1

1.0

1.1

1.25

1.4

1.6

1.8

2.0

2.2

2.5

2.8

3.2

3.6

4.0

4.5

5.0

5.6

6.3

7.1

8.0

9.0

10.0

11.2

12.5

14.0

16.0

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22.5

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28.0

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LIGHT SCATTERING AND SPECTROSCOPIC
STUDIES OF AGGREGATION PROCESSES
IN A WELL CHARACTERIZED FULVIC
ACID

by

C

Alan William Underdown, B.Sc.

A thesis submitted to the Faculty of
Graduate Studies in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Department of Chemistry

Carleton University

Ottawa, Ontario

March, 1982

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Abstract

The proton and metal ion induced aggregation of a well characterized fulvic acid has been studied by light scattering and spectroscopic methods.

Fractionation experiments confirm the material to be chemically heterogeneous with decreasing numbers of larger molecular species. The largest fraction of smaller species, accounting for 70% of the sample, has a weight average molecular weight of 10^4 .

A pH dependent aggregation was observed which differed both qualitatively and quantitatively in the presence and absence of background electrolyte. Both hydrogen bonding between un-ionized acidic groups and screening of charge on ionized groups are necessary for aggregation. The increasing stability of Cu(II) fulvate complexes with fulvic acid concentration results from a co-operative effect between binding and aggregation which probably involves the proximity of carboxyl groups from separate molecules.

Scattering measurements at different angles were analysed using a two parameter log normal (ZOLD) distribution of spheres as a theoretical model. Accurate estimates of the distribution parameters were not possible with the present level of experimental precision due to their inherent sensitivity on the data. Comparison with theoretical aggregation models showed that more than one aggregating species is involved. Selective aggregation among the smaller species is induced by both Cu(II) and proton binding. The behaviour of light scattering with proton and Cu(II) binding suggests that the maximum

binding capacity coincides with a gel point region in which aggregates are linked together by minute increments in cation binding.

Fluorescent emissions at 480 nm and 520 nm were observed with excitation maxima at 370 nm and 465 nm respectively. Both proton and Cu(II) complexing quench the 520 nm emission more effectively than the 480 nm emission. Occupation of the stronger Cu(II) binding sites quenches more efficiently. Both emissions show a maximum in their pH dependence which is shifted to high pH with addition of background electrolyte indicating a different order of ionization of acidic groups.

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CHAPTER 1

The Nature of Humic Substances

The humic substances are a broad class of naturally occurring polymers which result from the modification of plant-derived organic matter by chemical and microbial action in soil.⁽¹⁻⁴⁾ Their structures are thought to contain both aromatic and aliphatic units linked together in an irregular manner by covalent linkages of varying strengths and hydrogen bonding in which several groups may participate. They are characterized by both a variation in physical and chemical properties in samples derived from different sources and within a single sample itself. In particular, properties such as optical emission and absorption spectra, particle size, the content and reactivity of various functional groups vary with the source of the sample and during sample reaction with a simple species such as H^+ , or metal ions.

In most studies, these samples represent the fractions of organic matter produced from a specific soil type and location (or natural water source) by any of several specific fractionation schemes. Regardless of the specific procedure, the fractions known as humic acid, fulvic acid and humin conform to the standard operational definitions which are detailed in Figure 1. Shown below the three fractions of soil organic matter are the trends in various properties among these fractions which have been verified by a considerable number of researchers.

Fig. 1. Classification of Soil Organic Fractions¹

The molecular structure of these materials is poorly understood, due, to their extensive heterogeneity. A large number of studies have involved chemical degradation followed by separation and identification of the degradation products. These investigations, have employed, oxidative methods such as treatment with alkaline permanganate (5), nitrobenzene (6), or cupric oxide (7), as well as reductive methods using sodium amalgam treatment (8), and distillations and fusions with zinc-dust (9).

— These methods, which yield mostly single ring aromatic phenol-carboxylate degradation products, have not resulted in an exact assignment of any humic molecular structure. This could be due either to the wide variety of monomers and linkages present or to ambiguities in interpretation where the polymer may be altered by degradation beyond replacing linkages with specific types of functional groups.

The comparison of degradation studies and upper estimates for the molecular weight of the simplest (non-aggregated) independent structural units of a well characterized fulvic acid shows (10) that it must be a mixture. In this comparison all of the products of mild degradation could not be included into a single unit with a molecular weight of approximately 10^3 . A similar conclusion was drawn by Christman and Minear (11).

In addition to these degradation studies, several methods have been used to analyse the type and number of oxygen containing functional groups. (12,13) These include tests, for total acidity, carboxyl group content, hydroxyl group content, both total and phenolic, and quinone and methoxyl group contents.

In spite of their failure to produce an exact molecular structure for the humic polymers (assuming a single one exists) the degradation and organic analytical methods have been valuable in providing an imprecise knowledge of structure which conforms reasonably well to most available experimental data and functions as a starting point in interpreting new experiments.

This knowledge can be summarized by the representative structure due to Gamble⁽¹⁴⁾, shown in Figure 2.

While organic structural analysis continues to provide valuable insights into humic molecular structure, there is considerable advantage to be gained in applying techniques which measure a weighted-average property of the whole sample. In principle, it should be possible to characterize all the distribution functions for any fraction and their interrelationships, including the formulation of conditional distribution functions. This can be accomplished by measuring properties which are differently weighted averages of each distribution. The next section reviews the recent progress in characterizing the most fundamental of molecular property distributions in humic materials, that of molecular weight.

FIGURE 2: REPRESENTATIVE FULVIC ACID MOLECULAR STRUCTURE

Studies on Molecular Weight Distributions in Humic Materials

There is considerable disagreement in the literature about most aspects concerning the molecular weights, shapes, and volumes of humic substances. Several reviews have appeared on this subject, (15,16,17) Most workers now agree that this variation is due to the following factors: a) differences in the sources of humic material and the extraction procedures used. b) differences in conditions such as concentration, pH, and ionic strength under which the measurements were made. c) differences in the bias peculiar to each method of measurement and d) differences in the type of average which each method measures on a distribution of molecular weights.

With regard to this last effect, most available evidence suggests that fractions of humic matter are polydisperse in that they possess a range of molecular weights. The term polydispersity, is also often used to indicate the variation in other molecular properties occurring in a sample. Implicit in this more general range is the fact that these other molecular properties such as functional group content or aromatic character are believed to correlate with molecular weight. (See FIG.1).

The problem of variation with source and extraction method is an unfortunate one which shows no signs of being alleviated. A systematic study of molecular size on a variety of humic materials using several techniques is sorely needed.

Errors which are peculiar to each method include lack of suitable

standards, for viscometry, solute-column interaction in gel filtration experiments, and the uncertainty in correction procedures for H^+ dissociation in colligative property methods such as vapour phase osmometry. Also, because our knowledge of particle shapes is, at best imprecise, sedimentation velocity and experiments involving the scattering of electromagnetic radiation cannot be interpreted completely rigorously.

Methods based on measurement of colligative properties will yield a number average molecular weight where each particle is counted with equal weighting regardless of size whereas light scattering and sedimentation velocity measure the weight-average molecular weight where each particle contributes to the average to an extent which is proportional to its actual mass. Viscosity measurements give a viscosity-average molecular weight which will always be intermediate between the number-average and weight-average values.

The number- and weight-average molecular weights are the first two members in a series of general expressions given by: (18)

$$\bar{M}_x = \frac{\sum n_i M_i^{(x+1)}}{\sum n_i M_i^x} \quad (1.1)$$

where n_i = number of molecules in the sample having molar mass = M_i .

The number-average molecular weight is given by Eq. (1.1) with $x = 0$:

$$\bar{M}_n = \frac{\sum n_i M_i}{\sum n_i} \quad (1.2)$$

The weight average molecular weight is given by setting $x = 1$:

$$M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \sum \omega_i M_i \quad (1.3)$$

where $\omega_i = [n_i M_i / \sum n_i M_i] =$ weight fraction of the i th type molecule in the sample.

It can be shown that the series of average molecular weights of successive orders is one way to specify the molecular weight distribution. The moments of the distribution can, in fact be expressed in terms of these averages. For example for the first and second moments of a distribution:

$$M_1 = \langle M \rangle = \bar{M}_n \quad (1.4)$$

$$M_2 = \langle (M - \langle M \rangle)^2 \rangle = (\bar{M}_w / \bar{M}_n - 1) \bar{M}_n^2 \quad (1.5)$$

The viscosity-average molecular weight, on the other hand, does not correspond to any of the aforementioned averages but is given by⁽¹⁹⁾

$$\bar{M}_v = \left[\frac{\sum n_i M_i^{(1+a)}}{\sum n_i M_i} \right]^{1/a} \quad (1.6)$$

where a is a function of both temperature and solvent and is related to the measured viscosity by the Staudinger Equation:

$$[\eta] = \lim_{c \rightarrow 0} \left[\frac{\frac{\eta}{\eta_0} - 1}{c} \right] = K M^a \quad (1.7)$$

where η = viscosity of the solution, η_0 = viscosity of the solvent.

The application of viscosity to the problem of particle size in humic matter has been reported in a number of studies including one

on essentially the same material used in the present study. It was found that the intrinsic viscosity, $[\eta]$, showed a minimum at a pH of 3 and at higher pH, was a decreasing function of concentration.⁽²⁰⁾ It was proposed that the slight increase in $[\eta]$ at higher pH was due to increased binding of water molecules to the highly charged molecules, although in a later paper⁽²¹⁾ it was suggested that homolytic bonding between free radical species may play a role. They applied their results to the Staudinger Equation (1.7) by choosing a to be 0.67 which is mid-range for a number of water-soluble organic polymers and by solving for K using an M value of 2,745 obtained from sedimentation velocity experiments.⁽²²⁾

By comparing their values of $[\eta]$ and M with theoretical calculations of $[\eta]$ for different shapes, they concluded that FA molecules were elongated with a major-to-minor dimension ratio of about 5 which changed little over the pH range from 2 to 8. These results seem to agree with those of other workers such as Reuter⁽²³⁾ and Visser⁽²⁴⁾ although Plaig and Beutelspacher⁽²⁵⁾ found no dependence of specific viscosity with concentrations and interpreted their results on the basis of globular shaped particles. It should be pointed out that in calculating major-to-minor axis ratios, none of these workers took the polydispersity of the system into account.

Another uncertainty in calculated particle shapes has recently been pointed out by Hayes,⁽¹⁶⁾ The ratio of experimental frictional coefficients to the theoretical values for compact spheres of the same molecular weight will deviate positively from unity because of both non-sphericity and expansion due to solvation. Studies which have calculated particle shape as a function of varying solution conditions have ignored possible variations in the degree

of solvation and estimate molecular volume from the density of the bulk solid. For a weak-acid polyelectrolyte, this is likely to be a physically unreasonable assumption.

Cameron et al,⁽²⁶⁾ found an approximately linear relationship between frictional coefficients obtained from ultracentrifugation and apparent molecular weights from gel filtration for a number of HA fractions. Such behaviour is consistent with a random coil structure having a spherical shape.

Ghosh and Schnitzer⁽²¹⁾, have recently reviewed the literature on particle shapes and suggested that much of the disagreement can be rationalized by assuming the humic molecules behave as spherocolloids at low pH and in the presence of neutral electrolytes. At higher pH, they behave like elongated rods according to the interpretation used. Such behaviour is consistent with a flexible linear polyelectrolyte.⁽²⁶⁾

Several techniques exist for measuring the molecular weights of polymer molecules, from their behaviour in an ultracentrifuge. In the sedimentation velocity technique, the molecular weight is calculated from the equation of Svedberg⁽²⁷⁾:

$$M = RTs/(1-\bar{v}\rho)D \quad (1.8)$$

where $\bar{v}$ is the partial specific volume of the solute, ρ is the solution density, D is the diffusion coefficient of the solute and s is the sedimentation coefficient calculated from the speed at which the solute/solvent boundary moves in the centrifuge cell.

In the equilibrium sedimentation method, the rotor speed is adjusted so that a steady state is reached in which the centrifugal force on a molecule is just balanced by the tendency for diffusion

under a concentration gradient. The molecular weight is calculated from the following equation:

$$M = [RT/(1-\bar{u}_p)] \left[\frac{dc}{dx} / w^2 \times c \right] \quad (1.9)$$

where w = angular velocity of the rotor, x = distance from the center of the rotor, $c(x)$ is the local concentration of solute molecules, and the other terms are as in equation (1.8).

Most of the studies of humic materials using the ultracentrifuge have employed the method of sedimentation velocity.

A molecular weight of 5893 has been reported by Schnitzer and Skinner⁽²⁸⁾, for the Armadale fulvic acid from this technique.

Several investigators have found that sedimentation velocity gives direct evidence of the polydispersity of humic materials. For example, Beutelspacher⁽²⁹⁾, interpreted his results on the basis of three fractions, the most abundant having a molecular weight of $3-5 \times 10^4$.

There was a small fraction of particles of much higher molecular weight which settled more rapidly and a straw yellow-coloured component which did not settle at all and was assumed to have a molecular weight of less than 1000. In theory, sedimentation velocity gives a weight-average molecular weight. In practice, however the situation is complicated by the fact that optical detection methods are used and the optical properties themselves are a function of humic particle size.⁽³⁰⁾ In addition, there is, at present, no way to estimate how aggregate sizes are altered in the presence of strong concentration and velocity gradients which occur during these experiments. Thus, care must be used in interpreting sedimentation velocity studies in which aggregation is studied as a function of such chemical variables as pH

and metal ion concentration as reported by Sipos et al., (31). The effects of velocity gradients could in principle also affect results obtained from viscosity studies. One use of the equilibrium sedimentation method which has been reported is the work of Posner and Creeth (32) on an unfractionated humic acid. They calculated $\bar{M}_n$ and $\bar{M}_w$ values from the data and hence estimated the polydispersity. They commented, however, that their results were not representative of the whole sample since the higher molecular weight components settled to the bottom and were not fully included.

Among the earlier studies of humic molecular weights were those employing vapour phase osmometry. This method, which relies on a difference in chemical potential between the solvent as a pure liquid and as a component of a polymer solution, yields the number-average molecular weight and is thus sensitive to low molecular weight components of a mixture. These components may be present in high concentration by number but low concentration by weight and hence will cause the $\bar{M}_n$ to be unrepresentative of the material on a weight fraction basis. Knowledge of both the weight-average and number-average molecular weight and hence $\bar{M}_w/\bar{M}_n$ provides a first-order approximation of the degree of polydispersity of a heterogeneous fraction of polymer molecules or aggregates. A V.P.O. study on the Armadale FA fraction reported by Hansen and Schnitzer (33), gave a number average molecular weight of 951. Care was taken in this study to correct for the dissociation of H^+ ions by using potentiometric data. The FA material was separated by gel-filtration into several fractions: the largest giving an $\bar{M}_n$ of 2110, represented 6% by weight of the total sample. Wilson and Webber (34) reported a value of 644 for a similar FA fraction which, while considerably lower, is in the same order of magnitude. On the

other hand, Wright et al⁽³⁶⁾ reported a value of 58,000 for the humic acid fraction extracted from the same podzol Bh horizon.

Studies of humic particle sizes using x-ray scattering techniques have been carried out by Wershaw and Pinckneff^(36,37,38). Data on the angular variation of scattering was analysed using the method of Guinier⁽³⁹⁾ which states that the log of scattering intensity will be proportional to $(2\pi \sin 2\theta / \lambda)^2$ with the proportionality constant equaling $\langle g \rangle^2 / 3$ where $\langle g \rangle$ is the radius of gyration, defined as the root-mean-square distance of all differential volume elements of the molecule, weighted by their densities, from the molecule's centre of gravity. For a polydisperse system, the Guinier curves will be non-linear and, in fact, slight curvatures were found for the humic solutions. Values of $\langle g \rangle$ were calculated, however, by treating the system as monodisperse. Results showed that particle size was greater at low pH and concentration, and in the absence of dissolved oxygen although no attempt was made to investigate the behaviour over a wide range of these variables. No mention was made of the use of filtration or centrifugation as possible pretreatment steps.

As a result of these studies, a generalized qualitative model of humic structure was formulated. The essential features of this model are the existence of more than one type of covalent unit which participates in both self- and mixed-aggregation resulting in a highly polydisperse mixture. The validity of applying this model to systems of low humic concentration has not been established since all of the scattering measurements had to be made in the range of several $g\ l^{-1}$. Proposed mechanisms for aggregate formation include hydrogen bonding of un-ionized carboxyl groups as well as charge transfer complexing, the formation

of ether linkages, and hydrophobic interactions.

Scattering studies using visible light have been limited to a handful including only one in the absence of heavy metal ions by Orlov⁽⁴⁰⁾. The molecular weights of HA fractions extracted from a sod-podzolic soil and a chernozem were measured to be 6.58×10^4 and 6.62×10^4 respectively.

Scattering measurements made during a titration with base and back-titration with acid showed that there is a reversible aggregation process which proceeds as the pH is lowered. No attempt was made to deal with absorbance and fluorescence effects and Orlov warns that a high concentration of inorganic matter, ~3%, may well have affected the results. Since scattering was measured at only one angle, 90° , possible effects of polydispersity and particle shapes could not be investigated. The remaining light scattering studies have been used to follow aggregation induced by metal ions such as Cu(II) and Pb(II).^(41,42) These studies have been performed without any of the above mentioned corrections and use only low instrumental sensitivity. Accordingly the data has been interpreted as describing a homogeneous solution up to the maximum binding capacity at which point "coagulation" occurs.

With the exception of Orlov's values for molecular weight⁽⁴⁰⁾, none of these studies have produced any information on the parameters governing the distributions of particle sizes.

As the application of light scattering to humic materials, the central issue of this thesis, is an almost un-tried technique, there are several theoretical and practical issues to consider. The underlying theory, including those aspects relevant to its application to humic substances, will be discussed in Chapter 3. As well,

suitable methods for data treatment will be developed. The practical aspects of the technique will be discussed in Chapter 2.

Gel filtration chromatography has been used extensively in the study of soil humic extracts and organic matter present in natural water. The underlying principle of the method is that the volume which a particular type of molecule must pass through in traversing the length of the column equals the column's void volume plus that fraction of the gel's volume through which the molecule can penetrate. The molecule will emerge from the column at an elution volume, $v_e = v_o + K(v_t - v_o)$ where v_o = void volume of column, v_t = total volume of column. It has been shown by Ackers⁽⁴³⁾ that K will be a function of the Stokes' radius of the molecule and the pore size distribution of the gel.

In order to minimize the effects of particle shape and solute-gel interaction, which are not accounted for in the theory, it is desirable to run a series of standards having a similar composition to that of the molecule under study and for which molecular weights have been determined by one of the absolute methods. Unfortunately no such series exists for the humic substances. Schnitzer found that molecular weights obtained by gel filtration are two to five times higher than those found using any of the other methods.⁽²⁸⁾ Gjessing⁽⁴⁴⁾ has shown that raising the pH or lowering the ionic strength increased the apparent molecular weight obtained by the method. He explained this result on the basis of polyion coil expansion under conditions of high charge and small screening effects from counter-ions. An alternative explanation is that the negative charge on humic molecules causes them to be excluded from regions of the gel which would be

accessible to them on the basis of molecular volume only. Raising the pH or lowering the ionic strength will increase the effective charge and hence aggravate the problem. This reasoning might also explain Schnitzer's observations on the discrepancy of results when compared to other methods as well as an experiment by Wershaw and Pinckney⁽⁴⁵⁾, in which they determined that the radius of gyration of molecules eluted at the void volume could not be explained on the basis of molecular exclusion alone.

In spite of continuing controversy over the use of gel filtration for the determination of humic molecular weights, the technique has proven itself a useful method of separating humic substances into fractions with less variability in composition. The technique could be exploited more fully by detailed examination of the properties of fractions produced under systematic variation of the conditions under which fractionation occurs.

Ultrafiltration membranes have also been employed in attempts at fractionation by molecular weight. Kwak et al⁽⁴⁶⁾, investigated the filtration of the sodium salts of fulvic acid and humic acid, from the same source as that used in the present study through a series of membranes having molecular weight cut-offs ranging from 10^3 to 3×10^5 . In all cases they found significant retentions of material whose molecular weights had been reported as lower than the specified molecular weight cut-off of the filters. Similar behaviour for other humic materials has been reported in the literature^(47,48). Buffle⁽⁴⁹⁾ has reported that retention is higher at lower pH, all other things being equal, and points to the aggregation effect as being responsible.

Relationship Between Particle Size Distributions and Chemistry In
Humic Substances

From the preceeding discussion it is clear that acidic dissociation and complexation with metals have a profound influence on humic substance particle size. This is of practical importance in a number of areas where particle transport is involved. Thus, the mobility of protons and metals in soils and natural waters will be controlled by such factors as particle size and surface charge on humic substances which will in turn be controlled by the extent of dissociation and metal complexation itself. In addition, humic particle size distributions will affect the outcome of man-made separation procedures including municipal water treatment and sample pre-treatment as occurs in agricultural and environmental testing laboratories.

Of considerable scientific interest is the effect which particle size will have on the apparent reactivity of a functional group of a given intrinsic reactivity.

In analysing this problem, one should keep in mind that neither the fraction of groups reacted nor the state of aggregation and/or conformation is truly the independent variable with the other one being the dependent variable. In reality, the independent variables relate to the composition of the system as specified by total metal ions, pH, total humic matter and temperature, etc. For a set of fixed values of these variables, the humic matter will adjust the extent of groups of each kind reacted, conformation, and degree of aggregation to minimize the free energy of the system as a whole. From a theoretical viewpoint, it would be interesting to know the variation of the apparent reactivity of each type of functional group with each parameter necessary to specify the tertiary structure (state of aggregation and/or

conformation) while holding the other parameters and the fractions of other types of functional groups constant.

To approximate these partial differentials, useful information on reactivity and particle size can be derived, by studying these properties over a wide range of the variables specifying total composition.

Considerable speculation about the effect of tertiary structure on chemistry has arisen in the interpretation of potentiometric studies. For example, Gamble⁽⁵⁰⁾, in commenting on the continuous decrease in the weighted average dissociation constant with the degree of ionization of the Armadale fulvic acid, raised the possibility that fractions of different molecular weight were successively dissociating. Measurement of the dissociation of this material at two different concentrations with the pH held constant indicated that the weighted-average equilibrium constant depends on hydrogen ion activity only and not fulvic acid concentration⁽⁵¹⁾. Except for fortuitous agreement, such an equality could only come about if a) the FA molecule has the same tertiary structure at both concentrations. b) The ionization constant for each type of group present is not sensitive to the molecule's tertiary structure. From a physical standpoint, this second assumption seems highly unlikely since tertiary structural changes will affect the charge density at a given degree of ionization which will in turn affect the electrostatic term's contribution to the apparent dissociation constant. Thus one is left with the remaining assumption that the molecule's tertiary structure does not change upon dilution. Such behaviour would be quite remarkable since many polyions are known to undergo expansion at lower concentrations.

Furthermore, lowering the concentration would be expected to shift any aggregation equilibrium present towards smaller particle size. As mentioned earlier, viscosity studies on this particular humic material and x-ray scattering studies on other humic acids indicate that particle size increases with decreasing concentration. In view of the apparent contradiction of these results and concepts, it was felt that a systematic study of the effects of concentrations as seen simultaneously by several different probes was needed. The results are reported in Chapter 2 of this thesis.

Potentiometric measurements recently made in our laboratory (52) on titrations of the Armadale FA fraction with copper (II) have shown that the maximum binding capacity and conditional stability constants are larger at an FA concentration of 0.100 g l^{-1} than at 0.0100 g l^{-1} . These investigations have also shown that the maximum binding capacity decreases with increasing ionic strength and decreasing solution pH. In addition, it has been verified that the conditional stability constants are decreasing functions of the fraction of sites occupied. In order to investigate the relationship between the above chemical results and tertiary structure, several titrations with copper (II) were carried out under identical conditions as the potentiometric studies but with average particle size in solution being measured by light scattering. The results of this investigation are reported in Chapter 4.

Weber has studied the complexation of both copper (II) and cadmium (II) by a fulvic acid similar to the one used in the present study at various pH's and concentrations. (53,54,55) His results indicate higher conditional stability constants with greater FA.

concentrations for cadmium but no difference for copper. He has postulated that the FA molecule has an expanded conformation at lower concentrations and this makes more sites available for cadmium. The lack of any concentration effects with copper (II) were explained by the fact that Cu(II) will complex with sites regardless of their availability based on molecular conformation.

Several studies have shown that particle sizes increase upon complexation with heavy metal ions or addition of large amounts of neutral electrolyte.

Viscometric studies by Stevenson⁽⁵⁶⁾ and Kribek⁽⁵⁷⁾ showed an increase in viscosity of humic solutions upon addition of heavy metal ions such as Cu(II) and Pb(II). These authors pointed to the possibility of covalent cross-linking. Recently Ghosh⁽⁵⁸⁾ has shown that while the relative viscosity of metal complexes of the Armadale FA fraction is higher at FA concentrations greater than 4.0 g l^{-1} , the intrinsic viscosity, $[\eta]$ is higher for the uncomplexed molecule. Since such increases upon dilution have been shown in many cases to be due to molecular expansion, these results were interpreted in terms of the complex being more rigid and hence undergoing less expansion.

Ong and Bisque⁽⁵⁹⁾ did a systematic study on the ability of electrolytes to coagulate uncomplexed humic acid and that complexed with Fe(III). The tendency to coagulate as determined by visual examination of series of solutions for turbidity, was much greater for the iron humate complex.

Their results also showed that the humic materials behaved like hydrophobic colloids in obeying the Shultz-Hardy rule⁽⁶⁰⁾, regarding the relative effectiveness of mono- and di-valent cations in causing aggregation.

Further evidence of hydrophobicity was provided by adherence to the prediction of the theory developed by Derjaquin, Landau, Verwey and Overbeek, regarding the effect of the hydrated cationic radius on effectiveness in inducing aggregation.^(61,62) (DLVO Theory).

Langford et al.⁽⁶³⁾ have used kinetic studies, filtration experiments and light scattering to show that the presence of the Armadale FA fraction causes an increase in particle size in a solution of precipitating hydrous ferric oxide at pH 6. Of particular interest was the observation that the humic material actually increased the speed with which iron would react with ferrozine, which forms an absorbing complex with iron (III).

Saar and Weber⁽⁴⁰⁾ as well as Pagenkopf and Whitworth⁽⁴¹⁾ have recently shown qualitatively that complexation with divalent metal ions causes an increase in particle size as seen by light scattering.

These latter authors performed titrations at pH 7.0 with a series of metals and found that the amount required to increase the intensity of light scattered at 90° to 50% of its maximum value, decreased the order Mg > Sn > Ca > Ni > Zn > Ba > Cd > Cu > Pb. This order was found to agree qualitatively with that of stability constants of these metals with ligands such as acetate and formate⁽⁶⁴⁾. The maximum increase in scattering, upon addition of excess metal ions was found to be roughly independent of metal ion. Although both effects have been suggested, it is not known whether this increase in particle size aids further complexation by entrapment within aggregates or whether complexation is subsequently hindered through the non-availability of sites within the interiors of aggregates.

Of central importance to the relationship between metal ion binding and aggregation is an understanding of the nature of the

bonding between metal and ligand and between one humic molecule and another. An obvious possibility is cross-linking by inner sphere complexing⁽⁶⁵⁾ of ligand sites from two different molecules to the same metal ion. This does not preclude, however, the operation of the mechanisms thought to cause aggregation even in the absence of metal ions. As these mechanisms such as hydrogen bonding, ether linkage formation and hydrophobic interaction are normally inhibited by the repulsion between similarly charged polymer molecules, one would expect them to become more effective upon charge neutralization through the binding of metal ions to the humic molecules. This will be true regardless of the mode of metal ion-humic binding. It is hoped that the present study, which employs particle size measurements on samples where proton and metal binding have also been measured, will help elucidate the inter-relationship between chemical reaction and aggregation..

Absorption and Fluorescence Spectroscopy as Auxilliary Techniques in Aggregation Studies

While the central aspect of this thesis is aggregation as seen by Rayleigh light scattering, there are at least two reasons why electronic spectroscopy could be used to provide auxilliary information.

Firstly, a correlation between chemical composition and the shape of the UV-visible absorption spectrum is known for humic materials derived from different sources. The absorption spectra of these materials, which are always more intense at shorter wavelengths

and show no distinct maxima, are thought to be due to the overlapping of transitions within the many types of chromophores present in the material. The slope of the spectrum, often reported as the ratio of absorbance at 465 nm and 665 nm, $E_{4/6}$, was interpreted by Kononova⁽⁶⁶⁾ as a decreasing function of the degree of condensation of the aromatic rings. The organic analytical work reported earlier in this chapter, however, tends to discount the presence of any significant proportion of highly condensed aromatic constituents. More recent work by Chen et al⁽⁶⁷⁾ has shown an almost linear decreasing relationship exists between the $E_{4/6}$ ratio and the intrinsic viscosity of both humic and fulvic acids derived from a number of locations. They showed that plots of $\log A$ vs $\log \lambda$ were almost linear and possessed a slope close to -4 in all cases. This is behaviour typical of apparent absorption by Rayleigh scattering rather than true absorption. While conceding that the relative contributions of scattering and true absorption in solutions of these materials are not known, they investigated the application of the equation derived by Doty and Steiner⁽⁶⁸⁾ describing the wavelength dependence of scattering from a solution of particles of maximum dimension λ :

$$\frac{d \log A_{\lambda}}{d \log \lambda} = 4 - \beta (1/\lambda) \quad (1.10)$$

Hence β is generally an increasing function of $1/\lambda$ due to the intra-particle interference effects explained in Chapter 3.

Remarkably, they found that the trend in β with intrinsic viscosity was consistent with a scattering mechanism. Furthermore, the correlation of $E_{4/6}$ with $[\eta]$ was found to be maintained, at least qualitatively in samples of the same humic fraction at different pH's. Significantly,

it was pointed out that there was a positive correlation between E 4/6 and the number of oxygen bearing functional groups in different fractions. Thus one of the goals of the present project is to establish the relative contributions of scattering and true absorbance in solutions of humic materials in order to gauge the validity of using equation (1,10).

The fluorescent emissions from humic substances have also been found to be decreasing functions of apparent molecular weight during fractionation experiments. (69,70,71) Stewart⁽⁷²⁾ showed that such relationships can, in principal, be used in environmental studies of dissolved humic materials. Earlier literature generally refers to one emission occurring anywhere from 455 nm to 540 nm with corresponding excitation maximum in the 355-365 nm range.^{73,74,75)} In fact, the emission maximum moves to higher wavelengths as the excitation wavelength is increased^(76,77). Such behaviour is consistent with that of a mixture of fluorescing compounds.⁽⁷⁸⁾ The variation which exists in the literature in the excitation and emission maxima is probably due to a combination of varying experimental conditions and sample origins.

Because the apparent molecular weight is affected by aggregation phenomena, it is not known to what extent the negative correlation of fluorescence with particle size is due to : segregation of non-fluorescing chemical structures within larger aggregates; quenching by aggregation agents which modify electronic structure; or quenching due to tertiary structural effects associated with environment of fluorescent chromophores in a large aggregate. The simultaneous study of particle size (through light scattering), fluorescent emission and the extent of reaction with protons and metal ions as reported in

this thesis, promises to clarify at least some of the ambiguities discussed above.

A second reason for considering absorption and fluorescence spectra in conjunction with light scattering studies is the possibility of using the responsible chromophores and fluorophores as internal probes to monitor the changes in molecular structure which accompany aggregation changes. For example, the dissociation of tyrosine phenolic groups to phenolate ions in proteins has been followed by absorbance changes in the 290-300 nm region⁽⁷⁹⁾. Dissociation of a specific type of functional group in different environments in a humic material could be followed by absorbance changes at different wavelengths. Indeed changes in the tertiary molecular structure of proteins such as the helix coil transition in poly-L-lysine have been followed by absorbance changes in the 190-210 nm region.⁽⁸⁰⁾

The absorbance behaviour of polymers containing chromophores associated with ionizing functional groups and those undergoing tertiary structural changes upon ionization can be affected subsequently. Thus it is worth considering alternative explanations to that of light scattering to explain the empirical relationship between the E_{4/6} ratios and apparent particle size previously discussed.

Fluorescence spectroscopy has been reported as a method to study metal ion binding to polymers such as Pt to DNA⁽⁸¹⁾ and recently several transition metals to a fulvic acid fraction.^(58,76) Such techniques have potential as analytical methods and possibly as means of studying polymer/metal ion binding from a fundamental viewpoint. However the fluorophores in humic acid are poorly characterized and spectral interpretations will be less quantitative. The fluorophores

are thought to be aromatic, and associated with electron donating functional groups.⁽⁷⁴⁾ Ghosh and Schnitzer⁽⁷⁷⁾ have noted the positive correlation of fluorescent intensity with free radical content in pH variation experiments.

One puzzling aspect of the absorbance behaviour is that humic substances almost universally obey Beer's Law over a wide concentration range⁽⁸²⁾. Such behaviour is related to the controversy described on p.18 between potentiometric and viscosity results. Assuming typical polyelectrolyte behaviour occurs and the humic molecules uncoil as their concentration is lowered, one would expect at least some of the chromophores to be sensitive to the changes in degree of solvation which accompany this process. In this regard, a recent study of zinc-porphyrin complexes reported by Reimer et al.,⁽⁸³⁾ showed that these materials, not unlike the humic substances, show deviations from Beer's Law which were explained in terms of aggregation effects.

Chapter Two

Methods and Materials

Fulvic Acid Preparation

The fulvic acid, used in the present study was extracted from the same soil under similar conditions as the Armadale FA1 batch which has been extensively characterized by Agriculture Canada (28,33,84). In each case, 2 kg of air-dried soil from the Bh horizon of a poorly drained podzol (The Armadale, Prince Edward Island, Canada) was extracted into 20 l of 0.5 M NaOH overnight with occasional shaking. Suspended solids were then removed by settling and centrifugation at 5000 rpm for ten minutes.

Further operations on the alkaline extract required several weeks to perform, during which time, it was repeatedly saturated with nitrogen gas and stored mainly in a dark cold room. The containers used for storage and extraction were large polypropylene jugs. Portions of the extract were passed through columns containing Dowex 50W-X8, 20-50 Mesh Size, cation exchange resin. The two columns used in succession were a roughing column, 1 m long x 8.5 cm in diameter, and a finishing column, 55 cm long x 4.6 cm in diameter. The flow rate for both columns was kept at about one drop per second. When the pH of the effluent from the roughing column rose above 7, the column was regenerated by 0.1 M HCl, followed by rinsing with doubly distilled water. The effluent from the finishing column was monitored by a flame test for Na^+ and required a second pass through the regenerated finishing column to give a negative result.

The ion exchanged solution was freeze-dried in a cabinet type freeze dryer in trays holding 2 l portions. The freeze-dried FA powder was subsequently stored in a tightly stoppered flask in the dark at room temperature. For experimental work, samples were weighed out from a vial containing several g FA which was stored in a dessicator in the dark. The ash content was determined gravimetrically to be $1.0 \pm 0.1\%$ of the sample by weight, in agreement with that of the FA1 batch. The levels of several metals as determined by graphite furnace atomic absorption spectrophotometry, are reported in Table (I). Comparison of the total metal content in mmol/g FA with the complexing capacity determined potentiometrically, shows a maximum of 2% of the complexing sites to be occupied. The higher Fe and Al content in Table (I) indicates that the residual metal content is due to the metals originally present in the soil rather than contamination during preparation. While Si was not analysed for, it is known that the 1% ash content is chiefly aluminosilicate mineral matter. Since this consists of colloidal particles with aluminium bonded to oxygen, the total content of complexed sites is probably much less than 0.12 mmol/g FA.

The carbon, hydrogen, and nitrogen content of a dessicated sample were analysed by Guelph Chemical Laboratories, Ltd and are tabulated below along with the corresponding values from the FA1 batch: (84)

	% C	% N	% H-
New Material	49.52	0.55	3.09
FA1	49.5	0.8	4.5

Table I

Metal Content of Fulvic Acid from Atomic Absorption

<u>Element</u>	<u>Concentration</u> (mmol g ⁻¹)
Al	1.95×10^{-2}
Cu	$< 10^{-4}$
Mg	3.7×10^{-3}
Fe	3.0×10^{-2}
Mn	$< 10^{-4}$
Cd	$< 10^{-4}$
Zn	$< 1.5 \times 10^{-3}$
Na	6.5×10^{-2}
K	$< 2.6 \times 10^{-3}$
TOTAL	0.12

Gamble⁽⁵⁰⁾ has pointed out that a useful method to characterize a mixture of weak acid polyelectrolytes is acidometric titration. In a careful study involving the statistical averaging of several titrations, he determined that the FA1 batch had equivalence points at 4.98 and 7.74 mmol gFA⁻¹. These equivalence points, determined using Gran's functions were reproducible in the present FA batch within 2%.

On the basis of the above results, it was decided that the present and FA1 batches are very similar materials. Comparison of the results obtained in the present study with the large body of data accumulated on the FA1 batch is therefore justified at least on a qualitative level.

Light Scattering Experiments

FA stock solution was prepared by dissolving 0.1000 g of FA powder in 100 ml. of water obtained from a millipore filtration system. It was then equilibrated overnight before using or filtering. Any stock solution older than one week was discarded although preliminary observations indicated that it was stable for several weeks. Solutions were prepared with Millipore water and reagent grade chemicals. All glassware was washed thoroughly with soap and water, rinsed, and soaked overnight in 6 M HNO₃. After rinsing several times with millipore water, burets and flasks were kept tightly stoppered and scattering cells inverted until use. Blank measurements were made on the millipore water used to prepare a specific set of samples. The addition of the maximum amounts of filtered titrants employed

in titrations caused the scattering from the blanks to increase no more than 3-6%. Since this scattering increase was less than 1% of the FA scattering signal, it was not corrected for. The FA stock solutions were filtered through polycarbonate filters directly into a buret with a pressure head of about six inches. The filters were soaked overnight in and flushed with millipore water prior to use to eliminate soluble impurities and stabilize pore size. The first 10 ml of FA solution was always discarded prior to collection of the remaining 90 ml. This was done to minimize the effects of partial fractionation by adsorption on the filters on reproducibility. In some experiments, the FA stock solution was not filtered although it was made up from millipore water. This was done to study the effect of the 1% ash content on aggregation processes. This ash content must be considered a part of the sample since it is intimately associated with the organic matter and is fairly reproducible considering that the FA is a soil fraction defined operationally by extraction conditions rather than a chemical compound.

Several methods of sample preparation were tried in order to maximize reproducibility and minimize artefacts. These involved either preparing separate samples at each set of pH, ionic strength, FA concentration, and metal ion content to be studied or changing one of these variables by a titration. The light scattering from metal-free FA solutions after several hours equilibrium near pH 7 was not reproducible to within an order of magnitude. Graphs of scattering intensity versus pH showed a continuous variation except

in this pH region where some points were off-scale. Viewing a laser beam through the highly scattering samples revealed visible turbidity. To avoid this problem, the data reported for concentration, pH, and ionic strength variation experiments were obtained on samples after a 20 minute equilibration time. The preparation schedule included the following steps:

- a) Solutions were made up to the required composition with filtered reagent solutions and millipore water. To avoid local excesses of reagent, the FA stock solution was added last before dilution to volume.
- b) Samples were purged for five minutes with N_2 gas passed through a millipore water scrubber and a cellulose depth filter.
- c) The sample was transferred to a scattering cell and covered with a tightly fitting teflon cap.
- d) After alignment, the scattering cell was allowed to stand in the dark for five minutes.
- e) The shutter allowing the laser beam through the sample was opened and the scattering intensity at specified angles was recorded.

The effect of equilibration time was studied at high pH where the reproducibility problem at long equilibration times did not exist. The resulting scattering variation, discussed in Chapter Six, is much smaller than that caused by pH variation.

The effect of Cu(II) on FA aggregation was studied using procedures which correspond closely to those used in our research group for potentiometric titration. Conditions were identical with respect to sample, titrants, temperature, pH and overall time.

The light scattering titrations, however, employed fewer additions of larger volumes of titrant. Titrations were carried out in triplicate on 100 ml aliquots of 10^{-1} g ℓ^{-1} or 10^{-2} g ℓ^{-1} FA solution placed in the scattering cell. The cell had a teflon cover with ports for a N_2 purge, a combination pH electrode, and titrant addition. This latter port was opened only momentarily to allow addition of titrant from either a buret or a syringe. The temperature was controlled to $25 \pm 1^\circ\text{C}$ by water circulating through a coil surrounding the upper portion of the cell and the cell holder. The titrations, which required about five hours to complete, were performed by following the sequence of operations given below:

- a) 100 ml of FA solution was adjusted to the required pH with 10^{-1} M NaOH. The solution was stirred by purging with N_2 .
- b) 0.50 ml of 10^{-2} or 10^{-3} M Cu(II) titrant was added dropwise with continuous stirring.
- c) The pH was readjusted to within ± 0.01 units of the specified value with 10^{-1} M NaOH and HCl.
- d) The N_2 purge was removed, and the scattering cell aligned and allowed to stand for five minutes in the dark.
- e) The shutter was opened and the scattering intensity at specified angles recorded.

Scattering measurements were made on a Brice-Phoenix Universal Light Scattering Photometer modified for use with a 5 mW Coherent Radiation helium-neon laser. The experimental set-up is shown schematically in figure (3). Data were acquired by measuring the

FIGURE 3. EXPERIMENTAL SET-UP
FOR LIGHT SCATTERING

output of a 1P21 photomultiplier tube with a Honeywell chart recorder. The PMT signal was recorded for three minutes at viewing angles of 0° , 45° , 90° , and 135° . Transient spikes due to macroscopic impurities were discarded. The PMT dark current was recorded between each sample measurement while equilibration of a new sample was taking place.

The signals at different angles, which varied widely in intensity, were kept on scale by inserting neutral density filters in the beam. The % transmittance of each filter was measured with the PMT at 0° scattering angle since the manufacturer specifications were not valid for the 633 nm He-Ne wavelength. The Rayleigh Scattering Ratio was calculated from the following formula:

$$R_\theta = K \left(\frac{\sin \theta}{1 + \cos^2 \theta} \right) \left(\frac{1}{(-R)^2 (1 - 4R^2)} \right) \times \quad (2.1)$$

$$\left[\left\{ \frac{G_\theta F}{G_0} \right\}_s - \left\{ \frac{G_\theta F}{G_0} \right\}_b \right] - 2R \left[\left\{ \frac{G_{180-\theta} F}{G_0} \right\}_s - \left\{ \frac{G_{180-\theta} F}{G_0} \right\}_b \right]$$

here K = instrumental constant

$$R = \left\{ \frac{\tilde{n}-1}{\tilde{n}+1} \right\}, \quad (\tilde{n} = \text{solution refractive index})$$

$$\left\{ \frac{G_\theta F}{G_0} \right\}_s, \left\{ \frac{G_\theta F}{G_0} \right\}_b \quad \text{are the recorded intensity ratios of}$$

scattering at θ and 0° for the sample and blank respectively. F accounts for the transmittance of the neutral density filters. The instrumental constant, K , can be calculated from known values of the beam cross-sectional area and the scattering volume. In the present case, it was evaluated to be 5.15×10^{-3} by applying equation (2.1) to

scattering measurements on doubly distilled benzene. The benzene gave a dyssymmetry ratio of 1.00 ± 0.01 indicating the absence of macroscopic scattering impurities.

The factor $\sin \theta$ is due to the variation of the scattering volume with angle of observation and the factor $(1 + \cos^2 \theta)$ accounts for the scattering of vertically and horizontally polarized components of the incident beam. The terms in R correct for the effects due to partial reflection at the solution-glass interface. After subtracting the blank, scattering intensities obtained from titrations were corrected for dilution.

Absorbance and Fluorescence Spectroscopy

UV-visible absorption measurements were made on a Cary 14 recording spectrophotometer using 1.00 cm quartz cells.

Fluorescence measurements were made on a Perkin-Elmer 204-S spectrofluorimeter with 1.00 cm quartz cells and detection of fluorescent radiation at 90° to the incident beam. The spectral band-pass of the excitation and emission monochromators was 10 nm. Sample preparation procedures were similar to those for light scattering experiments with unfiltered FA stock solution.

Fluorescence intensities were corrected for sample absorbance of the incident and fluorescent radiation by using the following formula:

$$F_{\text{CORR}} = F \frac{e^{1.151(A_{\lambda 1} + A_{\lambda 2})}}{1 + 1.02 d^2} \quad (2.2)$$

Here $A_{\lambda 1}$ and $A_{\lambda 2}$ are the absorbance measurements at the excitation and emission wavelengths on separate sets of samples. Because comparable formulas for absorbance correction of fluorescence are not mentioned in any of the standard texts,^(86,87) and appear rarely in the literature, the derivation of equation (2.2) is given in Appendix (A). The formula, which was derived independently, is similar to that of Leese and Wehry.⁽⁸⁸⁾

The wavelength dependence of the Xe arc light source and the PMT sensitivity were corrected automatically by the spectrofluorimeter. As no attempt was made to determine the absolute fluorescent intensity, only relative fluorescence values are reported.

Potentiometric Measurement

The pH during Cu-FA light scattering titrations was monitored using a Fisher 13639-92 combination electrode calibrated with the standard buffers and a Fisher model pH meter. Data on the fraction of bound Cu(II) as a function of total Cu(II) added were obtained from a parallel study in our research group. For this work, an Orion Model 94-29A Cu(II) ion selective electrode, calibrated with standard Cu(II) solution was used in conjunction with an Orion Model 701 digital ion analyser.

Potentiometric data corresponding to experiments involving pH, and ionic strength variation were obtained with a Metrohm AG 9100 Herisau combination electrode and the Orion meter. These measurements were performed on separate samples after the same equilibration time, 10 minutes, as used in the light scattering experiments. The combination

electrode was calibrated with HCl and NaOH standards with the appropriate KCl concentration just prior to use. The fraction of ionized acidic groups, α , was calculated as:

$$\alpha = \frac{(H^+) + (Na^+) - (OH^-)}{7.74 \times 10^{-3} C_{FA}} \quad (2.3)$$

Here C_{FA} is the fulvic acid concentration in $g\ l^{-1}$. In converting hydrogen and hydroxyl ion activities to concentrations, activity coefficients in the absence of background electrolyte were evaluated from the extended Debye-Huckel formula. For solutions with $10^{-1} M$ KCl background electrolyte, values due to Kielland⁽⁸⁵⁾ were used.

Gel Filtration Experiments

Approximately 20 g of Sephadex G-75 was allowed to swell in aqueous solution overnight. Suction was then applied to the suspension until no further air bubbles were released. After swirling, and left standing for 10 minutes, the supernatant was poured off to remove fine particles. This was repeated twice more after additions of 300 ml distilled water. The gel was poured into a 30 cm x 4 cm² column and conditioned for 24 hours by passing distilled water through it.

A 0.30 g sample of FA was dissolved in 15 ml of distilled water and left standing for 20 minutes with occasional stirring. This solution was applied to the column and eluted with distilled water under a 1 m hydrostatic pressure head. The flow rate was about 1 drop every three seconds. Samples were collected every 5 ml and stored at 5°C for 24 hours until the analysis.

After reconditioning the column for 24 hours with distilled water, the experiment was repeated.

Chapter 3

The Application of Rayleigh Light Scattering to Samples of Humic MaterialsPreliminary Experimental Problems

Three recent reviews on particle size measurements in humic substances devote a short section to light scattering^(15,16,17). These reviews particularly stress the potential use in characterizing molecular weights of humic polymers. While light scattering could be an extremely useful technique, many workers have been discouraged by numerous obvious difficulties including:

- a) The corrections needed to account for attenuation of both the incident and scattered beams in coloured samples. On many of the newer instruments, this tends to be difficult as they cannot view the incident beam at 0° through the solution.
- b) The problem of subtracting fluorescence from Rayleigh scattering since the detection element is a photomultiplier-tube with no wavelength discrimination. Correction procedures are available based on the difference in depolarization behavior, in fluorescent and scattered radiation.⁽⁸⁹⁾ They cannot be applied in all cases.
- c) The distribution of particle size and shape ranging from small molecules in true solution through the colloidal range to macroscopic particles.

Although the techniques leading to the operationally defined fraction in figure 1, tend to selectively remove larger particles, it is clear that the fulvic acids and humic acids contain a wide range of sizes including small quantities of suspended matter. The common operational definition for separation of suspension from solution in

geochemical samples is filtration through 0.45 μ pore size filters, hence the filtrates can contain colloidal material. To summarize, there is no certain procedure which separates the fulvic acid molecule from impurities analogous to the separation of proteins from cellular debris because of overlapping size distributions.

In the present experiments the problem of sample absorption (a) was solved immediately by equipping a Brice-Phoenix Scattering Photometer with a He-Ne laser light source. The incident beam, through suitable low transmissions filters, can be viewed at 0° through the actual solution and the observed ratio of scattered to incident radiation is automatically corrected, with a residual error no more than 2%. The 632.8 nm line of the He-Ne laser can satisfy this condition over a moderate sample concentration range because of its low absorption coefficient. A rigorous derivation of the correction appeared in appendix B.

The fluorescence problem, (b), was similarly solved by the use of the He-Ne laser. At 632.8 nm there was no detectable fluorescence from the most fluorescent samples used at a sensitivity which gave a full scale Rayleigh scattering signal. This was determined by measurements on a fluorimeter in which the Rayleigh signal was taken with both excitation and emission monochrometers set at 633 nm.

The problem (c) of absence of clear separation between the size distributions of humic polymer molecules and "impurities" was managed by performing experiments on samples filtered through different pore sizes as well as unfiltered samples. The details will be taken up in the chapters concerned with methodology and results.

Several other more subtle problems have emerged in interpretation of experimental data. These will be dealt with in the present chapter. They are best introduced by outlining the theory of Rayleigh light scattering and applying it to distributions of scatterers.

Light Scattering from Monodisperse Systems

When a molecule or particle scatters light causing no change in wavelength, energy is first absorbed from the oscillating electric and magnetic fields associated with the incident light beam. This induces internal electronic oscillations in-phase with the fields. The magnitude of these oscillations are governed by the polarizability tensor describing the molecule. The present discussion will limit itself to isotropic molecules or particles, that is, those in which all off-diagonal elements of this tensor are zero. These induced electronic oscillations correspond to a virtual excited state as often described in the theory of Raman spectroscopy and will decay by a first order process.⁽⁹⁰⁾ This decay, resulting mainly in the emission of radiation at the same wavelength as the incident light can be described adequately for the present purposes by classical electromagnetic theory as was done by Rayleigh⁽⁹¹⁾ in 1871 for dilute isotropic gases. This leads to the following equation:

$$i_{\theta} = \frac{8\pi^4 n}{\lambda^4} \left[\frac{i_0 V \alpha^2}{R^2} \right] \quad (3.1)$$

i_0 = intensity of incident radiation

λ = wavelength of incident radiation

V = volume of sample from which scattering is detected

n = # of scatterers/unit-volume

α = polarizability of scatterer; $P = \alpha E$

R = distance of detector from scattering volume

i_θ = intensity of radiation scattered at angle θ taken from the direction of the incident beam.

This equation applies to an incident beam polarized perpendicular to the plane defined by the intersection of the incident beam and the line from the scattering volume to the detector (vertically polarized). For an unpolarized incident beam, the right-hand side of equation (3.1) must be multiplied by $(1 + \cos^2\theta)$ to account for the scattered radiation from its horizontally polarized (in plane) component.

The most widely used quantity in presenting light scattering data obtained from polymer solutions is the Rayleigh Ratio: (92,93)

$$R_\theta = \frac{i_\theta R^2}{i_0 V} \left[\frac{1}{(1 + \cos^2\theta)} \right] = \frac{8\pi^4 n \alpha^2}{\lambda^4} \quad (3.2)$$

which will be independent of instrument conditions and scattering angle. This latter condition holds only for scatterers small relative to the wavelength of incident light. Otherwise intra-particle interference effects will make R_θ a decreasing function of θ . Equation (3.2) which applies to small, isotropic independent scatterers in a vacuum (94,95) can be adapted to molecules in solution where R_θ represents the excess scattering of the solution over that of the solvent and α corresponds to the difference in polarizability.

between the solvent and solute molecules. This polarizability difference can be related to measurable solution properties by the following equation: (94,95)

$$\alpha(\text{EXCESS}) \approx (2\pi n)^{-1} \tilde{n}_0 \left\{ \frac{dn}{dc} \right\}^2 c \quad (3.3)$$

where $\tilde{n}_0$, $\tilde{n}$ are the refractive indices of solvent, and solution respectively.

c = solute concentration in g cm^{-3} . Using the relationship that $n = N_A c / M$, ($N_A = 6.023 \times 10^{23}$, M = solute molecular weight) and combining equations (3.2) and (3.3), one can show that :

$$R_\theta = K M c \quad (3.4a)$$

$$\text{where } K = \frac{2\pi^2 \tilde{n}_0^2 \left\{ \frac{dn}{dc} \right\}^2}{\lambda^4 N_A} \quad (3.4b)$$

Two factors can operate to reduce R_θ from that given in equation (3.4). Interparticle interference due to non-zero temporal and spatial correlation functions can be eliminated by extrapolating results to infinite dilution. If the scatterers are of a size greater than about 5% of the wavelength of scattered light, radiation scattered from different points of the same particle will interfere destructively. This effect can also be eliminated by extrapolating results to zero scattering angle, at which in theory the interference vanishes. The simultaneous double extrapolation of scattering data to both zero angle and concentration is the well-known method of Zimm. (96) In practice, experimental limitations make it difficult to measure scattering at angles less than 30° . (97)

For most synthetic and naturally occurring polymers, the interference effect can be calculated by the Rayleigh-Debye Theory which will be outlined later in the chapter (98,99,100). The results are expressed by a modified form of equation (3.4b):

$$M = \frac{P^{-1}(\theta)R_{\theta}}{Kc} \quad (3.5)$$

where $P(\theta)$ is the particle scattering function relating the observed scattering to that which would occur in the absence of intraparticle interference. As the function $P(\theta)$ can be defined by scattering at any two complementary angles, for monodisperse systems, it has become customary to measure scattering at 45° , 90° , and 135° and calculate the dyssymmetry ratio : (94,98)

$$D_{45/135} = R_{45}/R_{135} \quad (3.6)$$

If the particle shape is known, equation (3.5) is employed to calculate M from the 90° scattering where $P^{-1}(90)$ is taken from tabulations of $D_{45/135}$, $P^{-1}(90)$, and ℓ/λ for the given particle shape. Here, ℓ refers to the major dimension of the particle. If particle shape is not known the results can be interpreted by successively assuming it to be that of a sphere, rod, coil, and disk and determining which pair of $\{M, \ell/\lambda\}$ is most physically reasonable. Systems which are sufficiently monodisperse to be treated by the above method include highly purified proteins and synthetic polymers which have been either highly fractionated or prepared by certain ionic reaction schemes.

Light Scattering from Polydisperse Systems

For polydisperse systems, such as the humic materials, the tabulations of $\{D_{45/135}, \ell/\lambda, P_{90}^{-1}\}$ are no longer strictly valid. Now both $D_{45/135}$ and P_{90}^{-1} are differently weighted averages of the distribution of particle sizes and the simple approach may no longer be a good approximation. The molecular weight can be obtained by extrapolation to zero scattering angle regardless of the polydispersity but the value so obtained is the weight-average one, $\bar{M}_w$, given by equation (1.3). A semi-quantitative measure of the polydispersity can be obtained by using this value along with the number-average value $\bar{M}_n$ in equations (1.4) and (1.5).

However, care must be used in relating $\bar{M}_w$ to the chemical properties of a polymer fraction. As an illustration, of this point, consider a hypothetical polymer mixture with the following composition:

# moles of each component:	10^{-3}	10^{-3}	10^{-3}
Molecular Weight :	10^2	10^3	10^4
Functional Groups/Molecule:	5	50	500

<u>Property</u>	<u>Measurement</u>
a) T_w TOTAL SAMPLE WT. = 11.1 g	gravimetric
b) T_F TOTAL FUNCTIONAL GROUPS = 0.555 mole	titrimetric
c) $\bar{M}_w = 9.10 \times 10^3$ g/mole	light scattering
d) $\bar{M}_n = 3.700 \times 10^3$ g/mole	V.P.O.

Such a mixture would be homogeneous to the extent that there would be one functional group per 20 Daltons on all molecules.

Quantities c) and d) were calculated from equations (1.2) and (1.3)

respectively but would in reality, be measured by the appropriate method. Estimation of the # functional groups/molecule by the common formula $f = T_F / (T_W / M)$ results in $f = 185$ for $M = \bar{M}_n$, $f = 455$ for $M = \bar{M}_w$. Clearly $\bar{M}_n$ gives a more reasonable answer; in fact it is the simple average one computes from the total number of molecules and functional groups. The value computed using $\bar{M}_w$ is a little less than that for the highest molecular weight fraction.

Such a calculation may be useful as a lower estimate of the functional group content of the highest molecular weight species but the amount by which it falls short of that value will depend on the actual shape of the distribution function.

From the preceding example, it is clear that for highly polydisperse systems as the humic substances are thought to be, $\bar{M}_w$ values must be used with care in interpreting chemical properties.

Evaluation of Molecular Weight Distributions

It is possible to use light scattering to obtain a more detailed description of the molecular weight distribution in highly polydisperse systems. The intensities of light scattered at different angles or from incident beams of different wavelengths are differently weighted averages of the distribution. The difference in weighting factors arises from the intraparticle interference effects already mentioned.

The Armadale fulvic acid fraction has been modelled in this work as a distribution of spheres. Similar behaviour is observed with hydrophobic, flexible, synthetic polymers in aqueous media. (98)

The observed scattering from a collection of non-absorbing spheres is given by:-

$$R_{\theta} = \frac{8\pi^2 n}{\lambda^4} \left[\frac{m^2 - 1}{m^2 + 2} \right]^2 \langle r^6 P_{\theta}(r) \rangle \quad (3.7)$$

where m = refractive index of sphere/refractive index of solvent.

The refractive index of absorbing spheres is complex with the absorption coefficient, k , being the imaginary part: $m^* = m + ik$. (101)

Equation (3.7) employs the real part m and the brackets denote averaging over the distribution of particle radii. Equation (3.7)

is similar to Equation (3.1) except that the substitution

$\alpha = \left[\frac{m^2 - 1}{m^2 + 2} \right] r^3$ has been made. (94) For a distribution of particle

radii $f(r)$, equation (3.7) can be re-written as:

$$R_{\theta} = \frac{8\pi^2 n}{\lambda^4} \left[\frac{m^2 - 1}{m^2 + 2} \right]^2 \int_0^{\infty} r^6 P_{\theta}(r) f(r) dr \quad (3.8)$$

Most particle size analysis procedures start with an assumed functional form for $f(r)$ which will be governed by a set of adjustable parameters $\{a_1 \dots a_n\}$. Then $P_{\theta}(r)$ is evaluated from light scattering theory. The right-hand side of equation (3.7) can be numerically integrated for any set of parameters $\{a_1 \dots a_n\}$. These results can be compared with experimental R_{θ} 's by computer search techniques to solve the equation: (102,103)

$$\sum_{i=1}^n [R_{\theta}(a_1 \dots a_n) - R_{\theta_i}^{\text{EXPT.}}]^2 = 0 \quad (3.9)$$

Equation (3.9) can be solved for the parameters (a_1, a_2, ρ) from experimental values of R_{45} , R_{90} , and R_{135} if the particle number, n , is known. If a direct measure of n is unavailable it can be eliminated by converting equation (3.8) to specific turbidity, R_θ/c using the substitution:

$$n = c / \left(\frac{4}{3} \pi \langle r^3 \rangle \rho \right) \quad (3.10)$$

where $\langle r^3 \rangle$ is the expected value of r^3 and ρ is the density of the solvated particle or molecule in g cm^{-3} calculated on the basis of weight of solute per volume of solvated particle.

If the refractive index of the particle or solvated molecule is not known, the specific index, m , in equation (3.8) must be determined by measurement of the differential refractive index increment, $\frac{dn}{dc}$, of the solution from the equation:

$$m = \left[\frac{4/3 \rho n_o \frac{dn}{dc}}{1 - 2/3 \rho n_o \frac{dn}{dc}} \right]^{1/2} \quad (3.11)$$

In order to evaluate n and m , it is necessary to know ρ . As this quantity is unknown for solvated humic particles, it must be evaluated as an adjustable parameter along with those of the particle size distribution. Assuming a minimum of two parameters, a_1 , associated with the moments of the particle size distribution, a computer search through three dimensional error space would be needed to find the set of (a_1, a_2, ρ) to satisfy equation (3.9). While such a procedure would provide simultaneous information on the particle size distribution and average composition of the particles or molecules in solution, it is not the most efficient way to analyse

the data obtained in the present study for several reasons.

In particular, the computer search techniques, while by far the most elegant methods, have been subject to problems such as apparent multiple solutions,⁽¹⁰³⁾ solutions of the theoretical parameters which are highly sensitive functions of the set of observable quantities, and round-off errors. These problems are amplified because uncertainties in the present data are greater than those seen in most light scattering studies in the literature (5-20% in this study vs. 1-5% in the literature).

One contributing factor to this problem is the necessarily lower solute concentrations (10^{-5} - 10^{-3} g cm⁻³) used in the present study compared with those employed routinely in experiments reported in the literature (10^{-2} - 5×10^1 g cm⁻³). For the case of molecular weight measurements in polymers, the lowest concentrations of samples used in the extrapolation to infinite dilution of R_θ/c are still higher than the highest FA concentration used in the present study. The conventionally high solute concentration in light scattering compared to other solution measurements is used to overcome the lack of specificity of the signal. All particles and solutes including impurities which may be quite large contribute to the scattered light⁽¹⁰⁴⁾. The FA scattering work was done in the low concentration range to avoid attenuation of the signal by absorbance at 633 nm and to allow experiments to be carried out at FA concentrations approaching values relevant to natural waters. ($\sim 10^{-2}$ g l⁻¹) (105.)

Other contributing factors are associated with variability in the FA scattering signal. Because the FA material is chemically

heterogeneous, there are limits to reproducibility of filtration procedures as well as to the aggregation process brought about by added reagents. The relative contributions of the above factors to the total uncertainty vary widely depending on sample composition and will be considered throughout the next three chapters.

Even the two parameter numerical search routines often encounter problems fitting data of much higher precision than those available in the current investigation. For this reason and since dn/dc measurements were not generally available to the program, it was felt that a 2 parameter graphical procedure would be more suitable for the initial quantitative attempt at interpreting the light scattering behaviour of a humic system.

These graphical procedures involve two experimental scattering values which are each ratios of scattering at two angles. As detailed later, this method avoids the uncertainties associated with the evaluation of ρ and hence m and n . The procedure, while not unique to the present study, ^(106,107,103) is far from routine and requires the generation of data from numerical integration. These data are not available from other studies in the literature. A further complication is that the application of light scattering theory involves the use of different approximations of $P_{\theta}(r)$ depending on the size of particle involved. ^(94,101)

Two parameter distributions in which the log of the particle radius is distributed normally can be used to successfully approximate the real shape of many naturally occurring particle distributions. The general form for these distributions is: ⁽¹⁰¹⁾

$$P_n(r) = C_n a^n e^{-\frac{1}{2}((\ln r - \ln r_m)/\ln \sigma)^2} \quad (3.12)$$

where C_n is a normalizing factor given by:

$$C_n^{-1} = \left(\frac{1}{2}\right)^{1/2} \ln a_n^{(n+1)} e^{\frac{1}{2}((n+1)\ln \sigma_n)^2} \quad (3.13)$$

The members of the series for which $n = -1$ and 0 have been used in the interpretation of light scattering data. They are the logarithmic normal distribution:

$$f(r)dr = \frac{1}{\sqrt{2\pi} \ln \sigma} e^{-\frac{1}{2}((\ln r - \ln r_m)/\ln \sigma)^2} \frac{dr}{r} \quad (3.14)$$

and the zero-order logarithmic distribution (ZOLD):

$$f(r)dr = \frac{1}{\sqrt{2\pi} \ln \sigma e^{(\ln \sigma)^2/2}} e^{-\frac{1}{2}((\ln r - \ln r_m)/\ln \sigma)^2} \quad (3.15)$$

The shape of the ZOLD distribution is shown in figure (4) for a single r_m and several values of σ . Both the skewness and width increase with the parameter σ . The log-normal distribution behaves similarly but peaks at successively lower values of r as σ increases while r_m is held constant. The physical significance of r_m is that half the population of spheres have r below this value. In the ZOLD distribution, r_m always corresponds to the maximum value of $f(r)$.

The log-normal distribution has recently been employed in the analysis of particle size in mineral suspensions by Yang and Hogg⁽¹⁰⁸⁾ and in on-line measurements of latex suspensions by Zollars⁽¹⁰²⁾. In both cases light scattering was determined by apparent absorption at different wavelengths. Some earlier reports of the use of the log-normal distribution in particle-size analysis actually involved use of the ZOLD distribution.^(107,109)

FIGURE 4. TYPICAL ZOLD DISTRIBUTIONS

Before discussing the determination of $P_{\theta}(r)$ from light scattering theory, it is worth deriving some useful properties of these distribution functions. Several of the important properties of a distribution of spherical particles will depend on the value of $\langle r^n \rangle$ where n is an integer. The brackets indicate averaging over the distribution. These properties include average particle diameter, surface area, and volume. These averages can be determined by considering the solution to the following integral involving the normal distribution¹⁰⁵:

$$\int_{-\infty}^{+\infty} e^{xt} \frac{1}{\sqrt{2\pi} s} e^{-\frac{1}{2}\left(\frac{x-\mu}{s}\right)^2} dx \quad (3.16)$$

$$= e^{\mu t + \frac{1}{2} t^2 s^2}$$

By making the following substitution:

$$\mu = \ln r_m$$

$$x = \ln r$$

$$s = \ln \sigma$$

$$t = n$$

it can be shown that:

$$\langle r^n \rangle = \int_{r=0}^{\infty} r^n f(r) dr = e^{(n \ln r_m + \frac{1}{2} n^2 (\ln \sigma)^2)} \quad (3.17)$$

Comparison of equation (3.14) with equation (3.15) shows that the log normal and ZOLD distributions are related by the following factor: $r/r_m e^{(\ln \sigma)^2/2}$

By combining this relationship with equation (3.17) one can calculate the value of r^n averaged over the ZOLD distribution as:

$$\langle r^n \rangle_{\text{ZOLD}} = r_m^n e^{\frac{1}{2}(n^2+2n)} (\ln \sigma)^2 \quad (3.18)$$

(See Appendix C)

The right-hand side of equation 2.9 can be evaluated if the function $P_0(r)$ is known. This function has been evaluated for spheres by Mie (111,112) in terms of an infinite series of J-type Bessel functions which correspond to the contributions to the scattering from the electric and magnetic multipoles of the particles. Extensive tabulations of these functions are available in the literature (112,113) so that one approach uses $P_0(r)$ as a function derived from curve fitting.

While the Mie theory is completely rigorous for spherical particles, a more convenient approximation of the interference effect was developed by Debye. The model is valid for particles which are not large relative to the wavelength of scattered light and possess a refractive index close to that of the solvent (98,99,100). Preliminary experiments measuring the angular variation of scattered light in a cylindrical cell at 5° intervals showed a continuous decrease in intensity with scattering angle. The absence of maxima and minima show that the scattering particles are not significantly larger than the wavelength of scattered light. Under these circumstances, the Debye theory is appropriate for solutions of organic polymers. (94)

The Debye approximation considers only electric and magnetic dipole oscillations from an infinite number of infinitesimal volume elements of the particle, that is, each element acts as a Rayleigh scatterer. (99,100) The function $P_0(r)$ is calculated

by considering interference effects between every pair of scatterers and averaged over all possible particle orientations.. The general result is:

$$P_{\theta} = \frac{1}{n^2} \sum_{i=1}^n \sum_{j=1}^n \frac{\sin \mu l_{ij}}{\mu l_{ij}} \quad (3.19)$$

where n = # of volume element in particle

l_{ij} = distance separating the i th and j th volume element

$$\mu = (4\pi/\lambda) \sin \theta/2$$

Equation (3.17) has been worked out for several different particle shapes including the case for spheres which is given by:

$$P_{\theta} = (3(\sin \mu r - \mu r \cos \mu r) / \mu^3 r^3)^2 \quad (3.20)$$

A computer program, which appears as appendix (D), was developed to evaluate equation (3.8) using equation (3.20) to calculate $P_{\theta}(r)$ and equations (3.14) and (3.15) to employ the log normal and ZOLD distributions respectively. It does not evaluate the functions of n and m which will cancel upon ratioing the scattering measured at two different angles. In comparing the theoretical and experimental results for a specific set of (r_m, σ) , without knowledge of either n or m , one can formulate the following two quantities as observables which will be functions of r_m and σ only. They are the ratios of light scattered at different angles as the observables of the system namely:

$$D_{45/135} = R_{45}/R_{135} \quad (3.21a)$$

$$D_{90/135} = R_{90}/R_{135} \quad (3.21b)$$

Families of theoretical curves for these quantities with r_m as the independent variable and each curve corresponding to a different value of σ were generated by numerical integration using Simpson's Rule. They are shown in Figures (5) and (6) in which the ZOLD distribution has been employed. The evaluation of (r_m, σ) from $(D_{45/135}, D_{90/135})$ can be demonstrated for a hypothetical sample in which $D_{45/135}$ and $D_{90/135}$ are measured to be 4.64 and 1.34 respectively. Pairs of (r_m, σ) which correspond to the measured value of each observable are read from figures (5) and (6) by moving along lines aa' and bb' respectively. The loci of points satisfying the measured values of each observable are plotted in figure (7). They intersect at $(r_m = 60, \sigma = 1.5)$, the values of the theoretical parameters which are consistent with both observables.

For this and several other hypothetical distributions examined, the theoretical parameters, r_m and σ , are extremely sensitive functions of the observable measurements, $D_{45/135}$ and $D_{90/135}$. In this respect, the present method suffers from the same type of uncertainties as those methods in the literature which determine (r_m, σ) by a computer solution of equation (3.9). The lack of precision in determining the theoretical parameters occurs whether the observable quantities are formulated from light scattering at different angles or at different wavelengths.⁽¹⁰¹⁾ Also, the large range of (r_m, σ) over which the $D_{45/135}$ and $D_{90/135}$ lines are close to coinciding is not the result of using ratioed scattering quantities as experimental data. In a recent study by Zollers⁽¹⁰²⁾ it was shown that the residual response surface generated by a two-dimensional

$D_{45/135}$

FIGURE 5.
CALCULATED DYSSYMMETRY RATIOS, $D_{45/135}$, FOR ZOLD DISTRIBUTION OF
SPHERES: VARIOUS b 's

FIGURE 6.
CALCULATED DYSSYMMETRY RATIOS, $D_{90/135}$, FOR ZOLD DISTRIBUTION OF
SPHERES: VARIOUS a 's.

FIGURE 7.
HYPOTHETICAL SOLUTION FOR SIMULTANEOUS VALUES OF $D_{45/135}$ AND $D_{90/135}$

computer search program using experimental data in the form of specific turbidities from a hypothetical log normal distribution had a gently sloping departure from the solution co-ordinates. The range of r_m and σ over which the residual function, equation (3.9), was extremely close to zero was comparable to the equivalent range of r_m and σ in figure (7).

Since the proposed data reduction method is inherently susceptible to experimental error, its application to a system with inherently large experimental error needs to be justified. The reason for its use is that only light scattering can give an estimate of the shape of a distribution of polydisperse labile aggregates without perturbing the system and hence changing the distribution. Such an estimate, even if only to within an order of magnitude, provides valuable complementary information to that obtained by other methods. Once developed, the method can be refined by cutting down on experimental errors through automated processing of a large number of samples. The data reduction may then be refined by switching to the computer search technique in which more than two parameters of the system can be evaluated.

In evaluating equation (3.8) by numerical integration, steps were taken to ensure that the result was not in error due to improper choice of either the limits of integration or the number of trapezoidal segments into which the area under the integrand curve was divided.

Detailed treatment of the properties of the log normal distribution, equation (3.14), has shown that 95% of the population is contained within the interval $(r_m/(1+\Delta) < r < r_m(1+\Delta))$ where ⁽¹¹⁴⁾

$$1 + \Delta = e^{4.513 \ln \sigma} \quad (3.22)$$

The choice of practical limits of integration in equation (3.8) is governed by the rapidly increasing contributions of increasing particle sizes to the total light scattering. Accordingly, the lower limit of integration was chosen to be $r_m/(1+\Delta)$. The upper limit was allowed to vary, being calculated by the function: $ZED \times r_m (1+\Delta)$ where ZED is an input parameter of the program. As ZED was increased, the number of trapezoidal segments, $2N$, was increased proportionately. Typical results for the ZOLD distribution, shown in Table (11), indicate that a ZED value of 2.0 is sufficient to achieve precision to better than 0.1% in the calculated results. For lower values of the parameter σ , a ZED value of 1.5 was sufficient to achieve comparable results. The behaviour of the log normal distribution, in which $f(r)$ is given by equation (3.15), was found to be similar.

Variation of the number of trapezoidal segments, $2N$, while holding ZED, and hence the limits of integration, constant resulted in a maximum difference of 0.05% when $2N$ was increased from 30 to 60. This % difference can be taken as an upper limit of the actual error incurred by using a finite number of segments since the actual error would be closer to a third of this value. (115)

The values of $D_{45/135}$ and $D_{90/135}$ used in analysing light scattering data in the present study were calculated using the ZOLD distribution with $ZED = 2.0$ and $2N = 60$.

A further complication in the interpretation of light scattering from aqueous solutions of humic materials remains to be discussed. Under such conditions they are partially dissociated and will behave as negatively charged polyelectrolytes. Most light.

Table II
Effect of Variation of Upper Limit of
Integration on Calculated Values
of $D_{45/135}$

ZED	r_m	1.7	1.8	1.9	2.0
1.0		2.29571	3.67684	5.99723	9.33412
1.5	20	2.52784	4.02472	6.34233	9.40475
2.0		2.55092	4.04337	6.34495	9.45481
2.5		2.55213	4.04369	6.35246	9.45652
1.0		4.17024	6.96908	10.74967	14.96185
1.5	30	4.32654	7.05785	10.78407	15.14994
2.0		4.32765	7.06582	10.79596	15.16751
2.5		4.32807	7.06671	10.79952	15.16943
1.0		6.74973	10.80766	15.54823	20.62177
1.5	40	6.78952	10.83553	15.68131	20.65935
2.0		6.79300	10.83959	15.69476	20.66399
2.5		6.79318	10.84079	15.69644	20.66512
1.0		9.90649	15.01134	20.53670	25.37215
1.5	50	9.91572	15.09595	20.56287	25.46191
2.0		9.91810	15.10176	20.56436	25.46378
2.5		9.91868	15.10187	20.56433	25.46623

scattering studies on polyelectrolytes are performed in the presence of neutral electrolytes as it is not clear whether the Rayleigh theory, as outlined in this chapter is otherwise valid.⁽¹¹⁶⁾ The estimation of accurate molecular weights using equations (3.4) and (3.8) is strictly valid only if the quantity $\tilde{dn}/dc$, and hence m , is determined when the polyelectrolyte with excess salt is dialysed against distilled water until the Donnan membrane equilibrium is reached.⁽¹¹⁷⁾

The method of particle size analysis developed in this chapter is based on the angular variation of scattered light rather than absolute scattering intensities. Hence it does not rely on the evaluation of $\tilde{dn}/dc$ or m . The particle sizes determined should be interpreted as corresponding to the solvated humic molecule or aggregate including its ionic atmosphere.

Chapter 4

Studies of Concentration Effects

Light Scattering Experiments

Before investigating the effects of protons and metal ions on the aggregation of the Bh horizon Armadale fulvic acid (FA) in aqueous solution, experiments in which the FA concentration varied were carried out. This was done primarily to learn if changes in tertiary structure (aggregation and conformation) occur and can be followed by light scattering, visible absorbance, and fluorescence spectroscopy. Previously reported studies of the concentration dependence of visible absorbance are at variance with viscosity studies⁽²⁰⁾ but in agreement with potentiometric results⁽⁵¹⁾. There are almost no reports in the literature on concentration dependence of light scattering on fluorescence.

Light scattered at 90° from the incident beam for unfiltered FA is shown in figure (8'). Runs on un-neutralized solution and with the pH adjusted to 7.0, as well as with FA filtered through 0.2μ at pH 7 are reported. In all three cases, no supporting electrolyte was present. These results, which are reported in arbitrary units, show a linear dependence of light scattering on concentration within experimental error. Similar results were obtained for light scattered at 45° and 135° as well and are reported in Table (VII). The experimentally accessible concentration range was controlled, at the lower limit, by sensitivity and, at the upper limit, by sample absorbance. The correlation coefficient, r , and standard error, S_E , obtained

FIGURE 8.
CONCENTRATION DEPENDENCE OF LIGHT SCATTERING, NO BACKGROUND ELECTROLYTE.

Table III

Concentration Dependence of Light Scattering*

No Supporting Electrolyte

C_{EA} mg-dl ⁻¹	pH ~ 3.6			pH ~ 3.6			pH = 7		
	NOT FILTERED			FILTERED, 0.2 μ			NOT FILTERED		
	45°	90°	135°	45°	90°	135°	45°	90°	135°
0	35.9	3.17	19.9	10.6	1.36	11.1	16.2	1.99	18.4
1.00	97.6	11.2	31.2	-	-	-	-	-	-
2.00	129	17.0	39.5	-	-	-	88.5	12.9	36.0
4.00	236	31.4	60.8	22.5	3.29	15.0	152	21.4	46.0
6.00	-	-	-	-	-	-	203	29.4	57.3
8.00	428	56.2	96.0	26.0	4.30	17.3	269	39.3	70.5
10.00	498	70.7	125	34.2	5.40	18.5	325	48.2	83.4
12.00	-	-	-	-	-	-	365	54.9	94.0
16.00	867	117	183	39.9	7.81	21.3	544	76.7	133
20.00	-	-	-	49.8	7.25	24.8	601	96.2	154
24.00	1120	159	255	49.2	8.92	27.3	-	-	-
28.00	1730	213	306	63.4	10.3	29.2	860	136	212
32.00	1740	219	335	-	-	-	1010	161	250
r	0.9890	0.9966	0.9995	0.9840	0.9823	0.9964	0.9984	0.9989	0.9982
S_E	101.6	7.19	4.11	3.30	0.61	0.57	18.7	1.33	4.66

* Arbitrary Units

from linear regression analysis are reported for each experiment.

In all cases fitting to second and third degree polynomials resulted in little improvement in r and no improvement in S_E .

The linear behaviour seen in figure (8) is a significant result in that it points to the absence or fortuitous cancelling out of three phenomena often seen in such experiments. The three phenomena, which occur in solutions of both uncharged and charged polymers (polyelectrolytes) are aggregational changes, conformational changes, and solute ordering through intermolecular interactions.

Aggregational changes in various types of macromolecules of biological and synthetic origin have been studied by the concentration dependence of light scattering. (118,119,120,121). Results are usually reported as an apparent molecular weight obtained by the use of equation (3.5), strictly valid at infinite dilution only, at finite solute concentrations. The present results strongly indicate that the function, K_c/R_θ , is constant in the absence of supporting electrolyte over the concentration range investigated. While the use of this function to evaluate accurate molecular weights in the present study is hazardous due to the uncertainties in evaluating K mentioned in chapter 3, the constancy of R_θ/c points to a lack of aggregational changes occurring. This can be due to the absence of aggregation or the maximum degree of aggregation being present over the concentration range explored. For the case of a closed association⁽¹²²⁾ where there is a limiting size to the aggregate, both these situations can result in concentration ranges in which light scattering behaves linearly.

In systems having no upper limit to the number of molecules participating in an aggregate, (open association),⁽¹²²⁾ the apparent molecular weight, or aggregate weight, is always a variable function of concentration.

The linear behaviour seen in figure (8) is in stark contrast to that seen in viscosity studies by Schnitzer's research group⁽²⁰⁾ and those of Smith and Lorimer.⁽¹²³⁾ These studies show that the reduced viscosity given by: $\eta_{RED} = (\eta/\eta_0 - 1)/c$, increases with solute concentration in aqueous solution. Such behaviour, caused by expansion of a flexible polyion upon dilution with subsequent loss of bound counter-ions, was observed over a concentration range of 1.0 to 10.0 g-l⁻¹. The results from both types of experiments can be reconciled by noting the concentration ranges over which each was performed. The polyion expansion, as seen by solution viscosity, takes place over a considerable range but is essentially complete at a FA concentration of 0.2 g-l⁻¹. This can be determined by examination of the behaviour of $(\eta_{RED})^{-1}$, as a function of $\sqrt{c_{FA}}$. According to theoretical work by Fuoss and Strauss,⁽¹²⁴⁾ these quantities should be linearly related for polyelectrolytes and this has been experimentally verified for a number of materials including the Armadale FA fraction.⁽²¹⁾ The extrapolated values of $(\eta_{RED})^{-1}$ at solute concentrations of 0 and 0.1 g-l⁻¹ differ by about 10% whereas the change over the total concentration range studied was more than 300%. Thus the light scattering results show that over the FA concentration range of 0.02 to 0.2 g-l⁻¹, where viscosity information is not experimentally accessible, the degree of

molecular expansion is not concentration dependent.

The final phenomenon which is known to cause plots of the type shown in figure (8) to deviate from linearity is that of solute ordering which has been shown to be due to attractive forces between solute molecules⁽¹²⁵⁾. These attractive forces, resulting from the charge separation between a charged polyion and its diffuse counter ion cloud are of a long range nature and are similar to the favourable interaction between permanent dipoles. While mainly observed in concentrated solution, the effect has also been seen in relatively dilute solution⁽¹²⁶⁾ where a dynamic equilibrium occurs between ordered and disordered regions. The present results show no evidence of solute ordering over the concentration range studied. This is not to imply, however, that molecular association due to short range forces is not present in the solutions investigated in figure (8).

A concentration study was carried out under conditions which preliminary experiments showed to favour a high degree of aggregation; those of low pH and high ionic strength.

Fig (9) shows c/R_0 , for light scattered at 45° , 90° , and 135° at pH 2.2 and $10^{-1}M$ KCl supporting electrolyte. This quantity, which is approximately proportional to reciprocal apparent weight average molecular or particle weight, shows a functional dependence on solute concentration which is characteristic of an open association equilibrium.⁽¹²²⁾ Here aggregates of all sizes are in equilibrium with each other and the non-aggregated species or unimer as shown by the following equation:

FIGURE 9.
CONCENTRATION DEPENDENCE OF c/R_0 ; pH 2.2, 10^{-1} M KCl BACKGROUND
ELECTROLYTE: ○ 45°C, □ 90°C, △ 135°C

The continuous decrease in c/R_0 with solute concentration, and the absence of a critical micelle concentration, is consistent with the above reaction scheme according to the theoretical treatments of a number of workers (127,128). Due to the chemically heterogeneous nature of the humic materials, it seems likely that the correct description of association actually involves a collection of reaction schemes like equation (4.1).

Of particular interest are the relative magnitudes of the equilibrium constants for association between chemically similar and different unimers. The present knowledge of the chemical structure of humic substances would indicate that an exact description of association in terms of a finite number of unimers is impractical. There are too many distinct unimers. The evidence for this statement is the large number of separation and structural analysis studies which have failed to yield any single molecular structure constituting a significant fraction of these materials. This does not imply, however, that the various functional groups and structural entities found in the degradation products of humic fractions are connected together in the parent mixture totally at random. Fractionation studies performed without prior degradation of the sample show that aggregates separated by apparent particle size have different physical properties even after equilibration. (28,69,70,71) This implies a non-randomness in both the structure of the unimers and in the way they are aggregated. It seems possible that if a sufficiently detailed description of the various conditional distribution functions describing unimer properties as a function of molecular weight were available, the open association

seen in figure (9) could be treated quantitatively. Such an analysis could be based on the following scheme:

where x_{ij} represents the j th property of the i th unimer in $(M)_n$ and y_j represents the j th property of the reacting unimer. In such a description of the material, the total concentration of a unimer having a given set of properties would be given as

$$C_{M(y_j; i=1, e)} = \int_{j=\lambda}^e p(y_j/y_{j-1} \dots y_1) p(y_1) C_T dy_1 \dots dy_e \quad (4.3)$$

Here $p(y_j/y_{j-1} \dots y_1)$ is the conditional probability distribution of property y_j given that each of the other $j-1$ properties are within the interval: $(y_{j-1} + dy_{j-1}, y_{j-1})$ and C_T is the total unimer concentration. The most suitable choice for y_1 is probably unimer molecular weight and the choice of the other properties would most likely include the various functional group contents. At present, a rigorous analysis is impossible since the required information on conditional probability distributions is not available. Even if this information were available, the large number of equilibrium constants needed for a complete description of the system may make the problem intractable. Before such an analysis is undertaken, it will be necessary to determine what simplifying assumptions can be made. In the meantime, it is important to be aware of the relationships described by equations (4.2) and (4.3). This realization increases our understanding of aggregation phenomena in the humic materials.

Comparison of figure (9) with concentration dependence studies in the literature is not possible as no experiments employing such a low pH, 2.2, have been reported. In this context, it should be pointed out that the present study employs these conditions to study the behaviour of a highly aggregated humic substance in the absence of strongly binding metal ions, not to duplicate the situation occurring in any aquatic or soil environment.

The only concentration study employing light scattering as a technique in the literature is that by Oslov and Gorskova.⁽⁴⁰⁾ They investigated the light scattering of podzol and chernozem humic acids in basic solution over a concentration range of 5 to $20 \times 10^{-4} \text{ g l}^{-1}$. In both cases, a linear dependence of Kc/R_{90} on concentration was found; the slope for the podzol being negative and that for the chernozem being positive. These small, constant, non-zero slopes in such light scattering data are usually caused by the non-ideality of the solution.⁽⁹⁸⁾ The high pH, extremely low concentrations, and high reported ash content of the samples $\sim 5\%$ implies that their experiments measured the scattering from very large inorganic particles. The authors, who reported no clarification procedures were aware of this problem and cautioned against indiscriminate use of the calculated molecular weights. The behaviour of the Rayleigh ratios of light scattered at 45° and 135° was qualitatively similar.

In figure (10), $D_{45/135}$ and $D_{90/135}$ exhibit a complex dependence on concentration including one minimum in the concentration range studied. In considering possible reasons why $D_{45/135}$ and $D_{90/135}$ should decrease over any range of experimental variable where the total scattering per weight of solute increases, one must deal with changes in particle

FIGURE 10.
CONCENTRATION DEPENDENCE OF R_{90} , $D_{45/135}$, and $D_{90/135}$; pH 2.2,
 10^{-1} M. BACKGROUND ELECTROLYTE.

shape as well as particle size.

While the Debye theory shows that particle shapes such as spheres, rods, disks, and coils of the same maximum dimension will produce different values of $D_{45/135}$, the tabulations of this quantity cannot be used to predict the changes in angular scattering behaviour when particles of a given weight change shape because such behaviour is accompanied by a change in maximum dimension as well. Equation (4.4), due to Guinier, indicates that the degree of angular variation of scattered radiation is an increasing function of the radius of gyration, $\langle g \rangle$.

$$\ln I = \frac{-h^2 \langle g \rangle^2}{3} + K \quad (4.4)$$

where $h = 2\pi \sin 2\theta$ and K is a constant. This relationship predicts that the dyssymmetry ratio, $D_{45/135}$, should decrease if a molecule or aggregate of fixed mass undergoes a transition to a more compact, less solvated conformation. Such behaviour is seen experimentally in polyelectrolyte solutions in which the addition of neutral salt causes a decrease in the radius of gyration.⁽¹²⁹⁾ Increasing the FA concentration could, in principle, cause the molecules to adopt a less expanded conformation as detailed in the discussion concerning figure (8).

At least two factors indicate that an explanation of the concentration dependence of $D_{45/135}$ and $D_{90/135}$ based on conformational changes is unsatisfactory. For one thing, at low pH and high ionic strength, one would expect the molecules and/or aggregates to adopt a compact conformation at any concentration due to their extremely

low effective charge density under these conditions. This is verified by the work of Rajalakshmi et al (130), and Smith and Lorimer⁽¹²³⁾ in which the effect of back-ground electrolyte on the concentration dependence of the viscosity of humic acid solutions was studied. Furthermore, in view of the increase in R_g/c as solute concentration is increased, it seems unlikely that conformational changes could outweigh changes in aggregate weight and cause a net decrease in the radius of gyration.

An explanation based on particle size alone is possible if one considers that the aggregation process involves more than one type of non-aggregated molecular species or unimer. The behaviour of particle weight distributions with the degree of aggregation when a single unimer is involved in open association has been treated theoretically^(127,128) and is similar to the problem of molecular weight distributions in condensation polymerization. Such behaviour is characterized by an increase in both the mean and standard deviation of the particle size distribution and a decrease in the total number of scattering particles as the degree of association increases. As will be shown on p. 90, the angular variation of scattered light, as characterized by $D_{45/135}$, is a monotonically increasing function of the degree of association for such a scheme.

The above constraints on the behaviour of the distribution moments with degree of association are relaxed if one recognizes the existence of more than one type of unimer undergoing aggregation. In this case it is possible that, as the degree of association increases, particle growth would take place mainly among the smaller particles

and increase their relative contribution to the total scattered light without much increase in their dyssymmetry ratio. In this context, inspection of figure (5) reveals that for particle size distributions with low values of r_m and σ , $D_{45/135}$ is a relatively less sensitive function of these parameters. The net effect on the observed measurement of $D_{45/135}$ would then be a decrease since it is a weighted average for which each particle's weighting factor is an increasing function of its size. This effect will be demonstrated for a hypothetical system containing two unimers on pp. 91-92.

To carry this reasoning further, it may be possible that under conditions which inhibit aggregation among some of the molecular species, a significant fraction of the sample exists as molecules so small as to not scatter light significantly more than that which occurs due to density fluctuations in the solvent. If conditions are changed to favour aggregation of these molecules, there would be a net increase in the number of colloidal particles scattering light from solution. The average particle size could actually go down as light scattering increases due to the effective concentration of colloidal solute increasing.

The evidence supporting the presence of a significant amount of low molecular weight, more or less non colloidal species in at least some humic fractions include the low values of number average molecular weight reported from vapour phase osmometry experiments⁽³³⁾ and the presence of a pale yellow colour remaining in solution after sedimentation velocity experiments.⁽²⁹⁾

The procedure for particle size analysis developed in Chapter 3 was applied to the data shown in figure (10) for several solution

concentrations.

The effect of experimental error was accounted for by obtaining two sets of (r_m, σ) values, corresponding to the measured value of the dyssymmetry property $\pm$ the estimated uncertainty. In all cases, a unique set of (r_m, σ) was not obtained. Typical results are shown in figures (11), (12), and (13). In figures (11) and (12) which correspond to an FA concentration of $0.0100 \text{ g } \ell^{-1}$, and $0.0200 \text{ g } \ell^{-1}$ respectively, the regions derived from the measured values of $D_{45/135}$ and $D_{90/135}$ intersect over considerable ranges of r_m and σ . In figure (13), corresponding to $C_{FA} = 0.0400 \text{ g } \ell^{-1}$, there is no intersection of the two regions over a range of r_m going from 2 nm - 120 nm. Results similar to those in figure (13) were obtained at concentrations between $0.0400 \text{ g } \ell^{-1}$ and $0.200 \text{ g } \ell^{-1}$.

The intersection of the regions corresponding to the two experimental scattering ratios over large regions of the theoretical parameters can be explained on the basis of two factors. These are the extremely high sensitivity of the solution (r_m, σ) to the experimental quantities $D_{45/135}$ and $D_{90/135}$ and the relatively large uncertainties (about 5%) associated with these experimental values. The sensitivity problem was discussed in Chapter Three in connection with the behaviour of diagrams of the type shown in figures (11) and (12) for hypothetical distributions of spheres. As well, the factors leading to the observed levels of experimental uncertainty were discussed. Thus the lack of a definite solution can come about for the above reasons even if the sample is accurately represented by a ZOLD distribution of spherical particles.

FIGURE 11.
 ATTEMPTED SOLUTION OF ZOLD DISTRIBUTION PARAMETERS: $C_{FA} = 10^{-2} \text{ g l}^{-1}$,
 $\text{pH} = 2.2$; 10^{-1} M KCl BACKGROUND ELECTROLYTE

FIGURE 12.
ATTEMPTED SOLUTION OF ZOLD DISTRIBUTION PARAMETERS; $C_{FA} = 2 \times 10^{-1}$
 g l^{-1} , $\text{pH} = 2.2$, 10^{-1} M KCl BACKGROUND ELECTROLYTE

FIGURE 13
 ATTEMPTED SOLUTION OF ZOLD DISTRIBUTION PARAMETERS: $C_{FA} = 4 \times 10^{-1} \text{ g l}^{-1}$,
 $\text{pH} = 2.2$, 10^{-1}M KCl BACKGROUND ELECTROLYTE

In figure (13), however, there is no indication that the regions corresponding to the two dyssymmetry measurements lying virtually parallel to each other have any tendency to converge over the ranges of r_m and σ investigated. It was decided not to extend these ranges in looking for a solution mainly for two reasons. Extension to higher r_m values would require using the Mie theory in which P_0 is a function of both r and the refractive index ratio m . Accurate calculation of m requires knowledge of the particle density, p , and dn/dc at each solution composition. (See equation 3.11). As previously explained, this was not possible in the present case. The extension of r_m to values lower than 2 nm with a corresponding increase in σ is possible but the extremely small angle of intersection between the regions corresponding to the two dyssymmetry ratios would make such a solution extremely imprecise. This increasing uncertainty as r_m decreases and σ increases results from the greater number of smaller molecules present. These molecules contribute proportionately less to the experimental observations.

Inspection of figures (11) and (12) shows that the intersection of these two regions occurs at lower r_m and higher σ values as the degree of association increases. In figure (13) there is no region of intersection but over the whole range of r_m and σ plotted, the $D_{45/135}$ region occurs at higher r_m than the $D_{90/135}$ region for a given value of σ . Inspection of figure (7), for a hypothetical distribution, shows that the $D_{45/135}$ region will be above the $D_{90/135}$ region for all values of r_m and σ for which the following inequalities are satisfied:

$$r_m > r_m (\text{TRUE})$$

$$\sigma < \sigma (\text{TRUE}).$$

(4.5)

In the range of r_m and σ for which the $D_{90/135}$ region is above the $D_{45/135}$ region, the opposite inequalities hold. This consideration of all three figures in sequence supports the conclusion that as the degree of association increases, the distribution of particle sizes responsible for light scattering shift to lower r_m and higher σ value. As will be shown in the next section, the open association of a single unimer is characterized by regions where r_m is both an increasing and decreasing function of the degree of association. Thus a falling r_m value as aggregation increases is not proof of a multiplicity of unimers being present. However, it will be shown in the next section that a multiplicity of unimers is required to have a falling dyssymmetry ratio while the degree of association increases.

In considering the applicability of a ZOLD distribution of spheres to the data, deviations of two types should be mentioned including those relating to particle shape and those relating to the nature of the particle size distribution.

With regard to particle shape, it should be mentioned that the assumption of a spherical shape was made as an approximation to render the problem tractable. This was done with the knowledge that the literature contains conflicting evidence on particle shape in humic substances, most of it based on indirect calculation employing the assumption of monodispersity with respect to particle size.

2

distribution analysis using different experimental techniques and the results are quoted as "equivalent spherical radii." (62,108)

The assumption of a single-peaked or unimodal distribution of particle radii was made, again, to make possible the estimation of the shape of the distribution from the observation of a limited number of experimental variables. As mentioned previously, the use of distribution functions in which the logarithm of particle radius follows a normal distribution has been successful in many cases. In particular, Willis⁽¹³¹⁾ showed that a ZOLD distribution could give a good approximation, as evidenced by large overlap, of a known distribution having two maxima. It was found that a second maximum can appear in an initially unimodal distribution of liquid droplet sizes in an aerosol. The second maximum developed as the result of coalescence of droplets, an observation which is particularly relevant to particle size analysis in humic materials. Gel filtration studies have, depending on sample origin and elution conditions, resulted in two and even three maxima in the relative concentrations of material separated by apparent molecular weight. (28,44,132) This does not constitute proof, however, that multi-peaked distributions of particle sizes were present. Solute-column interactions and possible shifts in aggregation equilibria make unambiguous interpretation impossible. In discussing the fitting of single distribution functions to real distributions having two maxima, Kerker points out that such methods would probably fail to find a solution if the maxima were spaced far enough apart. (101)

Another possible deviation from the model relates the use of a single parameter, σ , to account for both the width and skewness

of the distribution. Although these quantities would be expected to increase together for the association of a single unimer⁽¹³³⁾, evidence has already been presented which indicates that the association in the FA material is more complex. In particular it was shown that there is a strong likelihood of selective association among the smaller molecules being important. This could result in the width of the distribution increasing while at the same time the skewness would decrease or possibly change sign.

Light Scattering From Hypothetical Open Associations

While a rigorous analysis of the FA aggregation using equations (4.2) and (4.3) is not possible, the angular dependence of scattered light from specific aggregation schemes can be calculated theoretically. Comparison with experimental results will hopefully give some indication of the nature of the FA aggregation.

Consider an open association between identical unimers each having f functional groups of equal reactivity. Functional groups from different unimers can combine to form aggregates of the type:

Here the -AA- linkage may be taken to represent two hydrogen bonded carboxyl groups:

By assuming all groups have equal reactivity and neglecting intramolecular bonding, it can be shown that the number and weight average degree of polymerization are respectively⁽¹³³⁾:

$$\bar{x}_n = (1 - \gamma f / 2)^{-1} \quad (4.6)$$

$$\bar{x}_w = \frac{1 + \gamma}{1 - \gamma (f - 1)} \quad (4.7)$$

where γ is the fraction of groups involved in hydrogen bonding. We will examine the scattering behaviour of a ZOLD distribution having $\bar{x}_n$ and $\bar{x}_w$ which vary with γ according to equations (4.6) and (4.7). It can be shown by using equation (3.18) that the parameters of such a distribution are given by: (See Appendix E)

$$r_m = \frac{\bar{X}_n^{0.6111} \cdot r_u}{\bar{X}_w^{0.2778}} \quad (4.8)$$

$$\sigma = \exp\left[\frac{1}{3}(\ln \bar{X}_w / \bar{X}_n)^{1/2}\right] \quad (4.9)$$

Here r_u is the radius of the unimer which will be set equal to 20 nm for the present.

The behaviour of $\bar{X}_n$, $\bar{X}_w$, r_m and σ vs. γ are shown in figure (14) for $f = 3$. The sharp rise in $\bar{X}_w$ as γ approaches 0.50 corresponds to the well known gel point in the polymerization of a polyfunctional unimer. At this point, $\bar{X}_w$ is infinite since the largest aggregates reach macroscopic dimensions. $\bar{X}_n$ remains finite, however, because of the presence of a large number of small aggregates. The decrease in r_m after $\gamma = .3$ is initially surprising until one considers that r_m is one of two parameters describing the distribution and does not correspond to any average property. The dyssymmetry quantities $D_{45/135}$ and $D_{90/135}$ which were obtained from r_m and σ using figures (5) and (6) are shown in figure (15). Both $D_{45/135}$ and $D_{90/135}$ are monotonically increasing functions of γ . Comparison of figure (14) and (15) shows that $\bar{X}_w$ increases more evenly over the whole range of γ whereas, at low γ , the dyssymmetry ratios are relatively insensitive. It is clear that an open association model involving a single unimer is inconsistent with the behaviour seen in figure (10).

The second open association model is similar to the proceeding one except that another collection of spheres characterized by a ZOLD distribution with $r_m = 12$, $\sigma = 1.8$ is added. This distribution which will be invariant, represents the larger, relatively less

FIGURE 14.
BEHAVIOUR OF ZOID DISTRIBUTION PARAMETERS WITH DEGREE OF
ASSOCIATION, γ , FOR ASSOCIATION OF SINGLE UNIMER

FIGURE 15.
BEHAVIOUR OF $D_{45/135}$, $D_{90/135}$ WITH DEGREE OF ASSOCIATION, γ , FOR
SINGLE UNIMER OPEN ASSOCIATION.

reactive species. This is a reasonable first approximation since aggregation is greatest among the smaller species. Since the weight per unit volume of each type of unimer is constant, the relative amount of light scattered by the two distributions can be approximated by:

$$\frac{R_{a45}}{R_{b45}} = \frac{\langle r^3 \rangle_a}{\langle r^3 \rangle_b} \frac{\omega_a}{\omega_b} = \left[\frac{r_{oa} \omega_a}{r_{ob} \omega_b} \right] \left[\frac{e^{7.5(\ln \sigma_a)^2}}{e^{7.5(\ln \sigma_b)^2}} \right] \quad (4.10)$$

Here a and b refer to the labile and non-labile unimers respectively and ω_a , ω_b are the corresponding weight concentrations. The relation is only approximate since only the light scattered at 0° , which is free of intraparticle interference effects, is proportional to $\langle r^3 \rangle$ for a given weight of material in solution. Since the total scattering is the sum of that from the two distributions, one can easily show that: (see Appendix E)

$$D_{45/135} = \frac{(R_{a45}/R_{b45}) + 1}{(R_{a45}/R_{b45})/D_{a45/135} + 1/D_{b45/135}} \quad (4.11)$$

$$D_{90/135} = \frac{\frac{D_{b45/135} D_{a90/135}}{D_{b90/135} D_{a45/135}} (R_{a45}/R_{b45}) + 1}{\frac{D_{b45/135}}{D_{a45/135} D_{b90/135}} (R_{a45}/R_{b45}) + 1/D_{b90/135}} \quad (4.12)$$

The behaviour of these dyssymmetry ratios as a function of the degree of association of the labile unimers, γ_a , is shown in figure (16).

Particularly noteworthy is their decrease with γ_a . This is due to an increasing scattering contribution from the labile distribution while the associated dyssymmetry ratios, $D_{a45/135}$ and $D_{a90/135}$ are

FIGURE 16.
BEHAVIOUR OF $D_{45/135}$, $D_{90/135}$ WITH DEGREES OF ASSOCIATION OF
UNIMER A, γ_a , FOR TWO UNIMER ASSOCIATION MODEL

still relatively insensitive functions of particle size. Eventually these quantities increase and surpass those associated with the non-labile distribution. This results in a rapid increase in the observed dyssymmetry ratios approaching the same values as those of the labile distribution alone seen in Figure (15).

The second association model presented here is not necessarily a good approximation of the real sample's behaviour. It is unlikely that the larger species are completely unreactive or that no cross aggregation of different species occurs. Comparisons of the two association models shown here, however, proves that the presence of more than one type of unimer is a necessary condition for the existence of a minimum in the observed dyssymmetry ratios. Also, a greater degree of aggregation among the smaller species is a sufficient condition.

The particle size analysis method developed in Chapter Three was applied to the pairs of dyssymmetry ratios evaluated from equations (4.11) and (4.12). This involved modelling a bimodal distribution with maxima at 20 nm and 12 nm by a single ZOLD distribution. The solutions obtained had r_m values of approximately 2 nm up to $\gamma_a = 0.45$. The corresponding σ values were also close to 2.0 indicating a broad distribution. Beyond $\gamma_a = 0.45$, r_m increased rapidly towards 20 nm as the total scattering becomes approximately that due to the labile association scheme alone.

The trend of decreasing r_m and increasing σ seen in figures (11), (12) and (13) derived from experimental results does not correspond to either of the two hypothetical association models shown here. The behaviour of the dyssymmetry ratios is more complex

than that seen in figures (15) or (16). While the r_m and σ trends are the same as in figure (14) at $\gamma > 0.35$, this behaviour could result from several causes in a heterogeneous system.

UV-Visible Absorption Spectroscopy

The concentration dependence of the absorptivity on a weight of solute per volume of solution basis of the FA fraction in aqueous solution at pH 6.0, is shown in figure (17). Under these conditions, the specific absorptivities at 250 nm and 300 nm show no concentration dependence. Similar results shown in figure (18) were obtained for the un-neutralized FA except that a slight downward trend in absorptivity resulted from a small decrease in pH with FA concentration. It will be demonstrated in chapter 5 that the specific absorptivity is a sensitive function of both degree of ionization and the environment surrounding the absorbing chromophores over the UV-Visible wavelength range. The results in figures (17) and (18), therefore, constitute supporting evidence that, in the absence of background electrolyte, no aggregational or conformation changes occur over the concentration range studied.

Further evidence was obtained from the concentration dependence of the solution refractive index shown in figure (19). These measurements were made with a differential refractometer on the

FIGURE 17. CONCENTRATION DEPENDENCE OF SPECIFIC ABSORPTIVITY;
pH = 6.0, NO BACKGROUND ELECTROLYTE, DUPLICATE EXPERIMENTS

FIGURE 18.
CONCENTRATION DEPENDENCE OF SPECIFIC ABSORPTIVITY; pH ~ 3.6, NO
BACKGROUND ELECTROLYTE

FIGURE 19. CONCENTRATION DEPENDENCE OF SOLUTION REFRACTIVE INDEX: pH $\sim$ 3.6, NO BACKGROUND ELECTROLYTE.

un-neutralized FA in the absence of supporting electrolyte. As seen in equation (3.3), the slope of the lines in figure (19), $(\frac{dn}{dc})$, is proportional to the particle polarizability and should be dependent on tertiary structural changes.

Burch et al⁽⁵¹⁾ showed that if the hydrogen ion molality is held constant, the degree of ionization of the Armadale FA fraction was independent of concentration over the range of 10^{-1} - 1.0 g l^{-1} . This behaviour would be expected if no changes occur in the intrinsic dissociation constants of any of the acidic groups upon dilution.

That the overall dissociation constant will be a function of hydrogen ion activity alone if the FA molecular structure is independent of concentration can be shown by referring to the theoretical formulation of the weighted average dissociation constant, $\bar{K}_a$, developed by Gamble:⁽⁵⁰⁾

$$\bar{K}_a(\alpha) = \frac{1}{1-\alpha} \int_0^{1-\alpha} K_a^0(\alpha) \Gamma(\alpha) e^{-\Delta G_{el}(\alpha)/RT} d(1-\alpha) \quad (4.13)$$

here $K_a^0(\alpha)$ is the average intrinsic dissociation constant of those acidic groups dissociating between α and $\alpha + d\alpha$. $\Gamma(\alpha)$ is the ratio of activity coefficients for the reaction:

$\Delta G_{el}(\alpha)$ is the free energy required to overcome the electrostatic field due to neighbouring ionized groups.

As defined here, K_a^0 is a function of α including the effect of short range interactions which stabilize acidic protons. It is likely that hydrogen bonded structures such as:

play a key role in causing K^0 to decrease with α . This is supported by degradation studies which indicate significant amounts of carboxyl groups ortho to each other. (5-9) The stabilization of the remaining proton is apparent from the ratio of K_2/K_1 in model compounds being in the 10^{-3} - 10^{-5} range. An additional effect involves polarization through the highly conjugated FA molecular structure. It will also be important at short range only and will not be affected by screening from added electrolyte. These two short range effects are probably responsible for the drop in $\bar{K}_a$ over 6 orders of magnitude as α varies from .23 to 0.99 in spite of the presence of 0.100 M KCl supporting electrolyte. It is reasonable that structures like that shown above are not disrupted by K^+ ions since the binding constant of the remaining proton in model compounds, $(K_2)^{-1}$, is approximately 10^5 - 10^8 compared to 10^2 for K^+ -FA binding.

The functional dependence of $\Delta G_{el}(\alpha)$ depends on the states of conformation and aggregation since they affect the average distance between neighbouring acidic groups. Similar conclusions can be made regarding the function $K_a^0(\alpha)$ since the short range effects will also be dependent on molecular and aggregate structure. If this structure is independent of FA concentration, at a given α , $\bar{K}(\alpha)$ will also be

independent of concentration. Here the concentration dependence of $\Gamma(\alpha)$ has been neglected since the major effects have been dealt with explicitly and are not included in this term. Rearrangement of equation (4.14) gives:

$$\alpha = \frac{\bar{K}(\alpha)}{\bar{K}(\alpha) + (H^+)} \quad (4.15)$$

This equation defines α as an implicit function of (H^+) if $\bar{K}(\alpha)$ is independent of concentration. Experiments by Burch et al⁽⁵¹⁾ on the Armadale FA have shown this to be the case over the concentration range of 0.100 to 1.00 g ℓ^{-1} . The results obtained from absorption spectroscopy, potentiometric measurements, differential refractometry, and light scattering, therefore, all point to a lack of aggregational and conformational change with concentration in the absence of supporting electrolyte and at constant hydrogen ion molality. Conversely, the light scattering results in figures (9) and (10) indicate that aggregational changes would make $\bar{K}(\alpha)$ and hence equation (4.15) dependent on FA concentration in the presence of 10^{-1} M KCl background electrolyte.

Fluorescence Experiments

Shown in figure (20) are uncorrected excitation spectra obtained at pH 7 in aqueous solution. In each case, the emission was monitored at 520 nm. Notably, the relative size of the two excitation maxima is dependent on FA concentration. The apparent position of each maximum is a somewhat less sensitive function of

FIGURE 20. FLUORESCENCE EXCITATION
SPECTRA OF 520 nm EMISSION; pH = 7.0

concentration. Caution must be used in the interpretation of uncorrected spectra in a highly absorbing chemically heterogeneous solution. In particular, the spectra in figure (20) should not be taken as mirror images of the absorption spectrum of a single chromophore at 520 nm. Emission spectra obtained with a fixed excitation wavelength of 370 nm contained one peak at about 480 nm and not even a shoulder at 520 nm. When the excitation wavelength was set at 465-480 nm, however, a partially resolved emission maximum at 520 nm was observed.

These results show that the FA fraction has an extremely broad fluorescent emission at about 480 nm with corresponding excitation maximum at about 370 nm. In addition it has an apparent emission maximum at 520 nm with corresponding excitation maxima at 370 nm and 480 nm. The small shifts in the apparent maxima and the relative size of the maxima associated with the 520 nm emission may well be due to the variation in self-absorption with concentration since the shorter wavelengths would be more affected.

These results are in agreement with those recently reported by Ghosh and Schnitzer⁽⁷⁷⁾ for a separate batch of the Armadale FA fraction. A careful search for fine structure in these emissions was made using an Ar laser source and a laser-Raman system monochromator having a wavelength resolution of 10 cm^{-1} . The broadness of the emissions and the absence of fine structure is consistent with the emerging picture of the humic substances as being heterogeneous polyelectrolytes with continuous distributions of molecular properties; in this case, the inter- and intra-molecular environments surrounding

the fluorescent chromophores.

Shown in figure (21) are the uncorrected relative intensities of the 520 nm emission at excitation wavelengths of 370 nm and 465 nm and the 480 emission excited at 370 nm. All three curves show the characteristic self-absorbance effect. Similar results were obtained on un-neutralized FA at pH 3.6.

The absorbance corrected curves, obtained using equation (2-2), are shown in figures (22) and (23) for FA at pH 7 and 3.6 respectively.

At pH 7, there is an almost linear dependence on concentration characterized by correlation coefficients of .9926 and .9987 for the 370 nm/480 nm and the 465 nm/520 nm emission respectively. These curves show a slight upward deviation from linearity. Since most concentration effects, as discussed later, cause a downward deviation from linearity, it is likely that the deviations at pH 7 are due to the approximations involved in the correction procedure. These approximations are discussed in detail in appendix (B). Thus, the pH 7 results are further evidence that, in the absence of neutral electrolyte, the tertiary structure of FA molecules and/or aggregates does not change significantly with concentration in the range studied.

The low pH results show significant negative deviations from linearity even after correction for absorbance effects. Possible causes include self-quenching through collisional processes and aggregational/conformational changes. The latter cause is extremely unlikely on the basis of light scattering, absorbance, differential

FIGURE 21
CONCENTRATION DEPENDENCE OF FLUORESCENCE: UNCORRECTED INTENSITIES
AT pH = 7.0, NO BACKGROUND ELECTROLYTE

FIGURE 22
CONCENTRATION DEPENDENCE OF FLUORESCENCE : CORRECTED INTENSITIES AT
pH 7.0, NO BACKGROUND ELECTROLYTE

FIGURE 23
CONCENTRATION DEPENDENCE OF FLUORESCENCE: CORRECTED INTENSITIES
AT pH $\sim$ 3.6, NO BACKGROUND ELECTROLYTE

	EX. λ (nm)	EM. λ (nm)
Δ	465	520
$\circ$	370	480
$\square$	370	520

refractometry, and potentiometric results already presented. That collisional quenching would be effective at pH 3.6 but not at pH 7 is physically reasonable for two reasons, both associated with the charge density on the FA polymer molecules. At the lower pH, the net repulsive forces between FA aggregates will be less due to a lower average charge density. This will result in a closer approach when two aggregates collide and subsequently more opportunity for non-radiative energy transfer. In addition, the FA molecules and aggregates will be less rigid at lower pH due to a lower charge density. This will increase the effectiveness of inter-particle collisions in causing energy transfer from an electronically excited state in one molecule to vibrational modes in another molecule.

The above reasons for more effective collisional quenching at lower pH will apply even if there is segregation of the fluorescing chromophores to smaller, more fully ionized particles. Collisions will be more effective in quenching fluorescence if the charge density on even one of the colliding particles is reduced.

Chapter 5

Light Scattering and Spectroscopic Properties
of FA Sub-FractionsFiltration Experiments

In developing suitable methodology for studying light scattering from humic substances in aqueous solution, several filtration experiments were performed. This was necessary because the true and apparent molecular weight (particle weight) distributions are very broad and have not been accurately determined. For comparison, one should note that the sample clarification procedures used in light scattering studies of synthetic and biopolymers depend on the system under investigation. Such procedures are designed to effect a complete removal of extraneous scattering matter from the polymer solution without creating artifacts resulting from polymer degradation or fractionation. The goal of such studies is usually an accurate molecular weight determination.

The immediate goal of the present study is to determine the effect of filtration on the light scattering properties of the Armadale FA fraction from a phenomenological standpoint. Shown in figure (24) is the relative light scattering at 90° and absorbance at 465 nm of solutions whose initial FA concentration was 0.100 g-l^{-1} , after filtration through polycarbonate filters of different pore sizes. Along the horizontal axis, reciprocal average pore diameter in $(\mu)^{-1}$ is plotted. This method of presenting data was employed to relate the values for the filtered solutions to those of the unfiltered solution which has a reciprocal pore size of zero. No

FIGURE 24.

LIGHT SCATTERING AND ABSORBANCE OF FA SOLUTIONS FILTERED AT pH 3.6; EFFECT OF FILTER PORE SIZE

theoretical relationship regarding the dependent and independent variables is known and none is implied here. The 90° scattering ratio falls rapidly with filtration through $0.4\ \mu$ and $0.2\ \mu$ filters while filtration through the $0.05\ \mu$ filter results in a relatively smaller reduction. By contrast, the absorbance at 465 nm shows an apparently linear decrease with reciprocal pore size. Similar behaviour was observed at other wavelengths. Assuming that the visible absorbance is a rough measure of the solute concentration, one must conclude that the particles removed by the two larger pore size filters can be classified as mostly extraneous scattering matter. This is because they account for a small percent of the sample by weight while affecting the total scattering enormously. Filtration through the $0.2\ \mu$ filter appears to be the optimum procedure for removal of impurities since it results in the largest scattering decrease before any substantial fraction of the sample is removed. Such a choice is admittedly somewhat arbitrary and may be dependent on the nature of the sample being filtered. It can be said, however, that the unfiltered solution and the $0.2\ \mu$ filtrate are substantially different in content of large particles while being very close in composition with respect to organic matter. Because of this, these two procedures; no filtration and filtration through $0.2\ \mu$, were employed in the experiments reported in later chapters to evaluate the effect of larger particles on the observed scattering behaviour. The assumption of visible absorbance being roughly proportional to FA concentration was verified for filtration through $0.4\ \mu$ by weighing several used, dry filters and an equal number of

unused filters. The two estimates of % sample loss were 3.6% and 3.1% respectively.

A series of filtrations at pH 7.0 were also carried out. The resultant losses in light scattering and visible absorbance are compared with those of the experiment at a pH of 3.6 in Table (IV). The % loss in light scattering is less for each pore size at the higher pH. This is evidence that the solution at pH 3.6 and an FA concentration of 0.100 g-l^{-1} contains aggregates which dissociate to some extent when the pH is raised to 7.0. The % loss in visible absorbance at the higher pH is zero for the 0.4μ and 0.2μ filters indicating that the material removed by filtration contains virtually no absorbing chromophores. Given that the absorptivities of different humic fractions on a per weight basis are generally increasing functions of apparent particle size,⁽³⁰⁾ it is reasonable to conclude that the particles removed in these two filtrations are mainly inorganic. It is particularly noteworthy that unfiltered FA which contains organic matter in aggregated particles exhibits no concentration dependence of light scattering in figure (8). The degree of aggregation in these solutions in the absence of supporting electrolyte, varies with respect to pH but not concentration. Thus the aggregation processes occurring in the presence and absence of 0.100 M KCl supporting electrolyte are not only different in magnitude but are of a qualitatively different nature.

The concentration behaviour at the higher ionic strength is due to an open association whereas the aggregation in the absence of background electrolyte resembles that of a closed association in the region where the maximum degree of association has been reached.

Table IV

Comparison of Filtration at pH 3.6 and 7.0:

Light Scattering and Absorbance Losses

Filter	pH 3.6		pH 7.0	
	% Loss in Absorbance	% Loss in Scattering	% Loss in Absorbance	% Loss in Scattering
0.4 μ	3%	58%	0.0%	11.9%
0.2 μ	5%	90%	0.0%	34%
0.05 μ	30%	96%	15%	70%

Clearly, since the same unimers are present in both cases, there must be at least two species of unimer present: those which are fully aggregated in aqueous solution and those which will only aggregate in the presence of supporting electrolyte.

The description of a closed association involves an equilibrium of the type:

Here, it is seen that the unimers are in equilibrium with a single aggregated species of degree of association, n . Typical examples of such behaviour include the aggregation of proteins such as insulin^(134,135) where n can equal 2 or 6 and micelle formations in soap solutions^(136,137). Considering the irregular molecular structure present in humic materials, it seems unlikely that aggregates possessing a definite number and arrangement of unimers would be formed.

There are, however, two other possible reasons for the existence of a limiting number of unimers in these aggregates. Both reasons involve an initially favorable condition for aggregation which decreases as more unimers are added to the aggregate. These include the increase of electrostatic charge density on the aggregates with degree of association and the possibility that only certain types of unimer can aggregate in the absence of background electrolyte through specific interactions. Both reasons can be considered if it is assumed that the aggregates consist of a particle of inorganic matter surrounded by a coating of organic molecules. Regarding this, it should be noted that the sample has an ash content of about 1%. As the levels of metal ions measured by atomic absorption spectro-

photometry cannot account for this ash content, it seems likely that the refractory material is mostly silicate. The thickness of the shell of organic matter would be limited either because of the build-up of charge density as unimers with a higher degree of ionization are added, or because of a limited number of sites where sufficiently strong chemical bonding can take place.

The same arguments can also be made without considering the inorganic matter which is present. In this case, the most strongly bound unimers in the aggregate are molecular species with a low degree of ionization or the necessary functional groups to undergo preferential bonding. As discussed earlier, there is ample evidence to support the presence of a multiplicity of organic molecular species. It is also physically reasonable that both types of aggregates would be present.

The presence of inorganic material in the aggregates allows the possibility that aggregation in the absence of supporting electrolyte is limited by the finite amount of inorganic particle surface; the limiting mechanism being specific interactions, (electrostatic field effects or both). Thus the results of experiments with no supporting electrolyte alone do not constitute proof of a multiplicity of organic species. They are, however, consistent with such a situation. The concepts developed in the preceding paragraphs do not depend on the specific nature of the attractive forces between unimers. It is particularly noteworthy that both reasons for a limiting aggregate size involve the participation of more than one species of unimer in the formation of the aggregate.

Comparison of the data at both pH's in Table (IV) shows that as the pore size of the filter is lowered, the relative decreases in both light scattering and absorbance approach each other. These differences are due to a different extent of aggregation at the two pH's. The trend in the relative decreases is that which would be expected if the solution at pH 3.6 had a higher population of the largest particles present. This represents a fundamentally different type of aggregation than that seen with 0.100 M KCl supporting electrolyte. In the latter case, an increase in the degree of association resulted in an increase in the population of smaller particles of colloidal dimensions.

Figure (25) shows electron micrographs of 0.2 μ polycarbonate filters which were dried at room temperature after filtering 100 ml of 1.00 g ℓ^{-1} FA solution at a pH of 3.6 through them. The irregularly shaped particles ranging in size from 0.2 μ to 3 μ were shown by x-ray fluorescence to be at least twice as rich in Si as the entire filter surface. This surface is covered with a featureless coating of organic material which has plugged most of the pores. These results are consistent with the presence of both charge limited and specific interaction limited aggregates. Electron micrographs of Armadale FA, freeze dried from solution at pH 4, revealed a fibrous structure⁽¹³⁸⁾ in contrast to the present micrographs, showing globular and featureless material. Clearly, the morphology of these residues is highly dependent on the method of water removal and may not be representative of that in solution.

Shown in figure (26) is the % decrease in visible absorbance,

MAGNIFIED X
2000

MAGNIFIED X
20000

FIGURE 25. ELECTRON MICROGRAPHS OF FA SOLIDS ON 0.2 μ
PORE SIZE POLYCARBONATE FILTER

FIGURE 26.
WAVELENGTH DEPENDENT DECREASE OF VISIBLE ABSORBANCE SPECTRUM
CAUSED BY FILTRATION THROUGH DIFFERENT PORE SIZES;
 $\bullet$ 0.05 μ , $\blacktriangle$ 0.2 μ , $\blacksquare$ 0.4 μ

% ΔA , as a function of wavelength between 380 nm and 650 nm for filtrates obtained with each pore size filter at low pH. The higher % ΔA at longer wavelengths is consistent with the organic molecules in the larger aggregates having a relatively higher absorptivity at longer wavelengths. Such behaviour in a single humic fraction is analogous to that seen in fractions of different origin as verified, for example, by Schnitzer's viscosity results.⁽²⁰⁾ As the pore size is decreased, the % absorbance loss shows less of a wavelength dependence. This is because the composition of the material removed with smaller pore size filters is closer to that of the whole sample. If material with the average composition of the sample were removed, the % ΔA curve would be a horizontal straight line. Possible reasons for the larger aggregates having smaller $E_{4/6}$ ratios include different composition in terms of unimer properties and spectral changes dependent on the degree of ionization.

In order to obtain a rough estimate of the weight-average molecular weight in the FA fraction, the filtration results, along with the measured values of dn/dc , were applied to equation (3.4). Table (V) presents results in which the molecular weight of the material removed by each filtration has been calculated. The concentrations of each fraction was estimated from the absorbance changes at 465 nm. The fractions I and II which failed to pass through the 0.4 μ and 0.2 μ filters have molecular weights of 2.4×10^7 and 1.5×10^7 respectively, consistent with the electron micrographs of figure (25) showing the presence of macroscopic particles. Fractions III and IV are in the medium to

Table V
Effect of Filtration on M.W.

FRACTION	% OF SAMPLE	M.W.
$> 0.4 \mu$	3.8%	2.4×10^7
$< 0.4 \mu, > 0.2 \mu$	2%	1.5×10^7
$< 0.2 \mu, > 0.05 \mu$	24.1%	9.3×10^4
$< 0.05 \mu$	70.3%	1.3×10^4

low molecular weight range for single polymer molecules. It will be shown in Chapter 6 that these two fractions are not aggregated. Comparison of these weight-average molecular weights with the number average values of 960, therefore, indicates a high degree of polydispersity even in the absence of aggregation. This supports the conclusion regarding the heterogeneity of the unimer or non-aggregated species.

The molecular weights in Table (V) are rough estimates for the reasons outlined in Chapter 3. Also, the precision in the calculated scattering ratios of fractions III' and IV is poor because of the relatively small differences in scattering ratio between the 0.2 μ and 0.05 μ filtrates and the blank.

As the scattering ratios in fig. (24) range from 10^{-5} to 10^{-7} with relatively small accompanying absorbance changes, the contribution of light scattering to the apparent absorption must be minimal. Further proof is that the attenuation per cm due to light scattering, $8\pi/3 R_{90}$, is about 10^{-4} whereas the total absorbance, as measured spectrophotometrically, is about 10^{-2} . It is therefore impossible to explain the approximate $1/\lambda^4$ dependence of the absorbance spectrum or the empirical relationship between viscosity and $E_{4/6}$ ratio on light scattering. An explanation based on true absorbance is needed. These conclusions were verified by obtaining a photoacoustic spectrum of a highly concentrated FA solution. The shape of this spectrum, due to absorbed radiation only, was similar to those obtained by conventional means.

Gel Filtration Experiments

The light scattering and spectroscopic experiments presented thus far have been interpreted in terms of a chemically heterogeneous mixture of organic polymers. In order to verify this picture and gauge the heterogeneity of the sample, the un-neutralized FA material was fractionated on a G-75 sephadex column using distilled water as the eluant. Fractions were collected roughly every 5 ml, diluted to a pale yellow colour, and subjected to visible absorbance and fluorescence measurements at several wavelengths. Shown in figure (27) is the absorbance at 465 nm after correction for dilution as a function of elution volume, V_e . The presence of two peaks does not indicate the existence of a bimodal molecular weight distribution for a number of reasons. Among them, the small peak at $V_e = 43$ ml is due to molecules or aggregates which are completely excluded from the pores of the gel on the basis of molecular size. Their particle weight is most likely greater than 7.5×10^4 based on the gel's specified exclusion limit for globular proteins. The detection of this high molecular weight fraction here is by its absorbing chromophores verifying the presence of significant amounts of organic material in these high molecular weight particles.

The $E_{4/6}$ ratio and relative light scattering at 633 nm measured in the fluorescence spectrometer are shown in figure (28). The 370 nm/480 nm and 465 nm/520 nm emissions are shown in figure (29). The two fluorescent emissions and the light scattering intensity at 633 nm have been corrected for internal absorbance and, also divided by the sample absorbance of the incident radiation.

FIGURE 27

GEL FILTRATION: ABSORBANCE AT 465 nm vs. ELUTION VOLUME

FIGURE 28
 GEL FILTRATION: VARIATION OF PROPERTIES DEPENDING ON PARTICLE SIZE
 WITH ELUTION VOLUME; ○ 90° LIGHT SCATTERING AT 633 nm, ▲ ϵ 4/6
 RATIO

FIGURE 29
GEL FILTRATION: CORRECTED FLUORESCENT EMISSIONS, VS. ELUTION VOLUME;
▲ 370 EX./480 EM., ○ 465 EX./520 EM.

They are, therefore, intensive properties of each fraction as opposed to the total absorbance plotted in figure (27). From the light scattering at 633 nm, one sees the expected correlation between elution volume and particle size for $V_e < 130$ ml. Beyond this point, however, the scattering intensity increases with elution volume. This is not surprising in view of the well known adsorption effects of aromatic molecules on sephadex gels.⁽¹³⁹⁾ The minimum in scattering intensity at an intermediate elution volume reflects the competing effects of exclusion and absorption chromatography which occur simultaneously. There is a roughly inverse correlation between Rayleigh scattering, on the one hand, and the $E_{4/6}$ ratio and emissions at 480 nm and 520 nm on the other. This confirms the conclusion, based on figure (26), that the relationship between particle size and the shape of the absorbance spectrum among different humic fractions applies to the particles in a single humic fraction. It is evident that most of the FA fluorescence originates from the smaller particles in solution. This is in agreement with the results of Stewart⁽⁷²⁾ who measured the fluorescence of natural water samples after gel filtration, using elution volume alone as a particle size index. As the samples were not de-ionized, it was not known if the larger particles were less fluorescent due to specific quenching effects from complexed metal ions. The present results are less ambiguous since the FA fraction was analysed to be essentially metal free. The lower fluorescence intensity seen among the larger particles here may be due either to segregation of the chromophores to smaller particles or the quenching mechanisms

associated with a low charge density mentioned earlier. The 480 nm and 520 nm emissions peak at almost the same elution volume indicating little if any segregation of the differently fluorescing chromophores among particles of different size. The strong fluorescence of the smaller particles is consistent with the conclusion of Datta *et al*⁽⁷⁴⁾, i.e. the fluorophores are associated with oxygen bearing functional groups. In the absence of aggregation inducing species, it is likely that the aggregation present will be greatest among those molecules having un-ionized carboxyl groups. The more fully ionized molecules, having a higher charge density and less groups available for hydrogen bonding, will not be aggregated. For un-neutralized FA, having a degree of ionization of about 0.23, the ionized carboxyl groups will be those with higher intrinsic dissociation constants. Comparison of model compounds indicates the presence of oxygen-bearing functional groups on the same ring as an aromatic COOH increases K_o (140)

After reconditioning the columns by eluting distilled water for forty-eight hours, the fractionation experiment was repeated. The elution volumes corresponding to the maxima in figure (27), (28), and (29) differed by 3-6% from the first experiment but occurred in the same order. The height of the peaks was about 15% greater indicating that a more efficient separation was achieved. The fractions were combined into two lots; a low molecular weight, LMW, lot with $120 < V_e < 170$ and a high molecular weight lot, HMW, consisting of all other fractions. The two lots were freeze-dried. The LMW lot had a brownish black appearance while the HMW lot was a light brown. In this regard, the parent FA fraction was dark brown.

Upon redissolution, the HMW lot formed a cloudy suspension which persisted after standing 24 hours in the dark and one hour in an ultrasonic bath. Addition of a surfactant, (Triton-x-300) and raising the pH to about 7 were both effective in clarifying the solution. Portions of the HMW lot not freeze dried showed no tendency toward cloudiness over prolonged periods of standing. The LMW lot and the parent FA material behaved similarly in this respect.

Because of the labile nature of the colloidal particles in the reconstituted HMW fraction, degradation processes resulting in extensive ring fusion can be discounted. Less easily discounted is decarboxylation resulting in a more humic acid like material. The humic acid fraction of soil organic matter is not soluble below pH 7 due to an insufficient number of ionized carboxyl groups to interact favorably with solvent molecules. The forces preventing dispersion in a typical humic acid were somewhat greater than those in the HMW lot since the addition of a large amount of Triton-x-300 failed to disperse any humic acid at low pH.

There is no specific reason why freeze drying should cause decarboxylation. It is noteworthy that the present batch of FA and the original FA1 batch prepared by Agriculture Canada⁽⁸⁴⁾, both freeze dried, showed consistent results from acidometric titration. Another FA batch which was inadvertently exposed to air for several weeks before freeze-drying, gave an equivalence point about 10% lower when analysed by the same procedure as the other two batches. Significantly, all three batches gave identical light scattering ratios within experimental error at the three usual scattering angles in aqueous solution at low pH. The carboxyl

deficient batch had the same appearance as the other two batches as a solid powder and in solution. Thus it is very unlikely that decarboxylation resulted in a more hydrophobic HMW lot after freeze-drying.

A reasonable explanation of these observations is that the detergent effect of smaller hydrophilic molecules solubilizes the larger aggregates in the parent FA material. Such a mixed aggregate would be limited in size by the specific and field effects discussed in Chapter Four concerning aggregation in the absence of background electrolyte. The parent FA solid is soluble at low pH because it was freeze-dried in the presence of a high concentration of hydrophilic molecules. In the HMW lot, there are enough hydrophilic molecules present to ensure stability before freeze-drying. When freeze-dried, however, the FA concentration rises to a point where segregation of hydrophobic and hydrophilic molecules is favoured. The hydrophobic aggregates will not redissolve because of too low a hydrophilic content in the sample. This analysis does not indicate which state of the HMW lot at 0.100 g-l^{-1} is thermodynamically more stable; hydrophilic mixed aggregates or hydrophobic and hydrophilic aggregates in which unimers are relatively more segregated according to their hydrophilicity.

Chapter 6

The Effects of Bound Cations on Aggregation

Earlier chapters have examined the polydispersity of the FA fraction in terms of particle size and unimer properties. Evidence was provided of both closed and open association phenomena occurring. It was shown that growth among the smaller particles was important in the open association concentration dependence. In this chapter, the role of the acidic functional groups will be examined by studying the aggregation behaviour induced by bound protons and metal ions.

Light Scattering Experiments

The dependence of the 90° scattering ratio on degree of ionization, α , is shown in figure (30). Results are shown for 0.100 g l^{-1} FA, both unfiltered and filtered through 0.2μ , in aqueous solution and 0.100 M KCl background electrolyte. Clearly evident is the much higher degree of aggregation at low pH caused by KCl. This aggregation results in a 30 fold increase in scattering from the filtered FA compared to 5 fold for the unfiltered solution confirming that growth among the smaller particles is the dominant feature in the presence of KCl. Thus the particles removed by filtration do not act as nuclei for the particle growth seen with background electrolyte. They are, however, responsible for the smaller amount of aggregation in the absence of KCl. Here the factors which limit particle size, resulting in closed association behaviour, cause a leveling off of light scattering ratio as α falls below 0.4 corresponding to pH's lower than 5.0. The small rise in scattering below $\alpha = 0.05$ is the beginning of FA precipitation

FIGURE 30.
DEPENDENCE OF 90° LIGHT SCATTERING, R_{90} , ON DEGREE OF IONIZATION, α ;
 ○ NOT FILTERED/ $10^{-1}M$ KCl,
 △ FILTERED, $0.2 \mu 10^{-1}M$ KCl;
 ● NOT FILTERED/NO KCl,
 ▲ FILTERED, 0.2μ /NO KCl

in strongly acidic solution. It is not known if the addition of KCl allows the aggregates present in aqueous solution to participate in the open association, although it seems likely. What is known, however, is that the major participants in this open association are the molecules which are not aggregated in the absence of KCl.

Possible interactions causing aggregation in the presence of KCl to be thermodynamically favourable include the following:

1. intermolecular hydrogen bonding
2. hydrophobic interactions
3. ether linkages between quinoid structures
4. co-operative K^+ binding
5. "salting-out" due to competition for solvent molecules.

Co-operative K^+ binding, can be represented by the following structure:

Similar structures without bound solvent, called ion clusters, have been documented in ionic polymers in the solid state. (141) Localised binding of Group II metals to octanoate micelles in aqueous solution has been observed by N.M.R. relaxation experiments. (142) The binding mechanism involves hydrogen bonding of bound water molecules to octanoate end groups. It seems likely that ion cluster formation in humic aggregates in aqueous solution involves a similar mechanism. While cooperative K^+ binding will contribute to the stability of the aggregates, it is not sufficient to cause aggregation by itself.

This is evident from the low light scattering at high α where most acidic groups are ionized.

The term "salting out" has been used generally to describe the decrease in solubility of many polymers in aqueous solution due to simple electrolytes.⁽¹⁴³⁾ It has also referred to competition for water molecules as a specific precipitation mechanism. This mechanism, based on the unavailability of water in the electrolyte ions solvation shells to aid polymer solubility, is too simple to explain many solubility effects. Specific interactions are responsible in some cases including solubility enhancement phenomena. Figure (31) shows the effect of NaCl on FA aggregation to be much smaller than that of KCl. Since Na^+ binds more water molecules than K^+ , competition for solvent is not important. Also since the largest scattering increase occurs when the KCl concentration is about 0.10 M versus a solvent concentration of 55 M, a shortage of solvent molecules cannot be an important factor.

The relative effectiveness of K^+ and Na^+ in promoting aggregation is consistent with DLVO theory⁽⁶²⁾ and the Schulz-Hardy rule⁽⁶¹⁾. This in turn implies that the cation's role is to screen charge or decrease the electrical double layer allowing a closer approach in which short range attractive forces can operate. While H^+ binding also enhances this effect, it is involved in the short range forces themselves.

Wershaw et al,⁽³⁷⁾ in discussing the possible role of weak covalent bonding in aggregation, refer to the possibility of an association equilibrium between radicals as shown below:

FIGURE 31
RELATIVE EFFECT OF K⁺ AND Na⁺ ON 90° LIGHT SCATTERING AT LOW
pH; ● K⁺; ▲ Na⁺

Steric hindrance was cited as the reason for the reversibility of the reaction. As several studies^(21,144,145) have measured an increase in free radical content with pH, it is possible that electrostatic repulsion from ionized carboxyl groups shifts equilibria like equation (6.1) to the left. Other reasonable explanations for the pH variation of free radical concentration do not involve changes in aggregation. For example, Wilson and Weber⁽¹⁴⁴⁾ suggested reactions of the type:

Depending on the relative pH sensitivity of the above two reactions, the net effect of both occurring could be to increase or decrease the number of ether linkages as the pH is lowered. Ghosh and Schnitzer have suggested that the increase in free radicals at high pH may cause a small increase in aggregation which increases solution viscosity.⁽²¹⁾ Other causes mentioned were increased binding of water molecules and polyion expansion.

The so-called "hydrophobic bond"⁽¹⁴⁶⁾ results from interactions of solvent molecules with solute molecules and with each other. Aggregation of non-polar organic molecules in water minimizes the destabilizing effect on the surrounding solvent molecules by reducing the solvent-solute contact area. This destabilization is actually an increase in long-range order.⁽¹⁴⁷⁾ It is not possible to evaluate the contribution of hydrophobic bonding to humic aggregation but it must be relatively small. This is because even unionized molecules possess polar groups capable of favourable interactions with solvent and each other.

Intermolecular hydrogen bonding has been cited as the cause of pH dependent aggregation in aqueous solutions of neutral polymers such as mixtures of unionized poly-(acrylic acid) and poly-(ethyleneoxide)⁽¹⁴⁸⁾. For molecules having large numbers of eligible groups, co-operative effects similar to the chelate effect favour hydrogen bonding between polymer molecules over solvent-solute hydrogen bonding. For FA aggregation, the number of eligible groups on the smaller molecules is approximately 7/molecule based on the number-average molecular weight. Later it will be shown that the number of linkages per unimer is between two and three making co-operative hydrogen bonding a possibility. Possible proton donor groups include unionized carboxyl, aromatic and aliphatic hydroxyl, amine, and hydroquinone groups. Acceptor groups include these as well as methoxy, quinone, and ionized carboxyl groups. Clearly the carboxylate groups are of central importance as seen from the pH dependence of R_{90} .

In examining the possible interactions responsible for aggregation, one must consider the sensitivity of R_0 to α . The

high ionic strength data in figure (30) could not be fit to a single polynomial of degree between two and six. For lower degrees, the polynomial had insufficient curvature while at higher degrees, it began to oscillate. The curves could also not be fit to a single exponential function. Evidently, this results from the rapid increase at low pH. While it is tempting to explain the existence of two regions of R_{90} vs α behaviour in terms of the ionization of different types of acidic groups, it would infer too much from the available data. In figure (14), it was shown that the weight-average degree of polymerization has a similar dependence on the degree of association. In that aggregation scheme, each unimer had three identical sites available for linking with other unimers. For FA aggregation, it is reasonable to assume a positive correlation between the fraction of unionized acidic groups, $(1-\alpha)$, and the average number of linkages per unimer. Later it will be shown that $(1-\alpha)$ varies almost linearly with pH. It is difficult to compare the idealized scheme in Chapter Four with FA aggregation because of the multiplicity of unimers already demonstrated. Nevertheless, the following rough calculation is useful. Considering the number average molecular weight of 960 and acidic group content of 7.7×10^{-3} moles- g^{-1} , there will be a large fraction of unimers with about 7 acidic groups per molecule. The maximum numbers of groups participating in each unimer-unimer link was calculated on this basis for $f = 7, 6, 5, 4$, and 3 and are tabulated in column B of Table (VI). Assuming that an acidic group must be unionized to participate in a linkage, the number of linkages per unimer at the apparent gel point is calculated as: $(1-\alpha) \times 7 / (\# \text{ of acidic groups/link})$.

Table (VI)

Comparison of pH Dependent FA Aggregation with Single Unimer
Association Model

A	$f_c =$	7	6	5	4	3
B	#acid groups/link	1.00	1.17	1.40	1.75	2.33
C*	links/unimer at gel pt.	4.80	4.11	3.43	2.74	2.06
D [†]	#links/unimer at gel pt.	1.16	1.17	1.18	1.33	1.49
E	$\frac{C-D}{E}$	3.18	2.51	1.91	1.06	0.34

* Calculated as (Column B) $-1 \times (1-\alpha) \times 7$, for $(1-\alpha) = 0.67$

[†] Calculated from Equation (4.7) Describing Gel Point Theory

The factor $(1-\alpha) = 0.67$ is the fraction of unionized acidic groups at pH 3.6 determined by potentiometric titration. The # of linkages per unimer at the gel point for each f value was evaluated by graphing equation (4.7) and is shown in row D. From the difference between rows C and D, shown in row E, an increasingly better fit is achieved as f varies from 7 to 3. For a gel point to occur, f must be greater on average than two, placing it between 2 and 3. Although this model is extremely simple, it leads to a physically reasonable picture: FA unimers linked together in linear segments between branch points due to effectively trifunctional unimers. Linkages involving two or three hydrogen bonded acidic groups will be more favoured than those involving only one due to the chelate effect. Linkages involving a larger number of acidic groups will be unfavourable because of steric hindrance.

In figure (30), there is a small upward trend in the R_{90} 's of filtered FA solutions with pH. Possible causes include the following:

- a) Expansion of the polyion to relieve the build-up of negative charge due to carboxyl group ionization.
- b) Increased binding of water molecules.
- c) Increased binding of K^+ counter ions.
- d) Increasing aggregation from the free radical combination according to equation (6.2).
- e) Greater intrinsic scattering power due to a larger refractive index, m , (See equation (3.7)).

a); While polyion expansion undoubtedly occurs, the scattering at 0° , given by equation (3.2), will remain constant since the particle weight does not decrease. Scattering at finite angles would actually

decrease due to increasing intraparticle interference effects. This does not conflict with equation (3.7) in which $R_0 \propto \langle r^6 \rangle$ since as the molecule expands, its composition approaches that of water so that $\frac{m^2-1}{m^2+1} \rightarrow 0$.

b);c); Increasing polyelectrolyte ionization increases the width of the molecules' hydration shell. (149) This region of more densely packed water molecules has a higher refractive index than that of the bulk solvent causing additional light scattering. The number of territorially bound K^+ ions will also affect the refractive index of the solvation region. Lowering the overall charge density will decrease the region but bound K^+ ions possess their own solvation shell.

d); The possibility of weak covalent linkages, as shown in equation (6.1) has already been discussed. Knowledge of the equilibrium constants associated with equations (6.1) and (6.2) are required to determine at what pH range these linkages are important. Cause e) is physically reasonable since the ionization of acidic groups transfers electrons from bonding orbitals to more diffuse non-bonding orbitals resulting in increased polarizability.

An increase in light scattering at high pH was also seen in a time study. The behaviour of light scattering and visible absorbance of $10^{-1}g-l^{-1}$ FA at pH 9 with no background electrolyte is reported in Table (VII). The slow increase in light scattering may result from increasing intrinsic scattering power or ether linkage formation. The former reason implies that further ionization

Table VII

Time Study: pH 9; No Supporting Electrolyte

Time (Min)	A_{465}	A_{650}	$10^5 R_{45}$	$10^6 R_{90}$	$10^6 R_{135}$
30	0.394	0.064	1.59	6.60	3.30
60	0.394	0.063	1.61	7.18	3.39
90	0.393	0.063	1.66	7.28	3.47
120	0.392	0.062	1.63	7.06	3.41
150	0.390	0.062	1.70	7.32	3.53
210	0.389	0.060	1.73	7.22	3.37
270	0.386	0.060	1.73	7.16	3.45
1170	0.378	0.055	1.78^{-5}	7.52	3.36

occurs on a long time scale through coupling to a slow process such as oxidation. It is unlikely that decarboxylation produced a more hydrophobic solute since the solution was kept in the dark under nitrogen between measurements. Electron spin resonance spectroscopy⁽¹⁵⁰⁾ has shown that the free radical content of the Armadale FA is at a minimum at about pH 6.5, increasing moderately at higher pH. At fixed pH, it decreases slowly with time. This decrease was accompanied by a decrease in visible absorbance similar to that seen in the present results. The E.S.R., absorbance, and light scattering experiments are consistent with reaction (6.1) shifting slowly to the right. An increase in intrinsic scattering and/or increased solvation from ionization coupled to slow redox processes is also possible and further research is needed in this area. Finally, it is important to note that the increase in R_0 with pH is due to an increasing rate for these processes since the measurements were made on separate solutions at a fixed time after raising the pH.

The dyssymmetry ratios, $D_{45/135}$ and $D_{90/135}$, of light scattered from solutions with and without 0.100 M KCl background electrolyte are shown in figures (32) and (33) respectively. These results on un-filtered FA solutions verify that the two aggregation processes are qualitatively different. The downward trends observed in 0.100 M KCl indicate increased aggregation among the smaller sized particles. The curves for no supporting electrolyte are less easily interpreted. At low pH, the dyssymmetry ratios show parallel behaviour to the 0.100 M KCl results. At high pH, however, they show the opposite trend. Unlike the angular variation of scattering discussed

FIGURE 32
 VARIATION OF DYSSYMMETRY RATIOS WITH DEGREE OF IONIZATION, α ;
 NO BACKGROUND ELECTROLYTE; $\circ$ $D_{45/135}$, $\square$ $D_{90/135}$

FIGURE 33. VARIATION OF DYSSYMMETRY RATIOS WITH DEGREE OF IONIZATION, α ; 10^{-1} , M KCl BACKGROUND ELECTROLYTE: O $D_{45/135}$, $\Delta D_{90/135}$

previously, the high pH, low ionic strength scattering is from highly charged particles making an interpretation based on size effects less reasonable. A possible explanation for the dyssymmetry ratios decreasing with pH is that the causes responsible for increasing R_{90} with pH, mentioned in the last section, affect the smaller molecules more strongly.

The dyssymmetry ratios for filtered FA in 10^{-1} M KCl, are also decreasing functions of pH. The trend was seen at low pH only, since at higher pH, R_{45} and R_{135} were too close to zero to produce meaningful results. The relative precision with decreasing scattering was observed in the relative deviations from fitted polynomials obtained from non-linear regression analysis. As α increases from 0.2 - upwards, these relative deviations increase from less than 1 to as much as a hundred percent.

Particle size analysis was applied to the low pH data. The resulting functions of r_m and σ are presented in Table (VIII). At pH 3.5, the results are similar to those in figure (13) showing no overlap between the regions associated with $D_{90/135}$ and $D_{45/135}$. As the pH is lowered from 3.6 to 1.96, the regions corresponding to the two dyssymmetry ratios converge at low r_m and high σ almost to a small overlap. This trend towards higher r_m and lower σ is the opposite of that seen in the concentration dependent association analysed in Chapter 4. This is not surprising in view of the dependence of r_m and σ on the degree of association for the hypothetical model involving a single unimer. Thus even if the polydispersity increases constantly, r_m may go through a maximum as association

TABLE VIII

EVALUATION OF PARTICLE SIZE DISTRIBUTION

PARAMETERS: LOW pH, 10^{-1} M KCl BACKGROUND ELECTROLYTE

$$\begin{cases} r_o & (\sigma, D_{45/135} + 5\%) \\ r_o & (\sigma, D_{45/135} - 5\%) \end{cases}$$

column a

$$\begin{cases} r_o & (\sigma, D_{90/135} + 5\%) \\ r_o & (\sigma, D_{90/135} - 5\%) \end{cases}$$

column b

DEGREE OF IONIZATION, α

σ	0.175		0.225		0.267		0.276		0.382	
	a	b	a	b	a	b	a	b	a	b
1.1	114.6	88.9	123.2	87.9	133.8	87.9	133.8	87.9	138.2	63.0
	108.0	71.7	117.5	70.7	129.0	70.0	129.0	70.0	133.4	34.0
1.3	76.8	58.7	83.3	57.9	92.3	57.9	92.3	57.9	96.0	40.5
	71.9	46.7	78.8	45.3	88.2	45.3	88.2	45.3	91.8	21.9
1.5	41.6	31.3	46.0	30.8	52.5	30.8	52.5	30.8	55.3	20.7
	38.5	24.2	43.0	23.5	49.6	23.5	49.6	23.5	52.2	10.8
1.7	19.5	14.5	22.2	14.0	26.3	14.0	26.3	14.0	28.2	-
	17.5	10.5	20.2	9.8	24.5	-	24.5	9.8	26.2	-
1.9	8.8	6.3	10.2	6.2	12.5	6.2	12.5	6.2	13.7	3.5
	7.8	4.4	9.2	4.2	11.5	4.2	11.5	4.2	12.5	1.8
2.1	3.8	2.8	4.5	2.7	5.7	2.7	5.7	2.7	6.3	1.5
	3.3	1.8	4.0	1.8	5.2	1.8	5.2	1.8	5.7	0.8

proceeds. For the association of a multiplicity of unimers, these parameters may show a complicated dependence on the degree of association. In principle, such values of r_m and σ will be useful for calculating properties of the distribution depending on $\langle r^n \rangle$. This will require a level of precision at least an order of magnitude better than that obtained in the present study. The large number of readings required to achieve such precision, $\sim 10^2$, could be handled easily by an automated instrument. Also, measuring scattered light intensity at a number of angles would allow the testing of more sophisticated theoretical models.

The greater aggregation among the smaller particles seen in the concentration and pH variation experiment with background electrolyte, is not merely a consequence of greater numbers of smaller particles being present. This can be determined by comparison with the hypothetical aggregation of a single unimer described in Chapter 4. When $0 < \gamma_a < 1$, the distribution of particle weights is given by:

$$N_x = \left[\frac{(1-\gamma)^2}{(1-\gamma f/2)} \right] \left[\frac{\gamma}{(1-\gamma)^{(2-f)}} \right] \left[\frac{(-x)!}{(x-2x+2)!x!} \right] \quad (6.3)$$

Here N_x is the mole fraction of aggregates containing x unimers. Because equation (6.3) is a decreasing function of particle weight, the most probable encounter based on random statistics will be between two small particles. If all encounters had equal probability of causing bond formation, the smaller particles would aggregate preferentially and the polydispersity would be limited. The polydispersity expressed as $\bar{x}_w/\bar{x}_n$, obtained by dividing equation (4.6) by equation (4.7), increases toward infinity as γ_a approaches

unity. The underlying reason for this is the greater relative reactivity of larger aggregates. The number of unreacted and hence available functional groups per particle is given by:

$$r = x(f-2) + 2 \quad (6.4)$$

This increasing reactivity with particle size is the dominant factor over the whole range of polydispersity, ie. for $1 < \bar{x}_w/\bar{x}_n < \infty$.

For the FA aggregation, the polydispersity does not increase indefinitely but shows a complex dependence on the degree of aggregation including a decrease over a considerable range of increasing aggregation. This is because the variation with aggregate weight of reactivity towards unimers differs substantially from equation (6.4). In particular, self association among the smaller unimers occurs to a greater extent. The most likely reason for this is that specific interactions such as hydrogen bonding and/or ether linkage formation are major factors. Since the smaller unimers have greater densities of oxygen bearing functional groups, co-operative phenomena such as chelate formation will occur more readily. Thus the pH dependent aggregation cannot be explained in terms of a charge neutralization induced "flocculation".

The effect of added Cu(II) on FA light scattering at pH 6.0 is seen in figure (34). A sample concentration of 0.100 g-l^{-1} FA filtered through a 2μ polycarbonate filter was employed. The results of three titrations included a small rate of scattering increase at low added copper (II) which increases continuously

FIGURE 34
EFFECT OF ADDED Cu(II) ON FA LIGHT SCATTERING; pH 6.0, $C_{FA} = 10^{-1} \text{ g l}^{-1}$, NO BACKGROUND ELECTROLYTE, 3 REPETITIONS

until the solution becomes visibly cloudy. The titrations were not continued farther since settling of large particles began to cause a notable decrease in signal in the time scale of a measurement. A continuous increase in particle size, albeit small at low copper levels, represents a different picture than that of a true solution which precipitates when the maximum binding capacity is reached. The concentration of bound copper, shown by the dashed line, is the difference between total and free Cu(II) determined by a Cu(II) ion selective electrode. The titrations were carried out under similar conditions to those followed by light scattering as detailed in Chapter 2. The greatest scattering increase occurs in a region where there is practically no increase in bound copper (II). This is similar to the experimental behaviour seen with proton binding in figure (30) and the theoretical behaviour of $\bar{x}_w$ in the open association model shown in figure (14). Although this model assumes a single unimer, this is not necessary for the gel point effect to occur. If some minimum fraction of the unimers is at least tri-functional, (capable of associating with three other unimers) three dimensionally cross-linked networks will arise at high degrees of conversion. Under these circumstances, minute increments of Cu(II) binding can connect aggregates which are already large; producing an extremely high sensitivity of particle size on complexation. This does not imply that the specific aggregation mechanism must be cross-linking by covalent bonding as suggested by Bresnahan et al⁽⁵³⁾:

(R^1 and R are separate FA polymer molecules)

Similar results would be expected if the major effect of Cu binding

was charge neutralization producing a more hydrophobic solute. Flocculation of hydrophobic colloids by simple electrolytes can result in similar behaviour. In this case, the electrolyte ions reduce the mutually repulsive double layers between particles allowing other attractive forces to operate. An increase in particle size with little or no increase in Cu(II) binding also suggests that aggregation may be the slower of the two processes. A series of samples with different amounts of added copper (II) gave qualitatively the same behaviour as in figure (34) but with the scattering values at high bound copper about twice as large. At present, it is impossible to tell whether this is a kinetic phenomenon or results from the further binding of trace amounts of copper. The change in copper binding is too small to be detected as seen in the comparison by Truitt et al of dialysis and potentiometric methods⁽⁵⁵⁾. In this comparison, no significant difference was found in Cu(II) binding between one month and a few hours of mixing.

The shape of the curves in figure (34), characterized by little scattering increase between 0 and 2 mmol Cu(II) added, is not the result of a slow aggregation process. This is seen in figure (35) in which scattering results from a time study are plotted. There is no increase in particle size either from increasing copper binding or slow aggregation processes at constant bound copper. The small decrease in scattering is greatest at 45° due to less scattering from the larger particles in solution. This may result from settling since the unfiltered FA solution was not

FIGURE 35. Cu(II)-FA LIGHT SCATTERING TIME STUDY; 2.00 mmol Cu(II)-gFA⁻¹, pH 3.6

stirred after purging with and sealing under N_2 .

The 90° scattering ratios for unfiltered FA at pH's 3.6 and 6.0 are shown as a function of bound Cu(II) in figure (36). These curves, and others involving Cu(II) induced light scattering, are averages of three experiments similar to those shown in figure (34).

Light scattering average functions and standard deviations are listed in Table IX.

The pH 6.0 curve is almost identical to the pH 3.6 curve but is shifted about $1.1 \text{ mmol Cu(II)-gFA}^{-1}$ to the right. Since potentiometric measurements with the glass electrode show that the number of bound protons is $2.24 \text{ mmol-gFA}^{-1}$ greater at pH 3.6 than at pH 6.0, it is evident that equivalent amounts of bound H^+ and Cu(II) have a similar effect.

This is reasonable on the basis of the hypothetical aggregation models in Chapter Four if both hydrogen bonding and Cu(II) cross-linking can form inter-unimer links. It is also qualitatively consistent with charge neutralization causing an increase in hydrophobic bonding. In either case, the scattering ratio at a fixed level of bound Cu(II) will be a decreasing function of pH as seen experimentally.

In considering a cross-linking mechanism, it should be noted that neither all of the bound protons or Cu(II) will participate in unimer-unimer linkages. It has already been demonstrated that interunimer hydrogen bonding results in an average of between two and three links per unimer and it is reasonable that the remaining protonated acidic groups participate in intraunimer and intra-aggregate hydrogen bonding. The presence of about $3.3 \text{ mmol-g-FA}^{-1}$

FIGURE 36. VARIATION OF R_{90} WITH BOUND Cu(II); $C_{FA} = 10^{-1} \text{ g l}^{-1}$,
NO BACKGROUND ELECTROLYTE: O pH = 3.6, ● pH = 6.0

TABLE (IX)
Cu(II)-FA TITRATIONS, LIGHT SCATTERING DATA

mmol Cu(II) 9FA	pH 3.6 C _{FA} =0.100g l ⁻¹		pH 6.0 C _{FA} =0.100g l ⁻¹		pH 8.0 C _{FA} =0.00g l ⁻¹		pH 6.0 C _{FA} =0.100g l ⁻¹		pH 6.0 C _{FA} =0.100g l ⁻¹	
	10 ⁵ R ₉₀	D _{45/135}	10 ⁵ R ₉₀	D _{45/135}	10 ⁵ R ₉₀	D _{45/135}	10 ⁶ R ₉₀	D _{45/135}	10 ⁶ R ₉₀	D _{45/135}
0.00	3.21 ± .08	5.92 ± .04	2.46 ± .17	5.40 ± .27	2.58 ± .20	5.05 ± .32	3.41 ± .45	6.60 ± .25	4.13 ± .53	1.85 ± .16
1.00	3.36 ± .15	6.36 ± .52	2.66 ± .25	7.05 ± .92	2.51 ± .17	6.16 ± .30	-	-	5.09 ± 1.98	2.16 ± .12
2.00	3.69 ± .14	7.02 ± .32	3.24 ± .12	5.98 ± .11	3.25 ± .31	5.47 ± .24	3.83 ± .21	7.58 ± .65	9.67 ± .64	2.20 ± .12
3.00	4.14 ± .16	6.58 ± .38	6.23 ± .13	5.38 ± .45	5.84 ± .24	4.88 ± .31	-	-	32.7 ± 5.6	1.68 ± .10
4.00	4.91 ± .19	7.06 ± .47	18.7 ± 3.1	4.30 ± .15	13.1 ± 1.6	4.26 ± .05	5.62 ± .11	4.38 ± .08	114 ± 13	1.52 ± .04
5.00	6.35 ± .41	6.52 ± .79	32.8 ± 7.2	3.96 ± .31	21.6 ± 2.2	4.12 ± .13	-	-	202 ± 21	1.50 ± .06
6.00	9.00 ± .59	6.18 ± .87	45.0 ± 5.2	3.87 ± .34	33.7 ± 3.4	3.86 ± .04	8.58 ± .93	9.00 ± .82	276 ± 37	1.48 ± .05
7.00	12.3 ± .70	6.13 ± .21	60.7 ± 4.0	3.46 ± 3.8	46.1 ± 4.9	3.80 ± .04	-	-	407 ± 54	1.42 ± .06
8.00	17.2 ± 0	6.19 ± 1.0	88.8 ± 15	3.61 ± .36	53.4 ± 0.5	3.64 ± .14	11.4 ± .40	7.98 ± .51	778 ± 58	1.76 ± .01
9.00	20.6 ± .40	5.95 ± .56	-	5.08 ± .48	73.1 ± 7.2	3.87 ± .20	-	-	883 ± 83	1.98 ± .03
10.00	-	-	-	-	-	-	15.8 ± 1.2	9.94 ± 1.0	-	-

12.00
17.5 9.22
± 1.2 ± .81

14.00
20.3 11.8
± .40 ± 2.5

of salicyclic type carboxyl groups provides a mode of Cu(II) binding which does not lead to unimer-unimer linkages. The Cu(II) bound to salicyclic type sites and the intraunimer hydrogen bonded protons will however neutralize the polyion charge density allowing both hydrophobic interactions and cross-linking to be more effective. In this respect, their effect is similar to bound $K^+(H_2O)_x$ at low pH. Here the bound cation cannot cross-link unimers but lowering the charge density allows the other causes of aggregation, including H bonding, to be more effective.

R_{90} , $D_{45/135}$, and $D_{90/135}$ for titrations at pH 6.0 on FA solution filtered through 0.2 μ polycarbonate filters are shown as functions of bound copper, in figure (37). The behaviour of R_{90} is qualitatively similar to that seen for the unfiltered FA solutions but the relative increase at the maximum binding capacity is about 6 times greater. A similar result was found with proton aggregation and is due to greater aggregation effects among the smaller species in solution. The dyssymmetry ratios show an initial increase followed by a decrease which extends over the region where most of the aggregation occurs. This is further evidence of selective aggregation among the smaller species. As with proton induced aggregation, the smaller particles will aggregate to a greater extent with copper (II) binding because of a greater content of reactive functional groups. It also seems likely that this behaviour would not occur if aggregation were due solely to non-specific hydrophobic interactions.

FIGURE 37. Cu(II)-FA LIGHT SCATTERING; FA FILTERED THROUGH 0.2 μ , pH = 6.0, $C_{FA} = 10^{-1}$ g-l⁻¹, NO BACKGROUND ELECTROLYTE;
 ● R_{90} , □ $D_{45/135}$, △ $D_{90/135}$

The dyssymmetry ratios for titrations of unfiltered FA solutions at pH 3.6, 6.0, and 8.0 are reported in figure (38). They behave similarly to each other and those for filtered FA shown in figure (37).

The decrease in polydispersity evidenced by a falling dyssymmetry ratio as the gel point is approached cannot go on indefinitely since at some point, the system will start to behave as if only one size of unimer were present. Thus in these figures, only the dyssymmetry ratio for the lowest total Cu(II) added is plotted at the maximum value of bound Cu(II). Further additions of Cu(II) caused no measurable increase in binding but did result in increased R_{90} and $D_{45/135}$. This is the gel point region where minute increases in binding greatly increase the average particle size and polydispersity.

Potentiometric titrations at pH 6.0 have shown that the Armadale FA has a Cu(II) complexing capacity of $3.3 \text{ mmol-gFA}^{-1}$ at 0.010 g-l^{-1} of FA compared to $4.7 \text{ mmol-gFA}^{-1}$ at 0.100 g-l^{-1} . Light scattering titrations were performed at both FA concentrations to see if this behaviour is reflected in the Cu(II) induced aggregation. The calculated dyssymmetry ratios at the lower FA concentration, seen in Table (IX), were subject to large errors due to the small difference in scattering between the sample and the blank. The large decreases seen in the other experiments were absent. In Table (X), values of R_{90} total and bound Cu(II)-gFA $^{-1}$, and the free Cu(II) concentration are tabulated for titrations at the two FA concentrations. The relative increase in R_{90} is larger at the

FIGURE 38: Cu(II) FA LIGHT SCATTERING: UNFILTERED
 $C_{FA} = 10^{-1} \text{ g l}^{-1}$, VARIATION OF DYSSYMMETRY RATIO, $D_{45/135}$, WITH
 BOUND Cu(II); ○ pH = 3.6, ● pH = 6.0, △ pH = 8.0; NO BACKGROUND
 ELECTROLYTE

TABLE (X)

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CuFA LIGHT SCATTERING-pH 6.0

FA CONCENTRATION

0.100 g l ⁻¹				0.0100 g l ⁻¹			
mmol Cu(II) gFA ⁻¹		10 ⁴ (Cu ⁺⁺)	R ₉₀ /R ₉₀ ⁰	mmol Cu(II) gFA ⁻¹		10 ⁴ (Cu ⁺⁺)	R ₉₀ /R ₉₀ ⁰
TOTAL	BOUND			TOTAL	BOUND		
0.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00
1.00	1.00	0.00	1.08	2.00	1.69	.031	1.12
2.00	1.99	0.01	1.32	4.00	2.39	.161	1.65
3.00	2.89	0.11	2.53	6.00	2.69	.331	2.52
4.00	3.62	0.38	7.60	8.00	2.85	.515	3.34
5.00	4.12	0.88	13.3	10.0	2.90	.710	4.63
6.00	4.49	1.51	18.3	12.0	2.90	.910	5.13
7.00	4.63	2.37	24.7	14.00	2.90	1.11	5.95
8.00	4.63	3.37	36.1				
9.00	4.63	4.37					

lower FA concentration at a given amount of bound Cu(II). This may be surprising in view of the greater binding at the higher FA concentration. These observations can be explained if one assumes that a fraction of the Cu(II) binding sites are involved in co-operative effects with a concentration dependent aggregation equilibrium. Complexation of other sites induces aggregation but not vice-versa. At lower FA concentration, the former aggregates will be more dissociated resulting in less bound Cu(II). At equal mmol Cu(II) bound-g FA⁻¹, this requires more complexation among the sites not involved in co-operative aggregation. Because of the non-linear behaviour of particle size with degree of complexation, the more uneven distribution of bound Cu(II) among these two classes of sites results in greater total scattering.

Since the extent of complexation to a given class of sites at equal concentrations of free Cu(II) depends on the appropriately weighted average equilibrium binding function, total binding is an increasing function of concentration. This is seen in equation (6.7):

$$(\text{CuLi}) = \frac{K_i(\text{HL}_i)}{(\text{H})} (\text{Cu}) \quad (6.7)$$

For the sites not involved in co-operative phenomena, the K_i are not sensitive to FA concentration but for those that are involved, the K_i are increasing functions of C_{FA} . This results in more total binding and more aggregation at constant $(\text{Cu})/(\text{H})$. This is verified experimentally in Table (X).

This simplified model is successful in explaining the qualitative behaviour of the maximum binding capacities and relative intensities of scattered light at two FA concentrations. A more rigorous description would have to account for the continuous variation in unimer properties. In addition, the unimers can form mixed aggregates although results have been presented earlier that show a non-random distribution of unimers among aggregates. This non-randomness is due to selective aggregation among the different unimer species. Equation (4.2) defines $K(x_{ij}, y_j, i = 1, n; j = 1, e)$ as a formal description of the relative preference of a specific unimer for all other types. The importance of specific interactions such as bridging by hydrogen bonding and Cu(II) has been demonstrated experimentally. These interactions are responsible for the mutual preference of unimers with high content of oxygen bearing functional groups.

To summarize, the greater copper (II) binding at the higher FA concentration is the result of co-operative effects between aggregation and complexation. These effects, which have been termed pseudochelation,⁽¹⁴⁾ result from the concentration dependence of aggregation of the copper complexed FA. The uncomplexed FA, in the absence of supporting electrolyte, is partially aggregated but is not affected by concentration variation at constant pH. These conclusions are shown in the following equations:

$$\frac{(Cu L_i) (H^+)}{(Cu^{++}) (HL_i^-)} = K_i \quad (6.9)$$

$$\frac{(Cu L_i L_j) (H^+)}{(Cu L_i) (HL_j^-)} = K_{ij} \quad (6.10)$$

The equilibrium function describing the binding to the i th type of site is given by:

$$\bar{K}_i = \frac{(Cu b_i) (H^+)}{(Cu^{++}) (HL_i^-)} = K_i \left[1 + \sum_j \frac{K_{ij} (HL_j^-)}{(H^+)} \right] \quad (6.11)$$

Here $(Cu b_i) = (Cu L_i) + \sum_j (Cu L_i L_j)$. This scheme describes only the simplest co-operative effect and does not account for the presence of several ligands on a single molecule. It does, however, demonstrate that aggregation induced by Cu(II) complexation can increase the stability of the complex since the partial equilibrium function, $\bar{K}_i$, is concentration dependent. For a polyelectrolyte with several ligand groups on the same polymer molecule, this effect can be enhanced by the formation of pseudo-bidentate ligands through complexation induced aggregation:

The degree of aggregation among the partially complexed molecules and hence the content of effectively bidentate sites per gFA will be concentration dependent.

The particle size analysis method developed in Chapter Three was applied to the results from titrations of filtered FA at pH 6.0. The variation of r_m with σ for the lines bounding the regions for each dyssymmetry ratio is shown in Table (XI). For each solution composition, the regions intersect over large ranges of r_m and σ producing diagrams similar to figure (11). As the bound Cu(II) increases from 0 to 4 mmol-gFA⁻¹, the two regions diverge causing the region of maximum overlap to shift to lower r_m and higher σ . As the bound Cu(II) increases beyond 4 mmol-gFA⁻¹, the regions converge causing a continuous shift to higher r_m and lower σ values.

The opposite trends in r_m and σ seen in the concentration and pH variation experiments result from information being available over a limited range of aggregation in each case. For Cu(II) induced aggregation the polydispersity, as described by σ , first increases and then decreases as aggregation proceeds. This may result from an initial small growth of the larger particles followed by most of the aggregation occurring among the smaller particles. Such a description is also consistent with the behaviour of D_{45/135} alone as seen in figures (37) and (38).

TABLE XI
CuFA LIGHT SCATTERING : THEORETICAL PARAMETERS
FROM PARTICLE SIZE DISTRIBUTION ANALYSIS
- pH 6.0, $C_{FA} = 0.100 \text{ g l}^{-1}$

σ	BOUND Cu(II), mmol gFA ⁻¹											
	0.00		1.99		2.89		3.62		4.49		4.63	
	a	b	a	b	a	b	a	b	a	b	a	b
1.1	123.3	137	166	147.4	152.9	126.5	139.5	111.7	131.7	109.7	142.7	125
	97.2	121	164.5	137	147.2	113.2	133.5	105.7	125.8	11.0	139.4	124
1.3	83.5	99.8	123.6	111	110	88.7	-	76.8	90.5	75.0	100.2	88.0
	63.5	66.0	127.4	102	104.3	77.9	92.0	71.6	85.4	68.2	97.2	87.3
1.5	46.2	63.5	78.6	75.3	66.2	53.5	56.4	43.5	51.3	42.0	58.5	53.0
	33.6	50.0	77.2	65.7	61.7	44.4	52.4	39.8	47.5	37.4	56.2	52.2
1.7	22.2	37.5	45.4	48.3	36.0	29.2	29.9	22.4	25.5	21.5	30.4	29.0
	14.8	26.6	44.2	39.2	32.6	23.0	25.2	19.9	23.1	18.5	28.7	28.2
1.9	10.2	21.1	24.9	29.7	18.4	15.4	14.0	11.0	12.0	10.4	15.0	15.0
	6.4	13.5	24.1	22.5	16.4	11.5	12.5	9.4	10.8	8.5	14.0	14.7
2.1	4.6	11.5	13.0	17.8	9.1	7.8	6.5	5.1	5.5	4.8	7.0	7.7
	2.7	6.8	12.6	12.6	7.8	5.4	5.7	4.3	4.8	3.8	6.5	7.4
2.3	2.0	6.2	6.8	10.4	4.5	3.8	3.0	2.4	2.5	2.2	3.3	3.8
	1.1	3.3	6.5	7.0	3.8	2.5	2.6	2.0	2.1	1.8	3.0	3.6

Visible Fluorescence and Absorption Spectroscopy

Fluorescence excitation spectra of the 520 nm emission are shown in figure (39). They were recorded in the absence of background electrolyte at various pH's. Proton binding causes quenching without a significant shift in wavelength. Similar results were obtained for the emission spectra and for spectra taken in 0.100 M KCl. Absorbance corrected intensities of the 370 nm/480 nm and 465 nm/520 nm emissions are shown as a function of the degree of ionization, α , in figure (40). Corresponding points from separate titrations reproduced within 0-5% of each other. These included titrations performed one month and two years after preparation of the FA batch, indicating that the material is stable under the storage conditions used. At both low and high ionic strength, the 465 nm/520 nm emission is the more sensitive one. In considering possible causes for this, arguments based on molecular or aggregate size must be avoided. This is because the fractionation experiments detailed in Chapter Five show that the fluorophores causing both emissions are distributed in an almost identical fashion among the different molecular sizes..

Uncorrected spectra, similar to those in figure (39), have been reported in the literature as increasing with pH.⁽⁷⁷⁾ The decrease in the 520 nm emission intensity, seen in figure (40), has not been previously noted. This decrease continues to OH^- concentrations as high as 10^{-1} M. Possible causes include collisional quenching by OH^- and effects arising from free

FIGURE 39. APPARENT FLUORESCENCE EXCITATION SPECTRA; EFFECT OF pH ON 520 nm EMISSION; A, pH = 3.6; B, pH = 4.1; C, pH = 4.4; D, pH = 4.7; E, pH = 6.0; F, pH = 8.0.

FIGURE 40. CORRECTED FLUORESCENCE INTENSITIES: DEPENDENCE ON DEGREE OF IONIZATION, α ; $\circ$ 480 nm EM./0.00 M KCl, $\bullet$ 480 nm EM./0.100 M KCl, $\triangle$ 520 nm EM./0.00 M KCl, $\blacktriangle$ 520 nm EM./0.100 M KCl

radical combination as in equation (6.1). Collisional quenching by Na^+ can be ruled out since, as seen in figure (40), the high pH fluorescence is actually an increasing function of ionic strength.

Collisional quenching by OH^- could involve excited state ionization of weakly acidic groups: (151)

here HA^* represents the FA molecules in an electronically excited state and ν, ν' are the frequencies of the absorbed and emitted radiation. The protons may be from phenol groups ortho to ionized carboxylates.

A Stern-Volmer⁽¹⁵²⁾ plot of F_{CORR} against $(\text{OH}^-)^{-1}$ in the high pH region, was non-linear showing that the quenching efficiency is a decreasing function of (OH^-) . Although this is contrary to the collisional quenching behaviour for a single fluorophore it is likely that the emission at a given wavelength is the sum of contributions from fluorophores in different molecular environments. In this case, the quenching efficiency would be a decreasing function of (OH^-) since the non-quenched fluorophores would be less sensitive.

A decreased quantum yield of fluorescence due to free radical recombination is possible in principle. This would result from changes in electronic structure or more available vibrational modes of relaxation to the ground state due to increased molecular weight. It is not possible to distinguish between these mechanisms on the basis of the present data.

The positive correlation between fluorescence and degree of ionization below $\alpha = 0.7$ may result from the following causes:

1. Changes in the electronic structure of fluorophores.
2. Changes in the environment of fluorophores resulting from increasing coiling of polymer molecules as the charge density is lowered.
3. Collisional quenching from free protons.
4. Proton induced aggregation providing a greater number of modes for relaxation to the ground state.

Experiments with Mn^{++} , Na^+ , and K^+ indicate that weakly bound cations have a minimal effect on FA fluorescence. It is unlikely, therefore, that free protons are responsible for the observed decrease with pH. Comparison of Figures (30) and (4) shows that R_0 increases more rapidly in 0.100 M KCl as α falls below 0.4, while the fluorescence quenching rate decreases. Since most of the fluorescence quenching occurs prior to aggregation, the aggregation process itself is not the major cause. Further work is required to determine the relative importance of 1 and 2.

The displacement to high α of the high ionic strength curves

Figure (40) is consistent with a difference in the order of ionization of acidic groups. Those groups to which fluorescence is most sensitive are ionized relatively later in 10^{-1} M KCl. Such a variation is reasonable considering that the FA fluorophores are concentrated among the smaller species in solution. This was determined by gel filtration experiments reported in Chapter Five. The acidic groups on the smaller species will be spaced more closely since numerous studies have shown that acidic group content increases with decreasing particle size.^(2,3) The effect of territorially bound K^+ ions will be to increase the acidity of unionized protons by screening of the electrostatic field through space. The short range, $(dK^0/d\alpha)$, effects involving hydrogen bonding between adjacent acidic groups and polarization will not be substantially affected. Since the field effect is of a long range nature, it will be relatively more important for the larger molecules having a larger average distance between acidic groups. In reducing the field effect, bound K^+ ions will selectively enhance the acidity of groups on the larger, less fluorescent molecules and delay the fluorescence increase to higher α_T . This argument is summarized schematically below:

While the above argument is consistent with the experimental data, other possibilities should be considered. These include a combination of quenching and enhancement effects operating in different regions of α . Such effects may be related to differences in counter-ion binding at different pH's and ionic strengths.

Figure (41) shows the absorbance-corrected emissions at 480 nm and 520 nm as a function of bound Cu(II) at pH 3.6 and 6.0. As with proton induced quenching, the longer wavelength emission is the more sensitive one. The continuously decreasing slopes result from decreasing quenching efficiency of Cu(II) bound to successively weaker binding sites. This may result from an intrinsic difference in the identity of the sites or a difference that results from Cu(II) binding to neighbouring sites. The approximate coincidence of curves at the two pH's indicates the order of site binding is roughly the same. The extra Cu(II) binding at pH 6.0 quenches additional fluorescence. The binding sites which are protected from Cu(II) by bound protons at low pH do not quench nearby fluorophores completely. The more efficient Cu(II) quenching may be due to its d^9 configuration since paramagnetic species can shorten the rate of relaxation to an electronic ground state⁽⁷⁸⁾. Preliminary experiments showed that a large excess of Mn(II) caused about half the maximum quenching obtained with Cu(II) although it has five unpaired electrons to Cu(II)'s one. This demonstrates that the quenching process is static rather than dynamic (collisional).

FIGURE 41.
 VARIATION OF CORRECTED INTENSITIES OF FLUORESCENT EMISSIONS WITH
 BOUND Cu(II): $G_{FA} = 10^{-1} \text{ g l}^{-1}$, NO BACKGROUND ELECTROLYTE.
 ● 520 nm EM., pH = 6.0; ○ pH ~ 3.6
 ▲ 480 nm EM., pH = 6.0; △ pH ~ 3.6.

Both Cu(II) and H^+ induced quenching of fluorescence were reversible within experimental error. For H^+ , this was shown by successive addition of 10 ml of 0.100 M NaOH and 0.100 M KCl to 100 ml of 0.100 g-l^{-1} FA. For Cu(II), the excess of metal ion was removed by passage through a cation exchange resin. Both experiments were carried out with continuous N_2 purging.

Figure (42) shows the light scattering, fluorescence, and bound Cu(II) variation with total Cu(II) concentration at pH 6.0. The fluorescence levels off as the rate of aggregation increases. This behaviour is similar to that seen in proton binding experiments and shows that the fluorescence of aggregating particles has already been quenched. In both cases, fluorescence quenching and aggregation are independent effects of cation binding.

Absorbance measurements on solutions with the same composition as those studied by fluorescence were made for use in equation (2.2) for absorbance correction. For all wavelengths, the absorbance was an increasing function of the degree of ionization. In some cases, the relationship was approximately linear while in others, various types of curvature were found. Because the spectrum is almost featureless, it is difficult to determine the relative contributions of intensity variations and wavelength shifts to this increase.

The $E_{4/6}$ ratio, defined as the ratio of absorptivities at 465 nm and 665 nm behaved in a similar manner to that reported by Chen et al. (67). This included a single maximum at approximately pH 7. Plots of $\log A_\lambda$ vs $\log \lambda$ were approximately linear in agreement with these authors. There was, however, a reproducible positive

FIGURE 42. COMPARISON OF LIGHT SCATTERING, FLUORESCENCE, AND Cu(II) BINDING AT pH = 6.0, NO BACKGROUND ELECTROLYTE.

portion gave a peak at approximately 465 nm. This apparent peak showed a pH dependence similar to that of the $E_{4/6}$ ratio.

The use of this apparent peak in humic structural analysis is risky since the empirical procedure for its resolution has no theoretical basis.

The increase in absorbance at all wavelengths is not due solely to ionization since the addition of a surfactant to un-neutralized FA resulted in similar increases. The degree of ionization did not vary measurably as determined potentiometrically. It is possible that ionization results in structural changes which affect the environment surrounding the chromophores. These changes would in turn affect the absorptivities.

Because both the fluorescent emission at 480 nm and the apparent absorbance peak at 465 nm both show a maximum at about pH 7, it is possible that they are associated with a common process.

✓ This may be the sequential ionization of two different classes of acidic groups. It is difficult to analyse this quantitatively since in a mixture of absorbing chromophores, one cannot easily calculate the true quantum yield of fluorescence.

Conclusions

The results in earlier chapters have demonstrated that light scattering and fluorescence spectroscopy can provide useful information about complexation equilibria between cations and humic substances. Since light scattering monitors molecular or aggregate size and fluorescence is specifically sensitive to cation complexing to smaller species, they are valuable as complementary methods to those involving the direct measurement of cation binding.

In selecting geochemical systems for light scattering studies one must compromise between two extremes. These are unfractionated samples which are the most relevant and highly fractionated ones, which are the most amenable to rigorous investigation. The present study, being the first quantitative attempt at light scattering on a humic system, used a well studied material of low ash content as a starting point. This was extended somewhat by filtration and fractionation experiments. It seems likely that further extensions in both directions may be useful. Studies on highly fractionated samples should allow a more quantitative study of aggregation mechanisms while measurements on unfractionated samples will reveal the distribution of particle sizes in natural water systems.

In analysing light scattering data, the initial strategy was to model the system as a two parameter distribution of spherical Debye scatterers. Determination of the distribution parameters would permit the calculation of average properties corresponding

to experimental variables. The high sensitivity of the theoretical parameters to the experimental data made this impossible. The limits within which the numerical values of the parameters may be estimated have been defined, however. This information could be used for the design of future experiments.

A greater problem involves the question of how realistic the model is. Possible deviations have already been considered in Chapter 4. These concern particle shape, the nature of the particle size distribution function, and the Debye scattering approximation.^(99,100) In principle, the first two can be handled by making measurements at several angles and applying a more sophisticated model. Because a significant portion of the scattering is from particles for which the Debye approximation is poor, the Mie theory^(94,111) should be employed. This will require extensive computation as well as specific refractive index increment measurements on all solutions.

The scattering from theoretical distributions has been used in conjunction with molecular association models to prove that selective association among the smaller species accompanies cation binding. Inter-unimer hydrogen bonding and Cu(II) inner-sphere complexing are more favoured among the smaller species and act to limit the polydispersity of the mixture. This is not trivial since intra-unimer hydrogen bonding and Cu(II) complexing to carboxyl groups on the same aromatic ring also take place. The importance of specific interactions justified the use of theoretical association models involving unimers of specific functionality. While these models are extremely crude, they have helped to identify fundamental properties common to both H^+ and Cu(II) induced

aggregation. This includes the presence of a gel point region in which a small increase in cation binding causes a large particle size increase by linking small aggregates together. Also, the number of linkages is significantly less than the maximum possible for both cations.

Recently, cation binding studies have referred to conformational and/or aggregational changes in humic polymer molecules in explaining pH and FA concentration effects. The present work confirms the importance of aggregation with respect to the concentration dependence of Cu(II) binding capacity.

Also, the absence of a similar effect with proton binding at low ionic strength is observed in both potentiometric and light scattering experiments. At high ionic strength, aggregation is concentration dependent. This predicts that the equilibrium proton dissociation function will be a decreasing function of FA concentration at constant pH. Care is required in relating the effect of particle size to chemical properties. There is a negative correlation between particle size and Cu(II) binding strength as characterized by the differential equilibrium function in samples with differing amounts of bound Cu(II) and/or protons. The contributing factors here result from differences in the extent of cation binding. These include occupation of the intrinsically stronger sites and induced structural changes. The particle growth induced by cation binding may contribute to complex stability but not to the extent of increasing the equilibrium binding function. A particle size increase at fixed fractions of bound sites, however, will increase the binding strength of the unoccupied sites.

While a rigorous description of humic aggregation is not yet possible, some of the properties of such a description were identified. In particular, it must account for the presence of mixed aggregates and the non-random distribution of unimer properties among them. The unimer molecular weight distribution and conditional distribution of important functional groups on molecular weight are necessary information.

Often, in the literature, metal-humic complexing data is interpreted in terms of a monodisperse ligand possessing distinct classes of binding sites. The present study has implications for the usefulness of this model. The distribution of unimer molecular weights is broad ($\bar{M}_w/\bar{M}_n \approx 10$) and contains increasing numbers of smaller species. Since functional group content is also a decreasing function of molecular weight, a large proportion of functional groups are situated on unimers with molecular weights near 10^3 . Thus explanations of chemical behaviour based on a single species with $M = 960$ may be fair approximations in this sense. These explanations do not, however, consider the effects of complexation on the metal binding strength of the remaining sites. These include the long-range and short-range effects discussed in Chapter 6. In this regard, the large variation in equilibrium binding function and the rates of particle size increase and fluorescence quenching as complexation proceeds would also occur if the FA unimer possessed a definite arrangement of equivalent sites. Complexation is largely responsible for the apparent non-equivalence.

3 3

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Of the spectroscopic methods employed, fluorescence, is more promising than UV-visible absorbance. This arises from the fluorophores being segregated on the smaller species and lack of spectral shifts accompanying cation quenching. It is unfortunate that fluorophores emitting at different wavelengths are not unevenly distributed among molecules of different size. In this respect light scattering is more selective since measurements at different angles are differently weighted averages of the molecular weight distribution. In addition, there is a theory which can, in principle, evaluate the weighting factors.

This research work is part of a recent trend over the last few years towards treating humic substances as mixtures of chemically similar but not identical polyelectrolytes. Further progress will be made by characterizing the associated distribution functions more rigorously. Light scattering can play an important role here since one can gain information on different fractions without disturbing the equilibria between them. This is a natural consequence of the fact that scattered light intensities at different angles or wavelengths represent differently weighted averages of these distributions.

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Appendix A

The Effect of Sample Absorbance on Light Scattering

Sample absorbance can affect light scattering experiments by two fundamentally different mechanisms. The first involves attenuation of incident and scattered radiation by the solution while the second relates to the scattering process itself.

The effects of sample absorbance on the intensities of incident and scattered radiation can be estimated from the figure below which shows the geometry of the scattering experiment:

The analysis makes the approximation of an infinitesimal scattering volume. For the semi-octagonal scattering cell, the length of absorbing solution travelled by the incident beam in reaching the scattering volume equals that travelled by scattered radiation in exiting from the cell at each angle of measurement. The apparent Rayleigh scattering ratio, using equation (2.1), is proportional to i_{θ}/i_0 where i_{θ} and i_0 are the intensities of light reaching

the PMT at angles θ and 0° respectively. These intensities are each related to the hypothetical intensities in the absence of sample absorbance by a factor of $10^{-2(\tau_a + \tau_s)}$. Here τ_a and τ_s give the attenuation due to true absorbance and scattering respectively.

Because both i_0 and i_θ are attenuated by the same factor, their ratio gives the Rayleigh ratio in the absence of sample absorbance within the limits of the approximation. The error incurred by the assumption of an infinitesimal scattering volume is negligible for the sample absorbances encountered in the present study. (89)

A more fundamental complication is that the Rayleigh equation (3.7) is not strictly valid for absorbing scatterers. The true scattering behaviour is described by a more complicated expression involving the complex refractive index: (101)

$$m^* = n + iK$$

Here n is the ordinary refractive index and K is related to the extinction coefficient per unit length by:

$$K = \frac{2.303A_\lambda}{4\pi} \quad (A.1)$$

It has been shown that the rigorous expression reduces to equation (3.7) providing that $n \gg K$. The absorbance of pure FA at 633 nm is approximately 500 cm^{-1} . Insertion of this value in equation (A.1) yields $K = 5.8 \times 10^{-3} \ll n = 1.36$, justifying the use of equation (3.7).

Appendix (B)

Equation (2.2) is derived assuming a perfectly collimated, rectangular, incident beam as shown in figure (43). Only radiation emitted at 90° to the incident beam is considered to reach the detector. The measured fluorescence originates from the rectangular region bounded by $x_1, x_2; y_1, y_2$ and having a thickness, h , in the z direction. Consider a thin section of the beam parallel to the x - z plane at a distance y from it and having a thickness dy . The intensity of the beam is given by:

$$\log_{10} \frac{i_0}{i(x)} = \epsilon_{\lambda_1} cx$$

since $A_{\lambda_1} = \epsilon_{\lambda_1} c(1.0 \text{ cm})$,

$$i(x) = i_0 10^{-A_{\lambda_1} x}$$

The power loss per unit area will be:

$$di(x) = -i_0 A_{\lambda_1} 10^{-A_{\lambda_1} x} dx$$

The total radiation absorbed in a small volume element at (x, y) is:

$$-h dy di(x) = h i_0 A_{\lambda_1} e^{-A_{\lambda_1} x} dx dy$$

The fluorescent radiation emitted from this volume element which is sent towards the detector is :

$$df_0(x, y) = qgh i_0 A_{\lambda_1} 10^{-A_{\lambda_1} x} dx dy$$

RIGHT-ANGLE APPROXIMATION FOR
SELF-ABSORBANCE CORRECTION
EQUATION (2.2)

where q = quantum yield of fluorescence

g = geometrical factor proportional to the solid angle subtended by the emission monochromator entrance slit.

The emitted radiation will be attenuated by a factor $10^{-A_{\lambda_2}y}$ before leaving the sample cell. The fluorescent radiation reaching the detector from the volume element at (x, y) is:

$$df(x,y) = qghi_0 A_{\lambda_1} 10^{-(A_{\lambda_1}x + A_{\lambda_2}y)} dx dy$$

The total radiation reaching the detector will be:

$$F = \int_{x_1}^{x_2} \int_{y_1}^{y_2} K A_{\lambda_1} q 10^{-(A_{\lambda_1}x + A_{\lambda_2}y)} dx dy$$

where $K = ghi_0$, an instrumental constant.

$$\begin{aligned} F &= K A_{\lambda_1} q \int_{x_1}^{x_2} e^{-2.303 A_{\lambda_1}x} dx \int_{y_1}^{y_2} e^{-2.303 A_{\lambda_2}y} dy \\ &= \frac{Kq}{(2.303)^2 A_{\lambda_2}} (e^{-2.303 A_{\lambda_1}x_2} - e^{-2.303 A_{\lambda_1}x_1}) \\ &\quad \times (e^{-2.303 A_{\lambda_2}y_2} - e^{-2.303 A_{\lambda_2}y_1}) \end{aligned}$$

Because the fluorescing volume is symmetrical:

$$x_1 = 0.5 - d \quad x_2 = 0.5 + d$$

$$y_1 = 0.5 - d \quad y_2 = 0.5 + d$$

$$F = \frac{Kq}{(2.303)^2 A_{\lambda_2}} e^{-1.151 A_{\lambda_1}} (e^{-2.303 A_{\lambda_1}d} - e^{2.303 A_{\lambda_1}d})$$

$$\times e^{-1.151 A_{\lambda_2}} (e^{-2.303 A_{\lambda_2} d} - e^{-2.303 A_{\lambda_2} d})$$

expanding the exponential terms as:

$$e^{-2.303 A_{\lambda_1} d} = 1 - 2.303 A_{\lambda_1} d + \frac{5.304 A_{\lambda_1}^2 d^2}{2} - 2.036 A_{\lambda_1}^3 d^3$$

etc.

$$F = \left[\frac{k_0 e^{-1.151(A_{\lambda_1} + A_{\lambda_2})}}{(2.303)^2 A_{\lambda_2}} \right] \left[\begin{array}{l} - 4.606 A_{\lambda_1} d - 4.072 A_{\lambda_1}^3 d^3 \\ - 4.606 A_{\lambda_2} d - 4.072 A_{\lambda_2}^3 d^3 \end{array} \right]$$

Simplifying and rearranging:

$$\frac{qA_{\lambda_1}}{F} = \left[\frac{(2.303)^3}{(21.22) d^2 K} \right] \left[\frac{e^{1.151(A_{\lambda_1} + A_{\lambda_2})}}{1 + 1.02(A_{\lambda_1}^2 + A_{\lambda_2}^2)d^2} \right]$$

(qA_{λ_1}/F) is the ratio of true fluorescence to measured fluorescence.

The second term on the right-hand side accounts for sample absorbance of exciting and emitted radiation.

$$F_{\text{CORR}} = F \frac{e^{1.151(A_{\lambda_1} + A_{\lambda_2})}}{1 + 1.02(A_{\lambda_1}^2 + A_{\lambda_2}^2)d^2} \quad (2.2)$$

This expression is approximate because of the simplified geometry in figure (43). In particular, the incident beam is not perfectly collimated. Also, some fluorescent radiation emitted at angles other than 90° may reach the detector. The factor, g , should strictly depend on x, y . Since $2d$ is about 2 mm compared to 25 mm from the centre of the cell to the emission monochromator entrance slit, g will be approximately constant.

Other correction procedures (87, 88) also assume the right-angle approximation and additionally assume that the scattering volume is infinitesimal. This leads to a correction factor of $e^{1.151 (A_{\lambda_1} + A_{\lambda_2})}$. Application of this correction factor to the data in figure (21) produces greater positive deviation from linearity than in figure (22). The correction procedure by Leese and Wehry⁽⁸⁸⁾ accounts for the finite band-pass of the emission and excitation monochromators but not finite fluorescence volume dimensions. It should be possible to combine these two procedures. Neither procedure accounts for the fluorescence from reabsorbed radiation. This effect may be responsible for the positive deviations seen in figure (22).

Appendix E

$\bar{M}_n$ and $\bar{M}_w$ for a ZOLD distribution of spheres are given by:

$$\begin{aligned}\bar{M}_n &= \frac{\int_{r=0}^{\infty} f(r) \frac{4}{3} \pi r^3 \rho \, dr}{\int_{r=0}^{\infty} f(r) \, dr} = \frac{\frac{4}{3} \pi \rho \langle r^3 \rangle}{\int_{r=0}^{\infty} f(r) \, dr} \\ &= \frac{4}{3} \pi \rho r_m^3 e^{7.5(\ln \sigma)^2}\end{aligned}$$

here ρ = density of spheres

$f(r)$ = ZOLD distribution given by equation (3.15)

The averages were evaluated using equation (3.17). Similarly,

$$\begin{aligned}\bar{M}_w &= \frac{4}{3} \pi \rho \langle r^6 \rangle / \langle r^3 \rangle \\ &= \frac{4}{3} \pi \rho r_m^3 e^{16.5(\ln \sigma)^2}\end{aligned}$$

Assuming the M_w of a unimer is $\frac{4}{3} \pi \rho r_u^3$, the number and weight average degrees of polymerization are given by:

$$\bar{x}_n = (r_m/r_u)^3 e^{7.5(\ln \sigma)^2} \quad (E.1)$$

$$\bar{x}_w = (r_m/r_u)^3 e^{16.5(\ln \sigma)^2} \quad (E.2)$$

These two equations can be solved for r_m and σ :

$$\sigma = \exp[(\ln \bar{x}_w / \bar{x}_n)^{1/2} / 3] \quad (E.3)$$

$$r_m = \bar{x}_n^{.6111} r_u / \bar{x}_w^{.2778} \quad (E.4)$$

Appendix (D)

ZOLD PROGRAM-DOCUMENTATION

The program, listed on the following pages, computes the relative light scattering at 45° , 90° , and 135° from a ZOLD distribution spheres by numerical integration of equation (3.8) using Simpson's Rule. As listed, the program is executable on Carleton's Zerox Sigma-9 computer using CP-V. The program computes scattering ratios for a two dimensional array of the distribution parameters, (r_m, σ) . The outer most loop controls r_m and the intermediate loop, σ . Nested in the intermediate loop are three sequential inner loops which add terms to the integral with the coefficients: 4, 2, and 1; where Simpson's Rule is:

$$S_n = \frac{h}{3} [f(a) + 4f(a + H) + 2f(a + 2h) + \dots] \quad (D.1)$$

The important variables are described by function below:

- I - controls outermost loop
- J - controls intermediate loop
- E - controls sequential inner loops
- IR50, DR50, R50 - initial value, increment, and current value of r_m
- ISIG, DSIG, and SIGMA, initial value, increment, and current value of σ
- K - constant to account for solute concentration
- R, RA, RB - current value, and integration limits of r in nm
- N, H - the area to be integrated is divided into 2N segments of width, H

ZED - constant used to vary upper integration limit according to $RB =$

$$ZED \cdot r_m (1 + \Delta)$$

S45, S90, S135 - used for summing the terms in equation (D.1) for scattering at 45° , 90° , and 135°

P - coefficient of terms in equation (D.1), = 4, 2, or 1

LAMBDA - wavelength of incident radiation in nm; 633 in present case

SCAT(ANG, X) - function subroutine which computes the scattering from a sphere of radius $r = \lambda x / 2\pi$ at angle $\theta = \text{ANG}$

LOGNOR - gives the relative number of spheres according to equation (3.15)

SUBROUTINE ARE (R, SIGMA, R50, S45, S90, S135, P, LAMBDA)

- this subroutine evaluates the scattering from all spheres of a given r in the distribution and adds it to S45 etc. For each pair of (r_m, σ) , it is called 2N times.

$$Z = \frac{4\pi r}{\lambda} \sin(\theta/2)$$

RAT45(I,J), RAT90(I,J), and RAT135(I,J) - After completing the integration for a given (r_m, σ) , the contents of S45, S90, and S135 are placed in these locations. Then I and/or J are incremented and S45, S90, S135 are reset.

D45(I,J), D90(I,J) - the dyssymmetry ratios, $D_{45/135}$, $D_{90/135}$, for a given distribution characterized by (r_m, σ)

L(J) - outputting column numbers

This program can be easily modified to handle other forms of two parameter distributions by changing subroutine ARE. The scattering function, SCAT could be replaced with a more rigorous one to handle scattering from particles whose maximum dimension is large compared to λ .

Appendix E

$\bar{M}_n$ and $\bar{M}_w$ for a ZOLD distribution of spheres are given by:

$$\begin{aligned}\bar{M}_n &= \frac{\int_{r=0}^{\infty} f(r) \frac{4}{3} \pi r^3 \rho \, dr}{\int_{r=0}^{\infty} f(r) \, dr} = \frac{\frac{4}{3} \pi \rho \langle r^3 \rangle}{1} \\ &= \frac{4}{3} \pi \rho r_m^3 e^{7.5(\ln \sigma)^2}\end{aligned}$$

here ρ = density of spheres

$f(r)$ = ZOLD distribution given by equation (3.15)

The averages were evaluated using equation (3.17). Similarly,

$$\begin{aligned}\bar{M}_w &= \frac{4}{3} \pi \rho \langle r^6 \rangle / \langle r^3 \rangle \\ &= \frac{4}{3} \pi \rho r_m^3 e^{16.5(\ln \sigma)^2}\end{aligned}$$

Assuming the M_w of a unimer is $\frac{4}{3} \pi \rho r_u^3$, the number and weight average degrees of polymerization are given by:

$$\bar{x}_n = (r_m/r_u)^3 e^{7.5(\ln \sigma)^2} \quad (E.1)$$

$$\bar{x}_w = (r_m/r_u)^3 e^{16.5(\ln \sigma)^2} \quad (E.2)$$

These two equations can be solved for r_m and σ :

$$\sigma = \exp[(\ln \bar{x}_w / \bar{x}_n)^{1/2} / 3] \quad (E.3)$$

$$r_m = \bar{x}_n^{1/3} r_u \bar{x}_w^{2/3} \quad (E.4)$$

The number and weight-average degree of polymerization for a single unimer association model were calculated from equations (4.6) and (4.7). These values were used in equations (E.3) and (E.4) to evaluate the ZOLD parameters. The dyssymmetry ratios were then evaluated using figures (5) and (6).

The observed scattering from a collection of spheres described by two ZOLD distributions, a and b, will be:

$$R_{45} = R_{a45} + R_{b45}$$

$$R_{90} = R_{a90} + R_{b90}$$

$$R_{135} = R_{a135} + R_{b135}$$

The observed dyssymmetry ratios will be:

$$D_{45/135} = \frac{R_{a45} + R_{b45}}{R_{a135} + R_{b135}} \quad (E.5)$$

$$D_{90/135} = \frac{R_{a90} + R_{b90}}{R_{a135} + R_{b135}} \quad (E.6)$$

Using equation (4.10):

$$\frac{R_{a45}}{R_{b45}} = \frac{r_{ma} \omega_a e^{7.5(\ell n \sigma)^2}}{r_{mb} \omega_b e^{7.5(\ell n \sigma)^2}}$$

and the relationships $D_{a45/135} = R_{a45}/R_{a135}$

$$D_{a90/135} = R_{a90}/R_{a135}$$

$$D_{b45/135} = R_{b45}/R_{b135}$$

$$D_{b90/135} = R_{b90}/R_{b135}$$

one can convert (E.5) and (E.6) to (4.11) and (4.12)

END

1 6 1 0 6 1 8 3

FIN