[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Influence of Concentration upon the Sedimentation Rate of Tobacco Mosaic Virus

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I. Introduction

It is a well established fact that macromolecular substances with high intrinsic viscosities have sedimentation rates in the ultracentrifuge which depend strongly upon concentration. Previous studies have shown that tobacco mosaic virus protein is among the materials exhibiting this behavior.[1] During the course of a study of the physical properties of a well-defined, essentially monodisperse preparation of the virus, an opportunity to examine in greater detail the dependence of sedimentation rate upon concentration was presented. The present report is concerned with the description of the results obtained and the discussion of a simple mechanism proposed to account for this behavior.

II. Experimental Results

Sedimentation studies were carried out on virus Preparation A, described in a previous publication,[2] dissolved at various concentrations in 0.1 *M* phosphate buffer at *p*H 7. A Bauer–Pickels[3] air-driven ultracentrifuge equipped with Lamm scale[4] and Svensson schlieren[5] optical systems was used. The data are presented in Table I.

TABLE I

THE SEDIMENTATION CONSTANTS CALCULATED IN THE USUAL MANNER AND THOSE CORRECTED FOR SOLUTION VISCOSITY OF TOBACCO MOSAIC VIRUS PREPARATION A AT VARIOUS CONCENTRATIONS

Concentration (g./100 cc.)	s^0_{20} (Svedberg units)	s'^0_{20}
0.05	181	184
.102	180	185
.20	173	183
.20	170	180
.20	170	180
.20	170	180
.204	173	183
.408	168	188
.816	153	189
1.02	146	189
1.43	134	188
1.43	131	184
1.84	125	191

It may be observed that the sedimentation constant s^0_{20} of virus Preparation A decreases markedly as the concentration is increased. As is shown graphically in Fig. 1, the reciprocal of the sedimentation constant is a linear function of concentration. The dependence of the sedimentation constant on the concentration of tobacco mosaic virus was reported previously, but, probably owing to complications arising from progressive aggregation of the samples used in that study, the linear dependence of the reciprocal of the sedimentation constant upon concentration was not observed.[1] Kraemer and Lansing[6] assumed the reciprocal of the sedimentation constant of ω-hydroxydecanoic acid polymers to be a linear function of concentration, and relationships of that sort have since been established by Signer and Gross[7] for polystyrene, by Kraemer[8] for gelatin in non-gelating solvents, by Carter[9] for sodium nucleohistone, and by Lauffer and Flory[10] for polybutylenes. The straight line fitted to the data of Fig. 1 can be described by the equation

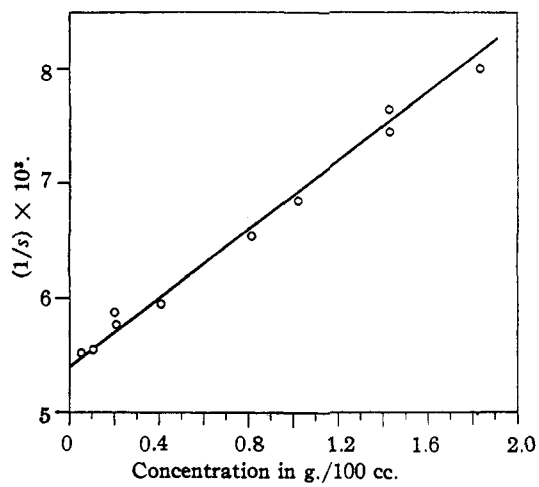

Fig. 1.—The reciprocal of the sedimentation rate in Svedberg units plotted as a function of the concentration of Preparation A of tobacco mosaic virus.

Eq. 1 $$10^3/s = 5.40\ (1 + 0.278C)$$

where s is the sedimentation constant in Svedberg units and C is the concentration of virus in grams per 100 cc. The straight line fitting the viscosity data[2] for Preparation A can be described by the equation

Eq. 2 $$\eta/\eta_0 = 1 + 0.285C$$

(1) M. A. Lauffer, *J. Phys. Chem.*, **44**, 1137 (1940).
(2) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).
(3) J. H. Bauer and E. G. Pickels, *J. Exptl. Med.*, **65**, 565 (1937).
(4) O. Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); **A143**, 177 (1929).
(5) H. Svensson, *Kolloid-Z.*, **87**, 191 (1939).
(6) E. O. Kraemer and W. D. Lansing, THIS JOURNAL, **55**, 4319 (1933).
(7) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 59 (1934).
(8) E. O. Kraemer, *J. Phys. Chem.*, **45**, 660 (1941).
(9) R. O. Carter, THIS JOURNAL, **63**, 1960 (1941).
(10) M. A. Lauffer and P. J. Flory, unpublished results.

where η is the viscosity of the solution and η_0 that of the solvent. It is thus apparent that the variation in the reciprocal of the sedimentation constant with the concentration of tobacco mosaic virus is almost identical with the variation of the relative viscosity of the virus solutions with concentration. The sedimentation data listed as s^0_{20} in Table I were reduced to a standard state corresponding to sedimentation in a solvent having the viscosity and density of water at 20° by correcting for the density of the virus solution and for the viscosity of the solvent in the manner accepted by ultracentrifugation investigators. The data under consideration indicate that, had the sedimentation data been corrected for the viscosity of the solution instead of for that of the solvent, the sedimentation constant would have been essentially independent of concentration. The data tabulated as s'^0_{20} in Table I are corrected in this manner. In making this correction it was assumed that the relative viscosity of the solution is equal to the product of the intrinsic viscosity and the concentration. This should give low values for the relative viscosities of concentrated solutions. The corrected sedimentation rates were obtained by multiplying the conventional values by the relative viscosities for the concentration under consideration. It may be seen that the use of this procedure does result almost in the elimination of the variation of sedimentation rate with concentration. It should be observed, however, that had the real relative viscosities been used for the corrections, instead of the fictional values calculated by multiplying intrinsic viscosity by concentration, the sedimentation rates at high concentrations would have been over-corrected somewhat. Sedimentation rates for tobacco mosaic virus corrected in this manner tend to increase slightly with concentration, but the effect is slight compared with the decrease observed when rates are reported in the conventional manner. In accordance with these results, it is proposed that sedimentation constants be corrected for the viscosity of the solution and not for that of the solvent. Further justifications for this proposal are presented in the discussion.

During the course of sedimentation in a sector-shaped cell, the concentration of the sedimenting material decreases according to the equation

Eq. 3 $$C_t = C_0(X_0/X_t)^2$$

where C_0 and C_t are the concentrations and X_0 and X_t are the positions of the boundary with respect to the axis of rotation initially and at the time t, respectively. Because of this decrease in concentration, the solution viscosity must decrease as sedimentation progresses. Sedimentation rate should therefore increase as the experiment progresses. This was pointed out by Sanigar, Krejci and Kraemer.[11] The data of Table I were not corrected for such an effect.

(11) E. B. Sanigar, L. E. Krejci and E. O. Kraemer, THIS JOURNAL, **60**, 757 (1938).

An experiment was carried out on Preparation D of tobacco mosaic virus described elsewhere[2] in order to test the reality of such an effect. A solution containing 13.35 mg. of the virus per cc. of 0.1 *M* phosphate buffer at *p*H 7 was centrifuged at a speed of 11,100 r. p. m. The temperature of the rotor was 25.3° at the beginning and 25.7° at the end of the run. The position of the boundary was recorded photographically at five-minute intervals. A total of 33 pictures was taken. Sedimentation constants were calculated for the successive intervals and are recorded in the third column of Table II. The sedimentation rate not corrected for temperature and solvent was found by the method of least squares to depend upon the time of centrifugation according to the equation,

Eq. 4 $$s = 144.30 + 0.1013t$$

where t is the time in minutes. The standard error of estimate, a statistic somewhat analogous to the standard deviation and one which measures the scatter of data about the best fitting graph, was 1.87% of the intercept. Statistical calculations were carried out which showed that the chances that the observed variation of sedimentation rate with time is due to errors of observation are about one in a million. This result confirms the decrease in sedimentation rate with increase in concentration (because of equation 3).

The viscosity of this virus preparation at concentrations up to that studied in the centrifuge was determined as described elsewhere.[2] It was found that the equation

Eq. 5 $$\ln \eta/\eta_0 = 0.305C$$

fits the data over the entire range studied. This would correspond to an intrinsic viscosity on a volume basis of 41.8, a value in good agreement with that of 39 found for Preparation A.

The virus concentration corresponding to each sedimentation interval was next computed by means of the equation 3. Then the relative viscosity of the virus solution for each interval was calculated by using the experimentally determined relationship between viscosity and concentration, equation 5. The results are tabulated in column 4 of Table II. The sedimentation constant for each interval was then corrected by multiplying by the relative viscosity of the solution, and the results are recorded in column 5 of Table II. The data were found by the method of least squares to fit the equation

Eq. 6 $$s' \times 10^{13} = 215.01 - 0.03115t$$

The standard error of estimate was 1.75% of the intercept and the probability that the observed negative correlation of sedimentation rate with time is due solely to random errors was evaluated as 0.047. This means that the chances are better than 20 to 1 that the observed decrease is real. However, the liklihood is less that the calculated value of the slope is nearly correct. This result confirms the small over-correction of sedimentation rate when actual viscosities are used.

TABLE II

VARIATION IN THE SEDIMENTATION RATE OF TOBACCO MOSAIC VIRUS DUE TO DILUTION DURING THE COURSE OF A SINGLE DETERMINATION

Exposure number	Distance from axis in cm.	s (Svedberg units)	η/η_0	s cor. for η/η_0	$\eta'/\eta_0 = 1 + C[\eta]$	s cor. for η'/η_0
1	5.8311	145	1.484	216	1.395	203
2	5.8654	144	1.477	212	1.390	200
3	5.8995	153	1.470	225	1.385	212
4	5.9360	147	1.462	215	1.381	203
5	5.9715	145	1.456	212	1.376	200
6	6.0068	146	1.450	212	1.372	201
7	6.0424	149	1.444	215	1.367	203
8	6.0788	146	1.438	210	1.363	198
9	6.1147	148	1.432	212	1.359	201
10	6.1514	148	1.426	211	1.355	200
11	6.1882	149	1.420	212	1.350	202
12	6.2256	153	1.414	217	1.346	207
13	6.2643	150	1.408	211	1.342	201
14	6.3024	150	1.402	211	1.338	201
15	6.3406	151	1.396	210	1.334	201
16	6.3795	154	1.390	214	1.329	205
17	6.4593	154	1.385	213	1.325	204
18	6.4193	159	1.379	220	1.321	209
19	6.5010	159	1.373	218	1.317	209
20	6.5428	155	1.368	212	1.313	204
21	6.5839	151	1.362	206	1.309	198
22	6.6244	154	1.357	209	1.306	201
23	6.6657	154	1.352	208	1.302	201
24	6.7073	157	1.347	212	1.298	204
25	6.7500	156	1.342	210	1.294	202
26	6.7927	156	1.337	208	1.291	201
27	6.8355	159	1.332	212	1.287	205
28	6.8796	160	1.327	213	1.283	206
29	6.9243	157	1.323	208	1.280	201
30	6.9685	163	1.318	215	1.276	208
31	7.0145	155	1.313	204	1.273	198
32	7.0586	166	1.309	217	1.269	210
33	7.1060					

The sedimentation data were next corrected by multiplying not by the relative viscosities, but by hypothetical viscosities, listed in column 6 of Table II, calculated by multiplying the intrinsic viscosity by the concentrations. The results are presented in column 7 of Table II. The data were found by the method of least squares to fit the equation

Eq. 7 $$s'' \times 10^{13} = 202.90 + 0.0011t$$

The standard error of estimate was 1.74% of the intercept and the probability that the observed low correlation between sedimentation rate and time is due solely to random errors was evaluated to be greater than 0.9. Thus, these data are consistent with the assumption that when corrected for a hypothetical viscosity rather than the real measured viscosity the sedimentation constant does not vary with time, that is, with the distance of the boundary from the axis of rotation, or with concentration. When the intercept is reduced to a standard state corresponding to water at 20°, a value of 187×10^{-13} is obtained. This is seen to be in excellent agreement with the extrapolated value of the sedimentation constant of virus Preparation A described previously.[2]

III. Discussion

The customary procedure followed by workers in the field of ultracentrifugation is to correct all sedimentation constants to a standard state by means of the equation[12]

Eq. 8 $$s_c = s_T \eta_T / \eta_0 \frac{1 - V_0\rho_0}{1 - V_T\rho_T}$$

where s_c and s_T are the corrected and observed sedimentation rates, η_T is the viscosity of the solvent at the temperature T and η_0 is that of the standard solvent at the standard temperature, V_T and V_0 are the partial specific volumes of the solute at the temperature T and the standard temperature, respectively, and ρ_T and ρ_0 are the densities of the solution at temperature T and of the solvent at the standard temperature, respectively. In making the viscosity correction, the generally accepted convention is to correct for the viscosity of the solvent, considered as the liquid plus any dissolved electrolytes or low molecular weight non-electrolytes.[12] This is the equivalent of making the assumption that a protein molecule sediments through a buffer solution and not through a protein solution.

In the present study it has been observed that, if the sedimentation constant of tobacco mosaic virus is corrected for the viscosity of the virus solution rather than for that of the solvent, it is no longer strongly dependent upon virus concentration. A similar observation was made by Lauffer and Flory[10] concerning the sedimentation rates of several butylene polymers. Making a viscosity correction of this sort is the equivalent of assuming that a protein particle sediments through a protein-buffer solution and not just through the buffer solution.

In Table III are listed the sedimentation constants of numerous macromolecular substances which show a dependence upon concentration of s corrected in the usual manner. The values reported as s_{20} are the authors' figures for the sedimentation constant reduced to a standard state represented by some solvent at 20°. In the reduction, the viscosity of the solvent was used. The data tabulated as s'_{20} were reduced to a standard state by correcting for the viscosity of the solution and not for that of the solvent. It may be observed that, in general, the sedimentation constants corrected by the latter method are nearly independent of concentration, while those corrected by the usual method show marked dependence upon concentration. The data obtained by Signer and Gross[7] on polystyrene fractions afford a particularly severe test of this procedure. In those cases for which the authors consider their viscosity data to be fairly precise, the constancy of s'_{20} is impressive. One of the polystyrene fractions was sedimented in several solvents, some of which were more dense than the polymer and some less dense. In some of the solvents the polymer had a negative intrinsic viscosity and in others a positive intrinsic viscosity. Yet, when reduced to a standard state represented by chloro-

(12) K. O. Pedersen, in "The Ultracentrifuge," by Svedberg and Pedersen, Oxford, 1940, p. 85.

form at 20°, s'_{20} is essentially a constant. Even the variation which is observed is almost within the limits of that allowed by the errors ascribed by the authors to their partial specific volume measurements. In the case of the studies on gelatins, however, it is evident that the application of the correction for solution viscosity overcompensates somewhat the dependence of s_{20} upon concentration. Two studies have been reported in which s'_{20} varies even more with con-

TABLE III

SEDIMENTATION CONSTANTS CORRECTED IN THE ORDINARY MANNER AND THOSE CORRECTED FOR SOLUTION VISCOSITY OF VARIOUS POLYMERS AND MACROMOLECULES

Material	Solvent	Concn. (g./100 cc.)	$s_{20} \times 10^{13}$	$s'_{20} \times 10^{13}$	Investigator
Polystyrene A	Chloroform	0.52	3.1±0.1	3.5±0.1	Signer and Gross[7]
		1.04	2.6±0.1	3.3±0.1	
Polystyrene B	Chloroform	0.104	4.6±0.1	4.9±0.1	Signer and Gross[7]
		0.26	4.4±0.1	5.1±0.1	
		1.04	2.8±0.2	4.8±0.3	
		2.08	3.3±0.1	8.6±0.3	
		4.16	1.0±0.1	5.5±0.5	
Polystyrene C	Chloroform	0.035	13.1±0.8	14.3±0.9	Signer and Gross[7]
		.104	10.7±0.1	13.7±0.1	
		.260	7.0±0.2	12.6±0.4	
Polystyrene D	Chloroform	.017	21.3±1.5	23 ±1.6[e]	Signer and Gross[7]
		.035	17.0±0.3	26.0±0.4	
		.104	11.8±0.1	18.3±0.2	
Polystyrene E	Chloroform	.0081	35.0±1.0	38.2±1.1[e]	Signer and Gross[7]
		.0162	27.8±0.6	30.6±0.7	
		.0324	21.5±0.3	28.0±0.4	
		.0649	16.3±0.5	29.4±0.9	
		.1298	10.2±0.1	35.7±0.4	
		.2596	6.5±0.1	52.0±0.8	
Polystyrene C	Decalene	.035	32[g]	9.6	Signer and Gross[7]
	Isobutyl acetate	.035	27	10.8	
	Ethylene bromide	.035	19	17.1	
	Cyclohexane	.035	14	14.0	
	Xylene	.035	16	16.0	
	Bromobenzene	.035	13	15.6	
Tobacco mosaic virus nucleate A	0.1 *M* phosphate buffer at *p*H 7	.36	7.5	8.52	Cohen and Stanley[13]
		.60	6.6	8.10	
		1.00	5.9	8.13	
Poly-ω-hydroxydecanoic acid	Tetrabromethane	0.108[f]	.865	1.08	Kraemer and Lansing[6]
		.199	.698	1.14	
Gelatin	KCNS solution	.261	2.09	4.6	Kraemer[8]
		.498	2.43	4.4	
		.888	2.71	3.9	
		1.230	3.04	3.6	
Eastman gelatin	0.2 *M* NaCl	0.4	2.96	4.02	Kraemer[14]
	1 *M* thiourea	.4	3.42	4.27	
	1 *M* thiourea[b]	.4	3.13	4.07	
	0.2 *M* NaCl	.2	3.43	3.92	
Schweinfurt gelatin	8% sodium salicylate	.5	2.93	3.93	Kraemer[14]
	2 *M* KCNS	.5	2.80	3.67	
Coignet gelatin	2 *M* KCNS	.5	3.06	3.71	Kraemer[14]
Allantoic gelatin	2 *M* KCNS	.5	2.86	3.78	Kraemer[14]
Gelatin A	Electrolyte solution	0.37	2.30	2.60	Sanigar, Krejci and Kraemer[11]
		2.10	1.50	3.36	
Gelatin B		0.39	2.35	2.82	
		1.94	1.59	4.07	
Gelatin C		0.41	2.56	3.30	
		2.09	1.25	4.41	
Gelatin D		0.40	2.40	2.98	
		.200	1.41	4.52	

(13) S. S. Cohen and W. M. Stanley, *J. Biol. Chem.*, **144**, 589 (1942).

(14) E. O. Kraemer, *J. Phys. Chem.*, **46**, 177 (1942).

TABLE III (*Concluded*)

Material	Solvent	Concn. (g./100 cc.)	$s_{20} \times 10^{13}$	$s'_{20} \times 10^{13}$	Investigator
Wood pulp	Copper ammonium solution	.04	14.3[a]	16.4[b]	Kraemer and Nichols[15]
		.104	9.6	13.0	
Purified cotton		.036	11.8[a]	13.1[b]	Kraemer and Nichols[15]
		.086	9.7	12.3	
		.178	8.25	12.8	
Cellulose regenerated from viscose		.038	8.9[a]	9.5[b]	Kraemer and Nichols[15]
		.096	7.8	9.1	
		.175	7.2	9.3	
		.173	6.9	8.9	
Milled crepe rubber	Chloroform	.05	16.5[a]	18.2[c]	Kraemer and Nichols[15]
		.10	11.7	14.1	
		.20	8.6	12.1	
Polychloroprene A	Chloroform	.05	11.8[a]	13.3[b]	Kraemer and Nichols[15]
		.10	9.8	12.3	
		.20	6.3	9.5	
Polychloroprene B	Chloroform	.05	8.6[a]	9.2[d]	Kraemer and Nichols[15]
		.10	8.6	9.8	
Polychloroprene C	Chloroform	.04	8.4[a]	8.8[d]	Kraemer and Nichols[15]
		.10	8.0	9.0	
		.20	6.3	7.9	
Polychloroprene D	Chloroform	.04	8.3[a]	8.9[d]	Kraemer and Nichols[15]
		.10	7.7	9.1	
		.20	7.3	9.9	
Polychloroprene E	Chloroform	.05	7.7[a]	8.0[d]	Kraemer and Nichols[15]
		.20	6.7	7.8	
Polychloroprene F	Chloroform	.05	5.7[a]	5.9[d]	Kraemer and Nichols[15]
		.20	5.7	6.5	

[a] Intrinsic sedimentation constants, $[s] \times 10^{13}$; $[s] = \eta Vs/(1 - V\rho)$. [b] Viscosities of solutions used in computing these values were estimated from intrinsic viscosities given by authors on the assumption that relative viscosity is a linear function of concentration. [c] Viscosities calculated from volume intrinsic viscosity and assumed value of specific volume of 1.47. [d] Viscosities calculated from volume intrinsic viscosity and assumed value of specific volume of 1.02. [e] Viscosity data inaccurate. [f] Weight per cent. [g] Standard state represented by chloroform at 20°.

centration than s_{20}. These are the experiments of Carter[9] and Carter and Hall[16] on sodium nucleohistone and those of Signer and v. Tavel[17] and Signer and Liechti[18] on methylcellulose. Nevertheless there are too many instances of approximate constancy of s'_{20} to dismiss without careful consideration the possibility that the dependence of s_{20} upon solute concentration is due in large part to the effect of solute concentration upon the viscosity of the solution.

Powell and Eyring[19] considered the sedimentation of macromolecules on the basis of the Eyring reaction rate concepts. They came to the conclusion that sedimentation rate ought to vary with the reciprocal of the *solution* viscosity. They then showed that when the sedimentation data of Signer and Gross[7] on polystyrene in chloroform are multiplied by the solution viscosities, approximate constancy is obtained. It was not emphasized that this use of solution viscosity was contrary to accepted procedure.

Supplementary evidence can be obtained from diffusion studies. Lauffer and Flory[10] showed that the diffusion constants of two butylene polymers are independent of concentration when corrected for the viscosities of the solutions. The data of Cohen and Stanley[18] on tobacco mosaic virus nucleate A also show that the variation with concentration of the diffusion constant corrected in the usual way can be attributed to the variation of the viscosity of the solution with solute concentration. On the other hand, Neurath[20] showed that when the diffusion constants of egg albumin, lactoglobulin, serum albumin and sucrose, which were allowed to diffuse at interfaces separating a concentrated from a dilute solution of the same solute, are corrected for the viscosity of the dilute solution, the diffusion constant is over-corrected and appears to increase with increasing concentration. Neurath showed that the data obeyed the empirical equation

Eq. 9 $$D/D_\infty = 1 - 0.466(\eta/\eta_0 - 1)$$

Onsager and Fuoss[21] deduced from theoretical considerations that the diffusion constant of a binary electrolyte should vary with the electrolyte concentration according to the equation

(15) E. O. Kraemer and J. B. Nichols, in "The Ultracentrifuge," by Svedberg and Pedersen, 1940, Oxford, p. 416 *et seq.*

(16) K. O. Carter and J. L. Hall, THIS JOURNAL, **62**, 1194 (1940).

(17) R. Signer and P. v. Tavel, *Helv. Chim. Acta*, **21**, 535 (1938).

(18) R. Signer and J. Liechti, *ibid.*, **21**, 530 (1938).

(19) R. E. Powell and H. Eyring, "Advances in Colloid Science," **1**, 183 (1942).

(20) H. Neurath, *Chem. Rev.*, **30**, 357 (1942).

(21) L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, **36**, 2689 (1932).

Eq. 10 $$D = D_\infty\left(1 + \frac{\mathrm{d}\ln\gamma}{\mathrm{d}\ln c}\right)$$

where D_∞ is the diffusion constant at infinite dilution, c is the concentration of solute, and γ is its activity coefficient. They concluded, however, that this equation was not adequate to account fully for the variation of D with c for certain electrolyte solutions. Gordon[22] and van Rysselberghe[23] independently proposed that the right-hand member be multiplied by the reciprocal of the relative viscosity of the solution, and Stearn, Irish and Eyring[24] showed on theoretical grounds that such a term should be included. Gordon and his associates have shown that the modified equation taking into account solution viscosity agrees with the observed results for potassium chloride, sodium chloride and potassium nitrate,[22] for hydrogen chloride at various temperatures in dilute solution,[25,26] for sulfuric acid over a wide range of temperatures for concentrations up to 1 M,[25,27] and for calcium chloride over a wide temperature range for concentrations up to 0.01 M.[28]

According to the development of the modified form of equation 10 by Stearn, Irish and Eyring, it should apply to Neurath's diffusion data on sucrose. Neurath found values of 42.8 and 39.9 $\times 10^{-7}$ cm.^{2} per sec. for the diffusion at 20° of 5% into 4.5% and 10% into 9% sucrose, respectively. He also recorded values of 1.116 and 1.280 for the relative viscosities of the 4.5 and the 9% sucrose solutions. It is possible to estimate the values of the activity coefficient factor for each concentration from the osmotic pressure values at 25° obtained from isopiestic ratios by Robinson, Smith and Smith.[29] Use can be made of the approximate thermodynamic relationship

$$\left(1 + \frac{\mathrm{d}\ln\gamma}{\mathrm{d}\ln c}\right) = \left(\phi + c\frac{\mathrm{d}\gamma}{\mathrm{d}c}\right)$$

where ϕ is the ratio of the observed osmotic pressure at a certain concentration to the ideal osmotic pressure calculated on the assumption that Raoult's law holds. The factors for 9 and 4.5% sucrose at 25° were estimated to be 1.0451 and 1.0206, respectively. D_∞ calculated by means of modified equation 10, using relative viscosities at 25°, turns out to be 48.8 and 46.8 $\times 10^{-7}$ for the 10 *vs.* 9 and the 5 *vs.* 4.5% solutions. This treatment of the data reduces the discrepancy between the measured diffusion constants by about 40%. More diffusion data in the dilute range must be made available before a critical test of the applicability of the modified equation 10 to the diffusion of sucrose can be made.

(22) A. R. Gordon, *J. Chem. Phys.*, **5**, 522 (1937).

(23) P. van Rysselberghe, THIS JOURNAL, **60**, 2326 (1938).

(24) A. E. Stearn, E. M. Irish and H. Eyring, *J. Phys. Chem.*, **44**, 981 (1940).

(25) W. A. James, E. A. Hollingshead and A. R. Gordon, *J. Chem. Phys.*, **7**, 89 (1939).

(26) W. A. James and A. R. Gordon, *ibid.*, **7**, 963 (1939).

(27) E. A. Hollingshead and A. R. Gordon, *ibid.*, **8**, 423 (1940).

(28) E. A. Hollingshead and A. R. Gordon, *ibid.*, **9**, 152 (1941).

(29) R. A. Robinson, P. K. Smith and E. R. B. Smith, *Trans. Faraday Soc.*, **38**, 63 (1942).

The situation with respect to electrophoretic mobility is comparable to that with respect to sedimentation and diffusion. Longsworth and MacInnes[30] found, 1, that the mobility of ovalbumin decreased with increasing concentration and, 2, that correcting for solution viscosity decreased the dependence but overcompensated slightly.

On the whole, it is possible to conclude that there is considerable evidence that in the diffusion process and in electrophoretic migration a solute molecule must be considered to move through a medium with the viscosity of the solution rather than through one with the viscosity of the solvent. This is evidence in favor of the justifiability of regarding sedimentation as taking place through the solution and not through the solvent, for the frictional interaction of a particle with its medium may reasonably be regarded as the same for all three translational processes.

The fact that the rate of diffusion of an electrolyte and possibly also that of sucrose depends to a considerable extent upon its activity coefficient, makes it seem reasonable that the failure to obtain perfect constancy when the sedimentation rates of some macromolecules are corrected for solution viscosity may be due in part to a factor of this sort. In fact, Bryce and Beckmann[31] made the suggestion that the activity coefficient factor in itself is sufficient to explain the dependence of sedimentation rate upon concentration. However, it is necessary to point out that no support for this idea can be derived from the theoretical treatment at present available.[19]

The essence of the contribution of this report is that, for many types of substances, the variation of the conventional sedimentation constants with solute concentration is closely correlated with the solution viscosity. From this, it follows as a practical expedient that sedimentation rates should be corrected for solution viscosity instead of for solvent viscosity. This appears to be contrary to hydrodynamic theory, which, as usually represented by Stokes' law, states that the frictional resistance to translation of a particle is proportional to the viscosity of the solvent. However, the contradiction is only apparent, for the usual form of Stokes' law was derived for a particle completely isolated from any influences, either thermodynamic or hydrodynamic, of its neighbors. Such a system would be an extremely dilute one. In such a case the distinction between solution and solvent viscosity ceases to exist as a practical consideration.

The fairly general correlation between solution viscosity and the reciprocal of sedimentation rate may mean that particles in concentrated solutions sediment through the solution instead of through the solvent. However, it can also be argued

(30) L. G. Longsworth and D. A. MacInnes, THIS JOURNAL, **62**, 705 (1940).

(31) H. G. Bryce and C. O. Beckmann, Paper read at the Pittsburgh Meeting of the American Chemical Society, September, 1943.

that the frictional resistance of a particle in a solution is modified in such a manner by its neighbors that the total resistance can be expressed as a power series of concentration, in which the first order term happens to have a coefficient roughly equal to the coefficient of the first order term in the relationship between solution viscosity and concentration. Should subsequent research show that the correlation between solution viscosity and frictional resistance is universal, this latter explanation and all others not postulating a fundamental connection between the two phenomena would lose all appeal.

IV. Summary

The reciprocal of the sedimentation constant, corrected in the usual manner, of tobacco mosaic virus preparations was found to be a linear function of virus concentration. It was shown that when the sedimentation rate is corrected for the viscosity of the virus solution instead of for that of the solvent this dependence upon concentration largely vanishes. There remains a small residual effect in the opposite direction which may be interpreted as being due to non-ideality of the solution. Data from the literature on the sedimentation of various polymers and macromolecules show that this close relationship between the apparent concentration dependence of sedimentation rate and solution viscosity is fairly general. Data from the literature on the diffusion of simple electrolytes also support the conclusion that solution viscosity rather than solvent viscosity should be considered in physical studies of this type.

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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY][1]

Preparation and Pyrolysis of Lactic Acid Derivatives. Production of β-Alkoxyethyl and Tetrahydrofurfuryl Acrylates[2]

BY M. L. FEIN, W. P. RATCHFORD AND C. H. FISHER

The corresponding acrylates or methacrylates have been obtained in moderate or high yields by pyrolyzing tetrahydrofurfuryl α-acetoxypropionate[3] and the β-methoxyethyl[4] and β-phenoxyethyl[4] esters of α-acetoxyisobutyric acid. These results suggested that an ether linkage on the β-carbon of the alkyl group increases the thermal stability of esters; the present work was done to ascertain the correctness of this premise and to determine whether β-alkoxyethyl acrylates can be made satisfactorily by pyrolyzing the corresponding α-acetoxypropionates.[5]

Using equipment previously described,[5,6] the pyrolyses were carried out by passing vapors of the esters through a Pyrex-glass tube heated at temperatures ranging from 475 to 525°. The claim of Claborn[3] that tetrahydrofurfuryl acrylate is the principal product of the pyrolysis of tetrahydrofurfuryl α-acetoxypropionate was confirmed.

Pyrolysis of the β-alkoxyethyl α-acetoxypropionates yielded the corresponding acrylates in yields of 26 to 47% on the basis of the starting material destroyed. Other products of the decomposition were acetic acid, acetaldehyde, carbon monoxide, carbon dioxide and hydrocarbon gases. Methanol was obtained in the pyrolysis of the β-methoxyethyl ester.

Vinyl alkyl ethers might have been formed during the pyrolysis of the alkoxyethyl esters, but these ethers were not found in the reaction products. Possibly the ethers were formed and subsequently decomposed,[7] or perhaps they were present in small quantities but not detected.

The results obtained in a preliminary study of the pyrolysis of β-ethoxyethyl acetate indicate that the β-alkoxyethyl group is relatively unstable to heat. The products identified were acetic acid, acetaldehyde (identified as the 2,4-dinitrophenylhydrazone), carbon monoxide, carbon dioxide and hydrocarbon gases. The production of acetaldehyde from β-ethoxyethyl acetate indicates that this aldehyde can be formed from the ethoxyethyl group as well as from acetoxypropionic acid when ethoxyethyl acetoxypropionate is pyrolyzed.

The considerable difference between the thermal stability of the β-alkoxyethyl and tetrahydrofurfuryl esters shows that the presence of an ether linkage on the β-carbon atom has little stabilizing effect. The ether linkage appears to have some stabilizing effect, however, since the yield of ethyl acrylate obtained by pyrolyzing ethyl α-acetoxypropionate under comparable conditions is only about 20%.[8] The stability of the tetrahydrofurfuryl group, as exemplified by the pyrolysis behavior of its acetoxypropionic ester, may be due to the presence of only one β-hydrogen atom, the

(1) One of the four Regional Research Laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) This paper was presented before the Division of Organic Chemistry at the 106th meeting of the American Chemical Society, Pittsburgh, Pa., Sept., 1943. Not copyrighted.

(3) Claborn, U. S. Patent 2,222,363, Nov. 19, 1940; U. S. Patent 2,229,997, Jan. 28, 1941.

(4) Burns, Jones and Ritchie, *J. Chem. Soc.*, 714 (1935).

(5) References to earlier work in this field are given by Filachione and co-workers, THIS JOURNAL, **66**, 494 (1944).

(6) Smith and co-workers, *Ind. Eng. Chem.*, **34**, 473 (1942).

(7) Wang and Winkler, *Can. J. Research*, **21B**, 97 (1943).

(8) Unpublished results from this Laboratory.