined directly from $\delta$, the weight ratio of BSA to PVP in the complex. Thus, by plotting $B$ as a function of $\delta$, values of $B$ could be found for all values of $\delta$.

The problem of obtaining values of $\delta$ from single points on the dissociating portion of the $\left(\frac{c_T}{R_G}\right)_{\theta=0}$ curves was resolved by the use of a trial-and-error
approach. Thus, lines were drawn through the single points at approximately the same slope as the extrapolated line used to determine $\delta$ for the complex, as shown in Figure 11. The intercepts of these lines with the ordinate axis were used to calculate values of $\delta$ in the usual manner. These values of $\delta$ were compared with the values of $\delta$ corresponding to the values of $B$ calculated from the slopes of the trial lines. The slopes of the trial lines were changed until values of $\delta$ calculated by the two methods coincided. The method is quite effective since, eventually, the correct value of $\delta$ has to be arrived at. Of course, this method is useful only if the $B$ vs. $\delta$ relationship has previously been determined, making it useless for the variable ionic strength and pH series of runs.

From Equation 36, a plot of $\frac{\delta}{r-\delta} \cdot \frac{M_{BSA}}{C_{PVP}}$ vs. $\delta$ is indicated. A definite proof of the applicability of the theory of multiple equilibria to the systems studied here would be given if all experimental points on this plot were co-linear. As can be seen in Figure 25, this is not the case. Points calculated from given light scattering runs form straight lines having negative slopes as postulated. However, the slopes are not identical, nor do the lines intercept the axis of ordinates in the same place. Of course, the data obtained are very sparse, so that a complete study of these regions of the light scattering curves, making more concentration measurements, might
Figure 25. $\frac{r}{r-\delta} \frac{M_{BSA}}{c_{PVP}}$ versus $\delta$
enable one to say more about the usefulness of the method of multiple equilibria here.

Another possibility of error in these measurements was introduced by the impossibility of proper thermostatting of the light scattering instrument. However, this variance of temperature should not be over a few degrees, since the temperature is mainly determined by the heat leak in the central compartment from the light source on one end and the power supply on the other, which leak should remain nearly constant despite the room temperature.

The values of the intrinsic association constant obtained by this method vary around $K=5 \times 10^5$ liters/mole, while the apparent number of sites varies with the mixing ratio, being about 4 sites at $r=1.1$ and rising to about 11 sites at $r=4.0$. The above results are confirmed, trendwise, at least, by the theory developed in a later section which concerns the actual mechanics of the interaction.

E. Interaction Measurement in the Ultracentrifuge

One ultracentrifuge run was made on an interaction mixture (pH=5.0, $\beta=0.025$, $r=1.1$), as has previously been mentioned, to determine if any extremely high molecular weight complexes were present in the interaction mixture. An attempt was made to utilize this run in another manner; that is, a determination of values of the binding constant, $\sigma$, from the relative areas of the peaks appearing in the
ultracentrifuge pattern was attempted. It should be possible to determine \( \delta \) from the area of either the free BSA peak, or the interaction complex peak, if the initial concentrations of BSA and PVP are known, and the specific refractive index increments of the two materials are also known.

Relationships were developed for the calculation of \( \delta \) from these peak areas, and are as follows:

\[
\delta = \frac{A_6}{K \Psi_4 c_2^0} - \frac{\Psi_2}{\Psi_4}
\]

\[
\delta = \frac{c_4^0}{c_2^0} - \frac{A_4}{K \Psi_4 c_2^0}
\]

where \( A_i \) = areas of respective peaks on ultracentrifuge photographic plates

\( K = \frac{\text{instrument constants}}{\tan \theta} \)

\( \theta = \text{angle between the inclined bar and the horizontal plane in the Philpott-cylindrical lens optical system in the ultracentrifuge} \)

and all other constants are defined as in Equation 25.

The above relationships were used to calculate values of \( \delta \), but nothing of concrete value was obtained, since the total area of the two peaks did not give the correct value for the total concentration of polymer in the system. To get reasonable values of \( \delta \), one would have to use apparent values for the specific refractive index increments for the two polymers, which substitution is not
supported by theory. It has been postulated by other experimenters in similar cases that a fraction of the polymer material moved to the meniscus at the beginning of the sedimentation, thus lowering the concentrations of the respective components in solution. However, the above surmise has not been validated by experimental evidence. Another possibility of error arises from the concentration measurements, as well as possible changes in the optical constants for the ultracentrifuge. A resolution of the above difficulties would be very valuable, since that method for obtaining values of \( \delta \) is quite simple, experimentally.

F. Molecular Shape and Distribution

An attempt was made to fit the experimental \( \Phi_\Omega \) functions measured in the light scattering experiments to a theoretical curve. In studying the interaction complexes, the interaction run at \( (pH=5.0, \frac{c}{\nu}=.001, r=1.1) \) was used since all of the BSA was found to be complexed under those conditions. Previous investigations of similar systems had indicated that the molecules would be random coils, so theoretical values of \( \Phi_\Omega \) were obtained from tables given by Doty and Steiner (43) for both monodisperse coils and polydisperse coils of distribution \( z=1 \), and radius of gyration=506 Å, as calculated. As can be seen in Figure 26, the experimental curve varies quite radically from
Figure 26. Comparison of Experimental and Theoretical Particle Scattering Factors for PVP-BSA Interaction (pH = 5.0, $B = 0.001$, $r = 1.1$)
either of these curves. However, the $P_\theta$ (experimental), if calculated from the points obtained by extending the
\[
\left(\frac{c^n}{P_\theta}\right)_{c=0}
\]
line to large angles, exactly follows the theoretical $P_\theta$ curve for the polydisperse coil with $z=1$ and $R_g=506 \text{ ft}$. Also, the true experimental $P_\theta$ curve approaches theoretical plots for monodisperse coils of smaller radius of gyration at higher angles.

It was mentioned above that the theoretical curves plotted for polydisperse coils were for distribution $z=1$. $z$ is a distribution parameter introduced by Zimm (44). This parameter is used for describing the spread of molecular weights in a polydisperse system. For every $z$ value there is a corresponding mononodal distribution curve. When $z=1$, a distribution is represented in which $M_w:M_w:M_z::1:2:3$. However, if the distribution of molecular weights is binodal or unsymmetrical, no $z$ parameter can be logically assigned.

As was shown in Equations 20 through 24, it is possible to obtain an idea of the polydispersity of a system if the plot of $\left(\frac{c^n}{P_\theta}\right)_{c=0}$ vs. $\sin^2 \frac{\theta}{2}$ is curved. This phenomenon was found in all interaction runs and PVP runs and indicated some polydispersity. A plot of $P_{\theta}^{-1}$ vs. $\sin^2 \frac{\theta}{2}$, which is similar to a $\left(\frac{c^n}{P_\theta}\right)_{c=0}$ vs. $\sin^2 \frac{\theta}{2}$ plot, is shown in Figure 27 for both the PVP-BSA interaction at
Figure 27. Plot of $P^{-1}_\theta$ vs. $\sin^2 \frac{\theta}{2}$ for PVP (pH = 4.5, $\frac{r}{2} = .1$) and PVP-BSA (pH = 5.0, $\frac{r}{2} = .001$, $r=1.1$)
(pH=5.0, \( \frac{r}{2} = 0.01 \), r=1.1) and the pure PVP at (pH=4.5, \( \frac{r}{2} = 0.1 \)). The initial and asymptotic slopes are different in the two runs, since the \( R_G \) values are different. However, the ratio of slopes for each run is almost identical.

From the interaction, it was learned that \( M_N : M_w : M_z : 1:2.16:4.47 \), while the pure PVP is dispersed so that \( M_N : M_w : M_z : 1:2.08:4.26 \). So, the polydispersity is slightly more pronounced in the interaction mixture, which is to be expected since a statistical interaction is occurring. However, the difference between distributions is small enough to be attributed to experimental error.

For the interaction mixture mentioned above, the radius of gyration had previously been calculated to be 506 Å. This is a z-average value. Using Equation 23, we can also calculate the number-average radius of gyration. It was found that \( R_G(N) = 238 \) Å. So, the radius of gyration of a molecule having a molecular weight = \( M_w \) is a great deal smaller than that radius for the z-average molecule.

One other experimental fact pertinent to the present discussion was noticed in the sedimentation patterns for PVP. As can be seen in Figure 5, the peaks at all concentrations and at all times are slightly skewed. Unsymmetrical peaks are quite common in sedimentation patterns since the sedimentation constants for most materials are concentration-dependent to some degree, causing a sharpening of
the sedimentation peak on the solvent or slow-sedimenting side of the peak. However, the opposite effect is noticed in the PVP sedimentation patterns. This phenomenon suggests that the PVP possesses a distribution with a large amount of high molecular weight material, but that it also possesses a low-molecular-weight tail of molecules which sediment more slowly. This unsymmetrical distribution causes the curvature to appear in the Zimm plots of the light scattering data. The distribution was caused by the particular method of polymer fractionation which was used.

G. Treatment of Errors

An errors treatment was made on the calculation of molecular weight from light scattering data, considering errors to arise in the determination of \( G_\theta \) and \( G_0 \), the specific refractive index increment, \( \frac{dn}{dc} \), the change in index of refraction, \( \Delta n \), and the instrument constant for the light scattering instrument.

Since \( M = \frac{1}{K \cdot R_\theta} \) and

\[
c = \frac{\Delta n}{\frac{dn}{dc}} , \quad K = \left( \frac{dn}{dc} \right)^2 , \quad R_\theta = S \cdot \frac{G_\theta}{G_0} , \quad \text{thus} \quad Kc = \int \left( \frac{dn}{dc} \right) \left( \Delta n \right)
\]

and \( R_\theta = f(G_\theta, G_0 S) \), where \( S \) = an instrument constant.

\[
\frac{\Delta N}{M} = \pm \sqrt{\left( \frac{\Delta G_\theta}{G_\theta} \right)^2 + \left( \frac{\Delta G_0}{G_0} \right)^2 + \left( \frac{\Delta \left( \frac{dn}{dc} \right)}{\frac{dn}{dc}} \right)^2 + \left[ \frac{\Delta (\Delta n)}{\Delta n} \right]^2}
\]

\[
= \pm 0.0535
\]

\[
= \pm 5.4\%
\]
The per cent error in the calculated molecular weights of the pure polymers was found to be ±5.4%, assuming that the per cent error in \( \Delta n \) measurements was ±5%. This large error was estimated, since the error in \( \Delta n \) for solutions in order of magnitude higher in concentration was found to be ±5% by Brice and Halwer (65). Thus, the major part of the per cent error in the calculation of \( M \) results from the error in measuring the concentrations.

The per cent error in \( s \) was also treated and found to be 9.1%. In this treatment, it was assumed that the per cent error in molecular weights was 5.4%, as determined above. Using Equation 28, we can solve for \( s \) explicitly by the binomial formula. Since \( s \) is a function of \( M_2 \), \( M_4 \), \( \Psi \), \( \Psi \), \( r \), then

\[
\frac{\Delta s}{s} = \pm \sqrt{\left[ \frac{\Delta M_2}{M_2} \right]^2 + \left[ \frac{2 \Delta M_4}{M_4} \right]^2 + \left[ \frac{2 \Delta \Psi}{\Psi} \right]^2 + \left[ \frac{2 \Delta \Psi}{\Psi} \right]^2 + \left[ \frac{2 \Delta M_4}{M_4} \right]^2}
\]

\[
\Delta s = \pm 0.091
\]

\[
= \pm 9.1\%
\]

The error in measurement of the slopes for the purpose of obtaining \( K \) or \( R_0 \) was estimated to be about 10% experimentally. All per cent errors were of the same order of magnitude normally encountered in light scattering studies. Similar values of per cent error were obtained by Outer, et al., for the solutions of polystyrene. (77)
In a similar manner, errors in $K$, the intrinsic equilibrium constant, were estimated to be about ±10%, since, again, the concentration measurements provided the most important sources of error.
VII. DISCUSSION

The investigations just described may be divided into two parts. In the following sections, the data from the titration of BSA with \( \text{PVP} \) will be used as the experimental basis from which a theoretical model for the interaction will be deduced. The light scattering data will be interpreted in terms of this model.

A. Theoretical Models for the Interaction

An analysis of the information obtained in the preceding investigations leads one to believe that the interaction between PVP and BSA is almost entirely electrostatic in nature. The titration of BSA by PVP releases \( H^+ \) ions, as shown by the lowering of the pH with the addition of PVP in Figure 2. The total lowering of pH for any one concentration is an inverse function of the initial concentration of BSA, which reflects the buffering capacity of the protein molecule. The fact that the \( H^+ \) ions are replaced at all indicates an electrostatic binding in which the PVP site replaces the \( H^+ \) ion on the BSA.

A striking difference between the titration curves in Figure 2 and normal acid-base titrations is apparent, since the pH decreases linearly with added PVP, but shows different rates of lowering in four different regions of the curve, separated by definite break-points. An attempt has
been made below to develop a theory to explain these break-points and the regions between them.

The BSA molecule has been determined to be ellipsoidal in shape. It is generally considered to be a prolate ellipsoid with axial ratio of about four to one (76). X-ray studies have shown the BSA to possess a unit cell with dimensions 22x45x145 Å. Thus, some investigators have even considered that the molecules of BSA resemble prisms in appearance. However, as a first approximation, we shall consider the shape to be ellipsoidal, with the short axis being about 30 Å long.

The PVP molecule is a long chain which is randomly oriented in solutions of moderate ionic strength. In solutions in which the added salt concentration is very small or zero, the PVP molecule would be fully extended into a rod, which would be about 4000 Å long, if the molecule has a molecular weight of 380,000 as previously determined, thus containing 1570 monomer units.

The three break-points in the titration curves must indicate that quite abrupt changes in the interaction are occurring at these points. A likely approach to the interpretation of these breaks might be based on the geometry of the BSA molecule.

Since the protein molecule, in this case, contains one long axis and one short axis, the arrangement of BSA
molecules around the PVP backbone which would allow the most BSA molecules to come the closest to PVP would be an end-on contact, with the BSA molecules sticking out from the PVP molecule as spokes from an axis. Presumably, as PVP is added to the BSA solution, all the BSA molecules possible would go on to each PVP, saturating it. At the first break-point, all of the BSA molecules would be bound at one end to the PVP.

The second region of the titration curve, according to this hypothesis, would then represent the distribution of the adsorbed BSA molecules among the new PVP molecules. As BSA molecules are removed from the original PVP molecules, more sites on the PVP would become available to each BSA molecule remaining on the chain, causing a shift of the BSA molecules from an end-on attachment to a parallel attachment, since the free energy of the new arrangement would be lower than the end-on arrangement. Thus, at the second break-point, all BSA molecules would be parallel to the PVP backbone and would surround it like a cylinder surrounding a central axis.

Following the above reasoning, one would postulate that the third region of the titration curve represents the range of mixing ratios in which the sheath of molecules breaks up, until, at the third break-point, only one BSA molecule occupies a given segment of the PVP backbone.
However, at this point, the theory must be modified.

Plots of the mixing ratios at the break-points vs. the concentration of BSA may be used to calculate the maximum number of molecules of BSA bound by one molecule of PVP. These plots are shown in Figure 28, and resemble adsorption isotherms. All three curves have a common intercept of about $r=6.2$. The curves for $r_1$, $r_2$, and $r_3$, approach maxima of 122, 51 and 26, respectively. Thus, if one considers the $M_{PVP}$ to be $3.8 \times 10^5$ and the $M_{BSA}$ to be $8.22 \times 10^4$, the maximum BSA/PVP at the first break-point is 565, at the second break-point is 236, and at the third break-point is 120. The number of BSA molecules bound at the common intercept is 28.6.

The BSA molecule possesses a long axis of about 145 Å, and a short axis which is approximately 30 Å in length. The PVP molecule, at full extension, is about 4000 Å long. Thus, in the first region of the titration curve, 565 BSA molecules can be bound, in an end-on manner, if about four ends surround the PVP at a radius of about 15 Å. In the second region of the curve, 236 BSA molecules can be bound per PVP if eight molecules are bound in a sheath around the PVP chain and parallel to the PVP, at a radius of about 30 Å. Finally, 120 molecules of BSA can be bound if the sheath decreases in size to only four BSA molecules surrounding each PVP segment, thus allowing the BSA molecules to again come closer to the PVP; thus, the radius
Figure 28. Mixing Ratio at Break Points of Titration Curves vs. Concentration of BSA
would again become about 15 Å. The fourth region of the curve represents the redistribution of BSA molecules in the sheath-of-four, until, at about \( r=6.2 \), only one BSA molecule occupies a given length of PVP. Evidently the last radius, about 15 Å, represents the distance of closest approach.

The above theory correctly predicts the pH variation found experimentally, since, in the first region of the titration curve, the pH would drop rapidly as initial contact is made. In the second region, the pH would drop less rapidly, since, although more BSA molecules surround a given length of PVP, and more sites on each BSA are involved in binding, the radius of the sheath becomes larger, lowering the rate of \( H^+ \) replacement. In the third region of the titration curve, the BSA molecules come closer to the PVP, again lowering the pH. Finally, no more increase of \( H^+ \) is noticed in the fourth region of the curve, since the distance of closest approach has been reached, and the BSA molecules are merely being redistributed on the new PVP chains.

The plots of \( r_i vs. c_{BSA} \) may also be used for the determination of maximum and minimum numbers of groups on the PVP involved in binding one BSA molecule. However, these results are less important than the actual numbers of BSA molecules involved in binding.

The above titration method might be used to determine
dimensions of protein molecules, if the PVP dimensions were accurately known. Of course, the shape and size of the protein molecule affects the resulting titration curves. An example of this is given by titrations of ovalbumin (36,37) which show only two break-points. Ovalbumin also has an axial ratio of about four to one. This phenomenon may be explained by the possible inability of the ovalbumin to form two different sheaths, or by the possibility that egg albumin might be an oblate ellipsoid, in which case the probability of strong end-on binding would be lessened, since the large number of charged groups on the disk-like faces would cause a large repulsion to arise between ovalbumin molecules.

Plots of $r_i$ vs. $c_{protein}$ for ovalbumin titrations are linear and horizontal, showing a constancy of $r$ at both break-points. This difference undoubtedly arises from the fact that ovalbumin is bound quite strongly to PVP, while BSA is bound weakly, as shown by the light scattering work. This will be discussed later. Thus, during the titrations of BSA by PVP, not all of the BSA is adsorbed on the PVP, even at zero ionic strength, until the concentration of BSA is increased to a value where the $r_i$ vs. $c_{BSA}$ curves become horizontal.

Titration curves for spherical proteins could conceivably only have one or two break-points, depending on the size of the spheres, while curves for proteins
possessing three widely different dimensions could show four or more break-points.

The above description is demonstrably qualitative, but, nevertheless, gives a reasonable picture of the interactions investigated. Further titration studies on different proteins could possibly be quite valuable in the interpretation of protein phenomena.

All light scattering experiments were carried out on solutions with mixing ratios of BSA to PVP which were lower than the critical value of $r=6.2$. In addition, the solutions considered were of moderate ionic strength, where electrostatic shielding would cause incomplete interaction. Thus, it was assumed that all complexes investigated by light scattering were composed of PVP backbones, with BSA molecules being bound to the chain in a parallel fashion, and that only one BSA molecule could be found at any one place on the chain, corresponding to the fourth, horizontal region of the titration curves.

B. Interpretation of Light Scattering Studies

The postulate that the interactions studied were mainly electrostatic in nature was further substantiated by the light scattering investigations. The binding is quite dependent on the ionic strength and $pH$ of the system. When the ionic strength is increased at constant $pH$ and mixing ratio, the extent of binding decreases. This in-
dicates that the ion atmosphere shields the electrostatic attraction forces between BSA and PVP. When the pH is increased at constant ionic strength and mixing ratio, the extent of binding also increases. This effect is caused by the increasing number of negative charges on the BSA molecules as the pH is increased above the isoelectric point. This, in turn, causes a greater attraction for the positively-charged PVP molecules.

In the light scattering investigations, the PVP was found to be quite polydisperse. The size distribution is quite broad with the majority of the molecules having high molecular weights, but with a fair number of relatively small molecules included. The average molecular weights for PVP varied in the following manner: $M_H: M_M: M_Z :: 1:2.08:4.26$. The molecular weights of the complexes formed at complete interaction varied in a similar manner: $M_H: M_M: M_Z :: 1:2.16:4.47$. Thus, the distribution of molecular weights is not greatly affected by complexing. Further evidence of polydispersity was found in ultracentrifuge patterns of the PVP. The sedimenting PVP formed peaks which were quite sharp on the sedimenting or solution side, but trailed off on the solvent side. This phenomenon indicated that a large amount of low molecular weight material was included with the faster-sedimenting high molecular weight material.

The BSA is probably quite monodisperse but contains a
very small amount of material which sediments faster and moves more slowly in an electric field than the major component. This material is possibly either serum lipoprotein or denatured BSA. However, the small amount present of this material will not affect the interaction.

The interpretation of the light scattering data on the interaction involves explaining the separate effects resulting from the variation of the ionic strength, pH, and mixing ratio in terms of electrostatic considerations. The variation of binding ratio with ionic strength at constant pH and mixing ratio (Figure 7) is characteristic of electrostatic binding, where the binding ratio decreases over a range of ionic strengths and finally approaches zero at high ionic strengths. The effect is caused by the greater shielding of electrostatic binding forces at high ionic strengths, and also possibly an actual competition for sites on the PVP between BSA sites and small negative counter-ions. The latter possibility was added since Bsharah found, from electrophoresis and diffusion studies on PVP, that, even in water solution, a maximum of only 50 per cent of the Br⁻ ions associated with PVP as counter-ions are completely dissociated in solution (73). Thus, at high ionic strengths, the counter-ions would be bound to an even greater extent.

As the ionic strength is increased, the radius of gyration of the complex decreases, as does the molecular
weight. This effect was shown in Figure 12, where the square of the radius of gyration was plotted versus the molecular weight of the complex. Two effects are probably involved in this variation. The shielding effect of the ionic strength could cause a shrinkage of the complex, since the repulsion between PVP chains decreases at high ionic strengths. Also, the spatial effect of decreasing the molecular weight could cause the decrease in radius. However, the second effect is not as important as the first, as shown in later experiments in which the ionic strength was held constant.

The interaction constant, B, is a measure of the solute-solute repulsion. As the ionic strength is increased, B decreases, very rapidly at low ionic strengths, and then asymptotically approaches zero as a limit (Figure 13). This behavior is again suggestive of an increased shielding of electrostatic repulsion forces between complex molecules as the ionic strength increases.

The binding ratio varies linearly with the mixing ratio, as is shown in Figure 16. Since the ionic strength and pH are held constant, no electrostatic effects should be noticed. The variation of the binding constant represents the redistribution of BSA molecules on the PVP molecules as assumed previously in the flat portions of the titration curves.

The variation of the square of the radius of gyration
as the mixing ratio is varied is shown in Figure 17. $R_g^2$ varies linearly with the molecular weight of the complex, but grows smaller as the molecular weight increases. Again, two effects are possible. However, in this case, the effects oppose one another. The spatial effect of increasing the molecular weight of the complex should increase the radius of gyration. However, the increased number of BSA molecules in solution and on the PVP could act to shield the repulsion forces in the manner previously described for small counter-ions. Evidently the second effect outweighs the first causing the molecules to decrease in size.

The quantity $B$ was plotted versus $\omega$, the fraction of PVP in the complex, in Figure 18. $B$ varies directly, but not linearly, with $\omega$, as shown by the small curvature which is slightly convex to the axis of the abscissa. This curve resembles the high-$\omega$ region of a similar plot by Heilweil (37) for PVP-ovalbumin interaction. A linear variation of $B$ with $\omega$ was found by Morawetz and Gobran (74). Thus, as the proportion of PVP in the complex increases, the repulsion forces also increase.

The plot of the variation of binding ratio with pH at constant ionic strength and mixing ratio, as shown in Figure 19, shows a nearly linear increase of $\hat{D}$ with pH at low values of the pH. However, at high values of the pH, the binding constant approaches asymptotically the
maximum value possible, the mixing ratio. This dependence is reasonable, since, at high values of pH, the net charge on the BSA molecule becomes negative, causing the attraction exerted by the PVP molecule on the BSA to become greater.

The square of the radius of gyration remains fairly constant as the pH is decreased, despite the decreasing molecular weight, as shown in Figure 23. However, at a pH of 4.8, the radius of gyration suddenly increases. This increase may be caused by the fact that, on the low pH side of the isoelectric point of BSA, the BSA molecules acquire net positive charges. Thus, the BSA molecules no longer shield the repulsion forces between the PVP molecules, and the radius of gyration increases as a result.

Finally, the variation of B with pH at constant ionic strength and mixing ratio shows an initial high value for B at pH=4.8, corresponding to the complex having the abnormally high radius of gyration (Figure 24). This justifies the previous assumption that the inter-particle repulsions are large at this low pH. As the pH is increased, the repulsion forces between particles decreases, causing B to decrease. Corresponding to the effect of pH on the radius of gyration, squared, the curve at high values of pH is nearly linear and slopes off quite slowly.

An interesting phase of this series of investigations developed out of the relatively weak complexing of BSA by
PVP at high ionic strengths. That BSA often forms rather weak complexes was found by Fuo, et al., (3), and Geiduschek and Doty (31). The fact that Heilweil (36,37) found strong binding in PVP-ovalbumin complexes is interesting, since it indicates that the weak complexing in these studies is merely a function of the BSA molecule and not the PVP. To generalize, we will state that probably all protein-polyelectrolyte interactions are of a type describable by a multiple equilibria treatment, with the apparent strength of complexing being a function of the intrinsic association constant and the sensitivity of the association to electrostatic effects, such as ionic strength and pH.

The intrinsic association constants calculated from different individual runs varied among themselves with no particular trend showing up. This variation is probably due to errors in concentration measurements, or to the fact that temperature equilibrium might not have been reached in some of the concentration measurements. However, the number of interaction sites calculated from the various runs also varied. This variation did show a trend, with one exception. The values calculated were as follows: 4.4 at \( r=1.1 \), 7.0 at \( r=1.8 \), 13.9 at \( r=2.5 \), and 11.0 at \( r=4.0 \). The change in the number of sites with the mixing ratio might represent an extension of the PVP molecule when the competition for sites becomes great,
making more sites available for interaction. If one investigates the multiple equilibria expression when charge effects are being considered (Equation 37), one notices that these effects can cause a change in the apparent association constant calculated, as well as the number of apparent sites. This effect might explain the variation of the number of sites, as well as the fact that all values calculated were well below the theoretical minimum number of sites available, which was about 30 sites, calculated from the degree of polymerization of the PVP (1569) and the minimum number of PVP groups bound per BSA molecule, which was 56.
VIII. SUMMARY

Curves representing the titration of BSA by PVP showed several linear decreases of pH with the volume of PVP added, indicating that the interaction of PVP with BSA is mainly electrostatic in nature. These titration curves were used to formulate possible models for the interaction complexes under various conditions, and also to calculate the number of BSA molecules bound by PVP at the extremes of BSA concentration.

The methods of light scattering were used to determine the binding ratio of BSA to PVP in the interaction complexes, the radii of gyration of the complexes, and the second virial coefficient under various conditions of ionic strength, pH, and mixing ratio. The above measurements verified that the interactions were electrostatic.

Certain light scattering runs at high ionic strengths indicated that the BSA molecules were only weakly bound. This appeared in the \( \frac{c_T}{R_\theta} \) versus \( c_T \) plots, which showed negative slopes at low concentrations. A method was developed for calculating intrinsic association constants and values of the number of PVP sites available for interaction from these measurements. The intrinsic association constants obtained by this method varied with the mixing
ratio, but with no definite trend, possibly because of experimental error. However, the average value obtained was $5 \times 10^5$ liters/mole/site in solutions of pH=5.0, $\frac{P}{2} = 0.025$. The number of sites on PVP increased as the mixing ratio was increased.
APPENDIX

Reduced Scattered Intensities for PVP and PVP-BSA Mixtures Measured under the Experimental Conditions Indicated
### TABLE VII
PVP (pH=4.5, $\frac{c}{2}=0.01$)

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<th>$G_0$</th>
<th>$\frac{c}{R_0}$</th>
<th>$G_0$</th>
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<td>94.4 (13)</td>
<td>94.4 (13)</td>
<td>94.4 (13)</td>
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</table>

The numbers in parentheses after galvanometer readings indicate which neutral density filters were used. If no such number appears, the same filters were in place as last mentioned above in the column.
### TABLE VIII

**PVP-BSA (pH=5.0, r=0.001, r=1.1)**

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<th>Buffer</th>
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<th>( c_T = 4.189 \times 10^{-4} )</th>
<th>( c_T = 6.233 \times 10^{-4} )</th>
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Values of buffer readings are averages and are of about the same size as those used in correcting all L. S. runs for solvent scattering, so will not be repeated.
TABLE IX
PVP-BSA (pH=5.0, \( c_T = 0.025, r=1.1 \))

\[
\begin{array}{cccccc}
\theta & G_\theta & \frac{c_T}{R_\theta} & G_\theta & \frac{c_T}{R_\theta} & G_\theta & \frac{c_T}{R_\theta} \\
0 & 92.8 (1234) & 93.75 (1234) & 93.25 (1234) & 92.05 (1234) & 92.05 (1234) & 92.05 (1234) \\
30 & 64.3 (13) & 5.366 & 64.55 (23) & 4.645 & 97.1 (23) & 4.463 & 92.05 (1234) & 4.416 \\
37 & 42.95 & 6.237 & 48.5 & 4.763 & 73.3 & 4.566 & 45.0 & 4.578 \\
45 & 75.5 (3) & 5.789 & 77.9 (13) & 4.906 & 55.5 & 4.670 & 71.85 (23) & 4.719 \\
55 & 53.8 & 5.992 & 56.4 & 5.091 & 86.4 (13) & 4.850 & 52.25 & 4.912 \\
65 & 40.0 & 5.956 & 93.4 (3) & 5.221 & 66.95 & 5.005 & 87.45 (13) & 5.021 \\
80 & 68.8 (2) & 6.239 & 70.75 & 5.521 & 51.05 & 5.257 & 66.85 & 5.261 \\
90 & 64.0 & 6.450 & 65.9 & 5.677 & 47.4 & 5.417 & 62.25 & 5.404 \\
100 & 66.1 & 6.522 & 67.75 & 5.842 & 48.5 & 5.580 & 63.55 & 5.570 \\
115 & 79.9 & 6.764 & 81.2 & 6.137 & 58.0 & 5.860 & 76.4 & 5.808 \\
125 & 47.0 (3) & 7.192 & 47.0 (13) & 6.249 & 71.0 & 5.992 & 43.55 (23) & 5.958 \\
135 & 60.0 & 7.441 & 60.0 & 6.441 & 90.8 & 6.157 & 55.85 & 6.102 \\
\end{array}
\]
TABLE IX, (continued)
PVP-BSA (pH=5.0, \( \gamma = 0.025 \), \( r = 1.1 \))

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TABLE X, (continued)

PVP-BSA (pH=5.0, $\frac{c}{z}=.025$, $r=0.5$)

c\_\_\_\_T=9.196x10^{-4} \text{ ml}$
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BIBLIOGRAPHY


34. R. F. Steiner, Ibid., 47, 56 (1953).

35. R. F. Steiner, Ibid., 49, 71 (1953).


AUTobiography

I, David Edward Erickson, was born in Grand Island, Nebraska, on July 15, 1931, and spent the first five years of my life in a small Nebraska town near there. My family then moved to Shenandoah, Iowa, where I attended primary school. In 1943 we moved to Rapid City, South Dakota, where I obtained my secondary education. I enrolled in the South Dakota School of Mines and Technology in Rapid City in 1948 and obtained the degree of Bachelor of Science in Chemistry from that institution in 1952. During the summer of 1952 I was employed by E. I. du Pont de Nemours and Co. as a chemist in their plant at Belle, West Virginia. Since autumn, 1952, I have been enrolled in the Graduate School of The Ohio State University, where I held the position of Teaching Assistant in general and physical chemistry during the academic years 1952 through 1956, and the position of Research Assistant during the summers of 1954, 1955, and 1956. I was married to Dorice Elaine Schneider of Lancaster, Ohio, in June, 1956.