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A New Method to Determine the Degree of Solvation of Macromolecules

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During the last two decades many physico-chemical investigations have been carried out to elucidate the mutual interaction between the solvent and the solute molecules. As a special problem the solvation of macromolecular substances has been closely studied.

I. INTRODUCTION

In a solution the solvent molecules generally form a solvate mantle around the solute molecules. Some investigators differentiate two kinds of solvation see 1, 2. The solvent molecules close to the solute molecule (»touching» it) are so firmly attached to it — either by secondary valences, for instance hydrogen bonds, or by van der Waal's forces — that they continually follow the movements of the macromolecule, forming a thin layer around it. This is called »primary solvation» (Moll[3]), »proper solvation» (Fikentscher and Mark[4]) or »chemical solvation» (Schulz[5]). In a wider sense the solvation comprises all solvent molecules within the range of the attractive forces from the solute molecule and also the solvent molecules mechanically immobilized in holes, micelles etc. of the high polymer molecules. The volume of the solvated particles in this sense, tentatively calculated from viscosity data, is of quite another order of magnitude, containing as much as several hundred ml of solvent per gram of solute.

Various methods have been employed for the elucidation of the solvation problem. The principles of the more general ones are briefly the following (for a complete review *cf.* Eirich and Mark[1] and Philippoff[2]):

1. Sorption of solvent vapours. The substance is swelled in the solvent vapour at various pressures and temperatures and the degree of solvation is calculated from the increase in weight.

2. Adsorption of solvent from a mixture of solvent and nonsolvent (Methode des nichtlösenden Raumes). The substance is immersed in a mixture, and the amount of solvent taken up is measured by observing the changes in the composition of the mixture before and after the addition of the substance.

3. Thermochemical measurements. From the heat of swelling, solution, and dilution of the substance, as functions of concentration, the degree of solvation can be calculated.

4. Thermodynamic calculations. The thermodynamic quantities free energy, heat content, and entropy of solution can be calculated from osmotic pressure, lowering of vapour pressure, and temperature dependence of the heat of dilution. From these data an approximate estimate of the degree of solvation of the dissolved substance is obtained.

5. Dielectric measurements. The dielectric constant of a solution of a given concentration is connected also with the degree of solvation of the solute molecules.

Furthermore conclusions on the solvation can also be drawn from viscosity data, X-ray diffraction, and in special cases colour change of swelling substances containing an indicator, for instance Cobaltsalts with water.

The first two of these methods are the simplest and the most commonly used. But the disadvantage of them is that the measurements must be made in solutions of high concentrations sometimes near to the jelly state. The method described in this article is similar to that of type two, but it has the advantage of being applicable even for dilute solutions.

II. THE NEW METHOD

The method is based on the possibility to remove the macromolecules with their primary solvate mantles from a solution by sedimentation in the ultracentrifuge (for a description *cf.* the monograph by Svedberg and Pedersen[6]). If the substance is dissolved in a mixture of a solvent and a nonsolvent, the solvent molecules alone forms a primary solvate mantle around the macromolecule and it can be assumed that the number of the solvent molecules taking part in the solvation of the solute molecule is independent of the nonsolvent used. During the ultracentrifugation the macromolecules with their primary solvate mantles are sedimenting in a mixture of solvent and nonsolvent. Thus the sedimentation gives a solute-free layer of solvent-nonsolvent mixture, above the sedimenting boundary, in which, due to the transport of solvent molecules in the solvate mantles of the sedimenting solute molecules, the concentration of the nonsolvent is higher than in the original solvent-nonsolvent mixture. If solvent and nonsolvent have about the same density and the same compressibility the centrifugal field does not cause any separation of the liquids by sedimentation. From the amount of solvent transported by the solute the solvation number (S), *i. e.* the number of solvent molecules per chain unit taking part in the primary solvate mantle, can be calculated. In the first experiments, we tried to analyse the solvent mixture by measuring the refractive index before and after the removal of the solvated macromolecules from it by sedimentation. Reproducible results were not obtained, however, probably because of evaporation from the small amounts of solution available from the experiment (the volumes of the ultracentrifugal cells used were only 0.5—0.6 ml) and the rather small changes in nonsolvent concentration, which can be obtained at the low solute concentrations applicable for this purpose. Likewise, it was not possible to obtain a solute-free sample, owing to the fact that at least a part of the solute, removed from the solution by sedimentation, was again mixed with the solute free layer on stopping the rotor. This disadvantage could have been avoided by using a so-called separation cell, in which a horizontal porous false bottom separates the upper part of the cell from the lower preventing the solutions in both parts from mixing with each other. For lack of a suitable separation cell another method was worked out, based on Lamm's scale method for measuring the refractive index gradient (dn/dx) at any point in the cell *cf.* 6, p. 253. By integration of the gradient curve between any two points we obtain the refractive index difference between these points.

$$n_2 - n_1 = \int_{x_1}^{x_1} \frac{dn}{dx} \cdot dx$$

Thus, the refractive index difference between the solute free solvent mixture layer and the sedimenting solution is directly proportional to the area of the maximum of the sedimentation picture. Since the refractive index of the later can be measured (= the refractive index of the original solution), the refractive index of the former can be obtained, and finally, the amount of solvent removed from the solution together with the solute molecules can be calculated from the refractive index difference between the solute free solvent mixture and the original solvent mixture.

For the application of this method for measuring the solvation, the following assumptions were made:

1. The refractive index of the solvent mixture is calculable from those of the components on a molar ratio basis (interaction between solvent and nonsolvent is not taken into account).
2. No nonsolvent molecules are absorbed by the macromolecules in their primary solvate mantles.
3. The refractive index increment of the solute in the mixed solvent is proportional to the concentration and independent of the ratio solvent — nonsolvent.

These assumptions are probably valid to a first approximation for the systems used for this investigation. The proof of their validity would require series of accurate measurements. The first experiments were made without the last assumption. The refractive index of each solution was measured by an interferometer, but evidently due to evaporation of the two liquids, reproducible results were not obtained. The refractive indices of all solutions were later calculated with the refractive index increment of the solute in pure solvent.

In the calculation the following symbols were used:

n_s = the refractive index of the solvent
n_n = » » » » » non-solvent
n_0 = » » » » » solvent mixture
n = » » » » » solution
n_0' = » » » » » solvent mixture after the solute has been removed by centrifuging
$\Delta n = n - n_0$
$\Delta' n = n - n_0'$
m_s = the molar percentage of solvent in the solvent mixture
m_n = » » » » non-solvent » » »
Δm = number of solvent moles sedimentated in the solvate mantles per 100 moles of original solvent mixture
M_s = the molecular weight of the solvent
M_n = » » » » » non-solvent
M_0 = » mean molecular weight of the solvent mixture
M = » basic molecular weight of the solute investigated (the molecular weight of the chain unit).
$\frac{dn}{dx}$ = refractive index increment
c = the weight of the solute in grams in 100 grams of solvent mixture

C = the amount of solute in moles of monomer units in 100 moles of solvent mixture

Then we have the following relations:

$$n_0 = \frac{m_s \cdot n_s + m_n \cdot n_n}{100}$$

$$C = M_0 \cdot \frac{c}{M}$$

$$M_0 = \frac{m_s \cdot M_s + m_n \cdot M_n}{100}$$

$$\Delta n = c \cdot \frac{dn}{dc}$$

$$(m_s - \Delta m + m_n)\ n_0' = (m_s - \Delta m)\ n_s + m_n \,.\, n_n$$

hence

$$\Delta m = m_s - m_n \frac{n_n - n_0'}{n_0' - n_s} \qquad (1)$$

and the solvation number is

$$S = \frac{\Delta m}{C} \qquad (2)$$

being the number of solvent molecules bound to the solute per chain unit.

Of the values required in these calculations those for n_s, n_n, M_s, M_n, and often also that for M, may be found in the literature. Δn can be calculated if the refractive index increment of the solute in the pure solvent in question is known and $\Delta' n$ can be measured from the sedimentation picture (see later).

Thus, on the basis of the above, the conclusion can be drawn that the substances used in the experiments must fulfil the following conditions:

1. The solvent mixture consists of two components, component 1 being a good solvent with respect to the substance to be investigated on which substance component 2 is quite incapable of adsorption *. The components must be soluble in each other in the ratios used for the measurements.

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* In the presence of a good solvent the danger of non-solvent adsorption on the solute molecules is evidently negligible.

2. The liquid components must possess as accurately as possible the same density and, in the solvent mixture used, about the same vapour pressure, but refractive indices which differ from each other as much as possible.

3. If the macromolecular substance to be investigated is polydispers, fractionated samples should be used, giving regular and well-defined sedimentation pictures for the calculation of the solvation numbers.

III. EXPERIMENTS

In the experiments carried out the solvation of cellulose nitrate in acetone, ethyl and butyl acetate was studied with benzene and xylene as non-solvents. The nitrogen content of the cellulose nitrate samples used varied from 11.7 to 13.2 % and all the samples were moderately polydisperse. Samples 682 and 708 were technical products of the Gyttorp factory and VF3 was studied by Jullander[7], while samples II 10 b and II 10 c were products of our own fractionation. The properties of the cellulose nitrate samples are evident from Table 1.

Table 1. The properties of the cellulose nitrate samples used.

Sample	N %	Degree of nitration	Basic molar weight	lim (η_{sp}/c)	lim s
II 10 b	13.19	2.63	280.5	2.3	11.4
II 10 c	13.19	2.63	280.5	1.2	
682	12.70	2.48	273.6		
708	11.75	2.18	260		
VF3	12.35	2.37	269	0.64	8.3

Table 2 shows the densities and refractive indices of the solvents and non-solvents used in the experiments and Table 3 the refractive index increments used in calculating the solvation numbers.

Table 2. The densities and refractive indices of the solvents and non-solvents used.

No.	Solvent	Density	Refractive index
A	Butyl acetate	0.882	1.39510
B	Ethyl acetate	0.901	1.37257
C	Acetone	0.792	1.35886
D	Benzene	0.894	1.50142
E	*m*-Xylene	0.868	1.49962

Table 3. The refractive index increments (dn/dc) of cellulose nitrate samples used in different solvents (concentration c = 1 g/100 g solution).

Sample	N %	Butyl acetate	Ethyl acetate	Acetone
II 10 b	13.19	74	(78)	84.5
II 10 c				
683	12.70	77		
708	11.75	80		

Of these increment values the one in acetone solution was measured by Jullander[7] and that in ethyl acetate is estimated on the basis of the increments in butyl acetate and amyl acetate.

Table 4 shows the results of the experiments. The composition of the solvent mixture is given in molar percents and the cellulose nitrate content of the solution in basic moles per 100 moles of solvent mixture.
The values in column $\Delta' n$ are generally mean values of the areas of the maxima of 3 to 4 sedimentation diagrams and the values in parentheses indicate the mean errors. In the two last columns the calculated solvation numbers per glucose unit and per nitrate group are given.

From Table 4 it is evident that the method gives reproducible results. The solvation numbers calculated from different parallel experiments differ not more then ± 10 per cent and often much less. The addition of nonsolvent does not seem to cause any association of the cellulose nitrate molecules; the sedimentation constants of the substances are the same both in pure solvent and solvent-nonsolvent mixtures (corrections for the change in density and viscosity must be introduced). Near the critical concentration association certainly occurs however. As the cellulose nitrate concentration and the proportion of nonsolvent added seem to have no effect on the solvation in the intervals used, the mean values of the solvation numbers S for each cellulose nitrate sample in the different solvent-nonsolvent pairs have been calculated, and collected in Table 5.

When calculating the values given in Table 5, experiment S 10, which gave a clearly erroneous result, and also experiment S 1, which was the only measurement with sample VF 3, were not taken into consideration. A mean value common for samples II 10 b and II 10 c has been calculated because these samples are subsequent fractions of a fractionation and apparently differ from each other only as to their degree of polymerization. The degree of nitration of these samples can differ from the value 2.63 used because this value has

Table 4. The data of solvation measurements.

Expt. no.	Solvent mixture	Cellulose nitrate	Degree of esterification	C	S	Δn	$\Delta' n$		Δm	S/gluc.	S/NO_3
S 3	42.74 A + 57.26 D	II 10 b	2.63	0.161	3.15	35.4	9.2	(1.1)	0.329	2.04	0.78
S 27	54.58 A + 45.42 D	II 10 b	2.63	0.110	3.34	23.0	11.2	(0.6)	0.225	2.05	0.78
S 28				0.0705	3.50	14.8	9.1	(0.2)	0.119	1.69	0.64
S 29				0.110	3.50	23.0	13.0	(1.3)	0.208	1.89	0.72
S 14		682	2.48	0.122	4.02	25.9	12.2		0.284	2.33	0.94
S 15				0.122	3.99	25.9	12.6	(2.1)	0.276	2.26	0.91
S 10				0.0986	4.67	21.0	7.4	(0.0)	0.282	(2.86)	(1.15)
S 16		708	2.18	0.0988	3.84	20.8	11.6	(0.2)	0.199	2.01	0.92
S 17				0.0657	4.95	13.8	7.4	(0.1)	0.133	2.02	0.93
S 18				0.0657	4.99	13.8	7.9	(1.0)	0.123	1.87	0.86
S 4	69.35 A + 30.65 D	II 10 b	2.63	0.222	2.58	44.2	22.4	(0.2)	0.604	2.72	1.03
S 5				0.151	2.99	30.0	18.5	(0.9)	0.351	2.32	0.88
S 6				0.0805	3.54	16.0	10.5	(0.5)	0.167	2.07	0.79
S 25	50.00 A + 50.00 E	682	2.48	0.100	4.11	18.9	3.7	(0.8)	0.290	2.90	1.17
S 26				0.100	4.07	18.9	4.0	(0.3)	0.284	2.84	1.15
S 23				0.0715	4.82	13.6	2.5	(0.3)	0.212	2.97	1.20
S 24				0.0715	4.86	13.6	1.2	(0.0)	0.237	3.31	1.33
S 19		708	2.18	0.0692	4.74	13.0	2.9	(0.8)	0.193	2.79	1.28
S 20				0.0692	4.53	13.0	3.2	(0.5)	0.189	2.73	1.25
S 21				0.0461	5.95	8.6	2.1	(0.4)	0.126	2.73	1.25
S 22				0.0461	5.74	8.6	2.4	(0.4)	0.120	2.60	1.19
S 1	46.99 B + 53.01 D	VF3	2.37	2.17	1.12	550	17.3	(2.0)	5.23	2.41	1.02
S 7	75.09 B + 24.91 D	II 10 c	2.63	0.121	4.14	30.8	17.8	(1.2)	0.405	3.35	1.27
S 8				0.0803	4.87	20.5	12.0	(0.0)	0.265	3.29	1.25
S 9				0.0601	5.04	15.4	10.7	(0.4)	0.148	2.46	0.94
S 33	70.00 C + 30.00 D	II 10 b	2.63	0.119	5.71	43.8	25.5	(2.5)	0.426	3.58	1.36
S 30				0.0736	6.72	27.1	13.0	(1.5)	0.329	4.47	1.70
S 32				0.0534	7.33	19.7	12.0	—	0.180	3.37	1.28
S 31				0.0445	7.56	16.4	7.6	(0.9)	0.205	4.61	1.75

Table 5. The mean values of the results of the solvation measurements.

Solvent mixture	Cellulose nitrate	Degree of nitration	S/gluc.	S/NO_3	Error
Butyl acetate	II	2.63	2.11	0.80	0.08
Benzene	682	2.48	2.30	0.92	0.01
	708	2.18	1.97	0.90	0.03
Butyl acetate	682	2.48	3.00	1.21	0.06
Xylene	708	2.18	2.71	1.24	0.02
Ethyl acetate Benzene	II	2.63	3.03	1.15	0.14
Acetone Benzene	II	2.63	4.02	1.52	0.20

been obtained for unfractionated cellulose nitrate, of which the samples II 10 b and II 10 c are almost the very lowest fractions. It is evident from the average errors (calculated as molecules of solvent per nitrate group) contained in the last column of the table that the data obtained from the measurements made in mixtures of butyl acetate and benzene as also of butyl acetate and xylene can be considered very reliable, while in other mixtures less accurate results have been obtained. This inaccuracy of the tests in the ethyl acetate-benzene mixture might possibly be due to the incorrect refractive index increment used, while that in the acetone-benzene mixture might be caused by the very different volatilities of the solvent components.

Summing up the solvation measurement tests given in Table 5, it is noted that the solvation number calculated as solvent molecules per glucose unit is dependent on the degree of esterification of cellulose nitrate. Above all, this is evident from the data obtained from the very successful test series made with samples 682 and 708. The solvation numbers of sample 708 calculated per glucose unit are 10—15 per cent lower than those for sample 682, but on calculating the solvation numbers per nitrate group this difference disappears. From this it may be concluded that solvation it apparently solely a phenomenon existing between the nitrate groups and the solvent molecules.

Moreover it is of interest to note that test S 1 has given a solvation number of the same magnitude as those of tests S 7—S 9, although the concentration of the former is about 7 per cent giving a gelatinous solution and those of the latter are only about 0.2—0.4 per cent. This shows that no difference exists in degree of solvation on transition from the gel-solution to the sol-solution.

It is evident that the solvation numbers given in Table 5 represent the minimum value of solvation, *i. e.* they report the smallest possible number of solvent molecules which on centrifuging are removed from the solution, together with the cellulose nitrate molecules to give the changes obtained in the refractive index measurements. If, on the other hand, the nonsolvent molecules also sediment with the solute molecules, the number of sedimenting solvent molecules must be greater than the values given in Table 4, and, indeed to an extent, which depends on the number of sedimenting nonsolvent molecules corresponding to the molar ratio of solvent mixture components.

On the basis of the data obtained from these tests the average solvation number of cellulose nitrate in butyl acetate is 0.85—0.91 when benzene is used as nonsolvent, but 1.23 in a solution containing xylene. It is difficult to explain the cause of this difference. Moll[3] has calculated, on the basis of the sorption investigation data given by McBain and collaborators[8], the solvation numbers for cellulose nitrate (the degree of nitration is not given) in different solvents and nonsolvents and reports that the solvation number in benzene is 0.33 molecules per glucose unit. Assuming that cellulose nitrate in butyl acetate solution also combines with this number of benzene molecules — which seems to be hardly probable — it would increase the solvation number only by 0.10—0.15 per nitrate group. Hence this does not explain the difference. The only possible explanation remaining is the suggestion that some nonsolvent molecules are involved in the primary solvate mantles mainly containing butyl acetate, and according to the results (Table 5) benzene molecules in a considerably greater quantity than xylene molecules. This can be an effect of association of solvent and nonsolvent molecules. If this is the case, both benzene and xylene should give too low solvation numbers and the actual solvation number for cellulose nitrate in butyl acetate would be at least 1.2 molecules per nitrate group.

Conclusions drawn from X-ray analysis infer that xylene, which as such is inert with regard to cellulose nitrate, associates with this if a small amount of acetone is present[9]. The X-ray diagram of this solvate is pronounced if the amount of acetone present is under 10 per cent but changes rapidly into a cellulose nitrate-acetone solvate diagram when the acetone concentration increases. On the basis of this it is obvious that the possibility of a nonsolvent action in the solvation of cellulose nitrate cannot be excluded. For a definite decision more experiments with other nonsolvents must be carried out.

Assuming Moll's report on the solvation number in butyl acetate conforms with the result, 1.23, obtained above, the degree of nitration of the cellulose nitrate used in McBain's investigations must be about 2. The solvation number 3.1 for cellulose nitrate in acetone solution reported by Moll then also conforms with the number 1.55 in Table 5. On the contrary the solvation number in ethyl acetate obtained in this investigation does not seem to agree with Moll's value. This may be due to an incorrect refractive index increment used. It is true, that McBain's test does not include measurements in ethyl acetate, but on the basis of the data obtained in butyl, propyl, and methyl acetate it is not improbable that for the solvation number in ethyl acetate a value of 3.2—3.3 might have been obtained. This would give 1.6 molecules of ethyl acetate per nitrate group which is about 50 per cent higher than that obtained from ultracentrifuge measurements.

SUMMARY

1. A method is developed for determining the degree of solvation of substances sedimenting in the ultracentrifuge. This method is based on the fact that on dissolving a substance in a mixture of solvent and nonsolvent the solute molecules adsorb solvent molecules which form a primary solvate mantle.

2. The conditions which the components of the solvent mixture and the solute must fulfil in order that this method should give reliable data are discussed.

3. Using the method described the degree of solvation of some cellulose nitrate samples in four different solvent mixtures have been measured. Thus, the fact that the solvation number of cellulose nitrate in organic solvents is proportional to the degree of nitration is confirmed, wherefore it is likely that the hydroxyl groups do not take part in the solvation process of cellulose nitrate with organic solvents.

4. The obtained solvation numbers are minimum values of primary solvation.

5. The solvation numbers calculated from the measurements are for the great part in conformity with the data obtained by other methods. The solvation number in butyl acetate (possibly also in other solvents) seems to be dependent on the nonsolvent used.

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