ON THE VISCOSITY OF SOLUTIONS OF HUMAN ALBUMIN AND GLOBULIN

by

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I. LIST OF SYMBOLS

A = serum Albumin (g/100 ml)
A′ = serum Albumin (g/100 g)
G = serum Globulin (g/100 ml)
G′ = serum Globulin (g/100 g)
a = index of A in equation (1)
K = equilibrium parameter in equation (1)
ν = kinematic viscosity coefficient ($cm^2/sec \cdot 10^{-2}$)
ϱ = density (g/ml)
μ = absolute viscosity coefficient ($g/cm/sec \cdot 10^{-2}$)
$= \nu \cdot \varrho$
μ_f = viscosity at zero mass
S = concentration of solute (g/100 g)
M_S = weight of solute molecule
M = weight of solvent molecule
M′ = "effective" molecular weight of solution
P, Q = constants in the "colloid calibration" of viscometers using human blood plasma

II. INTRODUCTION

In a previous communication[1] the present writers derived an expression

$$A^a \cdot G^{1-a} = K(A + G) \qquad (1)$$

based on the hypothesis of the existence of a combined-protein molecule in human blood serum. Here K is an equilibrium parameter with a small range of variation and a definite maximum value. To a first approximation a = 0.6 and the corresponding maximum value of K is 0.5102.

This expression was first found empirically from a study of the serum viscosity — protein relationships. If the Albumin and whole-Globulin fractions of a large number of sera be simultaneously plotted against the serum kinematic viscosity, a pair of "wave-patterns" is obtained, analogous to that between the erythrocyte sedimentation velocity and plasma viscosity (HOUSTON, HARKNESS, and WHITTINGTON[2], p. 157). There is no phase-difference between the Albumin and Globulin patterns, but the amplitudes of the Globulin pattern are inverted with respect to those of the Albumin pattern.

A first step towards the understanding of these wave-patterns and towards deriving

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some knowledge of the properties of the combined-protein molecule is the study of teh viscosity of solutions of the separated protein fractions.

In order to obtain viscosity-concentration data for such solutions in a viscometer about whose "colloid characteristics" something was already known, the following experiments were made.

III. EXPERIMENTS

800 ml of blood was collected from a physically-normal male subject, and the Albumin and whole-Globulin fractions separated by the method of ADAIR and ROBINSON[3]. This technique prepares the proteins in the form of their Sodium salts, the form in which they are generally considered to be present in the blood.

Solutions were then made up in distilled water and saline (0.9 % w/v) respectively. The kinematic viscosities were measured at 20.0° C in viscometer number V 6. Results are displayed in Tables I & II, both kinematic and absolute viscosities being given. The transformation from kinematic to absolute viscosity is effected by multiplying by the density. Densities were measured by the falling-drop method of BARBOUR and HAMILTON[4].

Technical details of the "V" type of viscometer, which is essentially a modification of the OSTWALD type, are given in a previous paper[2].

IV. CORRELATION OF RESULTS

If correctly calibrated, all viscometers having ordinary rates of shear will agree in their results for liquids having ordinary molecular dimensions. For colloids, however, the results are conventional, inasmuch as two viscometers having different rates of shear will give different results for the viscosity of a single colloid solution.

In a previous paper ([2] p. 155) we showed that any similar viscometer Vn could be related to Viscometer V5 (in terms of which the "wave-pattern" [2] p. 157 was obtained) by the simple formula

$$\nu_{V5} = P(\nu_{Vn}) - Q \qquad (2)$$

where P and Q are constants for Vn. By observing three plasmas, of widely differing viscosities, in the two instruments V5 and V6, we obtained the values for V6, P = 1.156; Q = 0.201.

Thus from the results for V6 given in the tables, corresponding values for V5 may be computed.

So far we have correlated the work done in different laboratories by referring all viscometers to the instrument V5. This is a perfectly arbitrary choice of standard, and it would be preferable if an "ideal" viscometer could be used as a general standard. As a preliminary step towards the indication of such an "ideal" instrument, the following analysis was attempted.

V. VISCOSITY OF SOLUTIONS

It is well-known that in certain homologous series of compounds the relation between the viscosity at a given temperature, and the molecular weight, is fairly uniform.

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For instance, in the aliphatic hydrocarbons, if log $\mu_{20°}$ be plotted against the molecular weights, the compounds lie close to a straight line, from pentane to octane.

Now it is possible that solutions with a given solvent behave in some such manner, i.e., display fairly regular dependence (at a given temperature) of log μ upon the molecular weight of the solution.

Before such a possibility can be explored, it is necessary to find a suitable criterion of the "effective" molecular weight of the solution.

If the weights of the molecules of solvent and solute respectively be M and M_S and if S (gm/100 gm) be the concentration of the solute, then the relative gm moles of solute and solvent, per 100 gm of solution are

$$\frac{S}{M_S} \text{ and } \frac{100 - S}{M} \text{ respectively}$$

Taking moments, therefore, we might define the molecular weight (M′) of the solution as

$$M' = \frac{100}{\frac{S}{M_S} + \frac{100 - S}{M}} \tag{3}$$

Evidently as $M_S \to \infty$, which will be the case with protein solutions,

$$M' \to \frac{100\,M}{100 - S} \tag{4}$$

or for aqueous solutions, taking M for water $\triangleq$ 18

$$M' = \frac{1800}{100 - S} \tag{5}$$

and for saline S $\triangleq$ 0.9 and $M_S \triangleq$ 58, so that from equation (3) we get M′ $\triangleq$ 18.1, and from equation (4)

$$M' = \frac{1810}{100 - S} \tag{6}$$

for saline solutions.

Solutions of Sucrose have been studied over the whole solubility range, and are therefore suitable for verification (or otherwise) of the formulae (3) and (5) above.

Although S/M_S is not negligible at the higher concentrations, expression (5) was taken as the basis for the "effective" molecular weight, so that the results might be more directly comparable with those for proteins.

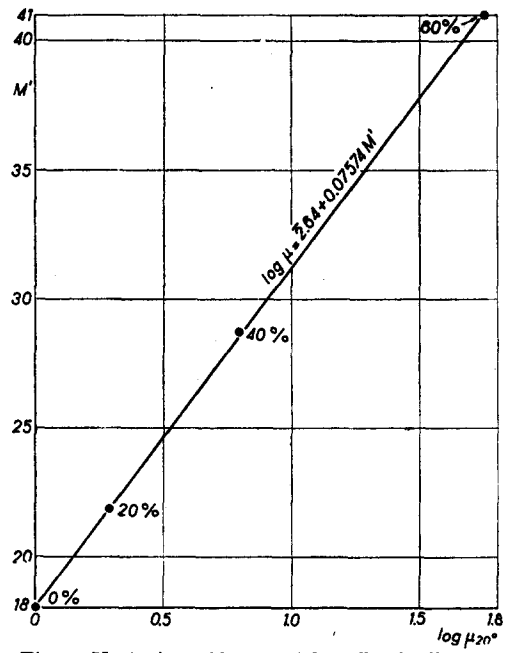

Fig. 1. Variation of log μ with "effective" molecular weight. Sucrose Solutions (BINGHAM and JACKSON), 20° C

$$M' = \frac{1800}{100-S}(1 + \alpha S);\ \alpha = -0.00143$$

Fig. I shows the remarkable adherence of the experimental results of BINGHAM and JACKSON[5] to the line

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$$\log \mu = 2.64 + 0.07574\ M' \text{ (at } 20^\circ \text{ C)} \tag{7}$$

where

$$M' = \left(\frac{1800}{100 - S}\right)(1 + \alpha S) \tag{8}$$

the value of α in this case being — 0.00143.

Thus equation (5), taking no account of the weight of the solute molecule, is modified by a factor $(1 + \alpha S)$. The quantity $\triangleq \bar{2}.64$ is of interest, being the hypothetical value of log μ at zero mass: if we denote this hypothetical viscosity at zero mass by μ_f, then

$$\mu_f \triangleq 0.043 \text{ to } 0.044 \text{ centipoises.}$$

Temperature-changes in the viscosity of aqueous Sucrose solutions can be represented by a rotation of the line (7) about μ_f. This matter is referred to again in the discussion.

VI. CONSIDERATION OF FIG. II

The Sucrose line is laid out in Fig. II, and equation (7) applied to the solutions of Sodium Albuminate. The analogous equation

$$M' = \left(\frac{1810}{100 - S}\right)(1 + \alpha S) \tag{9}$$

is applied to the saline solution of Sodium Globulinate. (We propose for convenience to refer inexactly to these solutes as Albumin and Globulin, except where the loose terminology would cause confusion). It is found that up to viscosities of about 1.4 the Globulin and Albumin points lie close to the Sucrose "ideal" line if

$$\alpha = + 0.025 \text{ (Globulin) approximately}$$
$$= + 0.008 \text{ (Albumin)} \quad ,,$$

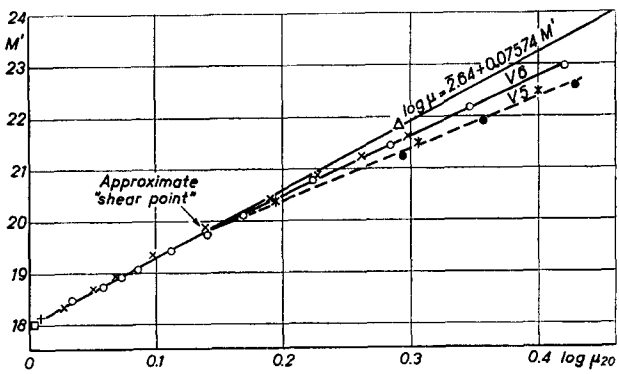

Fig. 2. Variation of log μ with "effective" molecular weight. $M' = \frac{100\ M}{100-S}(1 + \alpha S)$ where M = mol.wt of solvent; S = g/100g concentration of solute

△ Sucrose 20 % (BINGHAM and JACKSON) $\alpha = -0.00143$
\+ Sodium chloride 0.895 % $\alpha = -0.00496$
□ Water
× Sodium albuminate V6
* ,, ,, V5 (calculated) } $\alpha = + 0.008$
○ ,, globulinate V6
● ,, ,, V5 (calculated) } $\alpha = + 0.025$

The "lines" may, of course, be no more than good approximations to curved figures.

At higher viscosities, the Albumin and Globulin adhere to a single line (V6) which steadily diverges from the Sucrose line.

Equation (2) may be rewritten as

$$\mu_{V5} = P\mu_{V6} - Q\varrho \tag{10}$$

Using the predetermined values of P & Q, (1.156; 0.201) and the above values of α (+ 0.025 for Globulin; + 0.008 for Albumin), and inserting the values of ϱ appropriate to each concentration of Globulin and Albumin, we obtain the mean line marked "V5" in Fig. II. The Albumin points lie slightly above, and the

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Globulin points slightly below this mean line; but it is felt that in view of the many variables involved in the transformation, the agreement is quite good.

The V5 and V6 lines intersect the Sucrose line at log $\mu \triangleq 0.14$; and it is clear that only at viscosities greater than this ($\mu \triangleq 1.4$) does the effect of the individual variations of rate of shear in similar viscometers become perceptible.

A possible method of correlating viscometers for use with serum and plasma would be to run solutions of purified human sodium albuminate through each instrument, obtaining lines diverging from the Sucrose line at some point. The viscosity values could then be projected horizontally on to the Sucrose line, so that the viscometer would be calibrated in terms of the Sucrose line. This would appear to be a standard preferable to that of some arbitrarily-chosen known viscometer.

The range of viscosities in Fig. II covers almost all the values encountered in the viscometry of human plasma and serum. The "shear point" ($\mu \triangleq 1.4$) is about the lowest value found with citrate plasma; the upper value of about $\mu \triangleq 2.7$ is only rarely surpassed by sera and oxalate or heparin plasmas.

VII. VARIATIONS IN α

The observed viscosity of the saline at 20° C was $\mu = 1.021$. The concentration of Sodium Chloride was 0.9 % w/v = 0.895 % w/w.

For this point to lie on the Sucrose line, $M' = 18.08$ (equation (7)); and from equation (8), $\alpha = -0.00496$ for Sodium Chloride.

For the series of three dissociable Sodium salts we thus have

Sodium Chloride, $\alpha = -0.00496$
,, Albuminate, $\alpha = +0.008$
,, Globulinate, $\alpha = +0.025$
} approximately, see section (6)

Assuming the weights of the solute molecules to be respectively 58, 69000, and

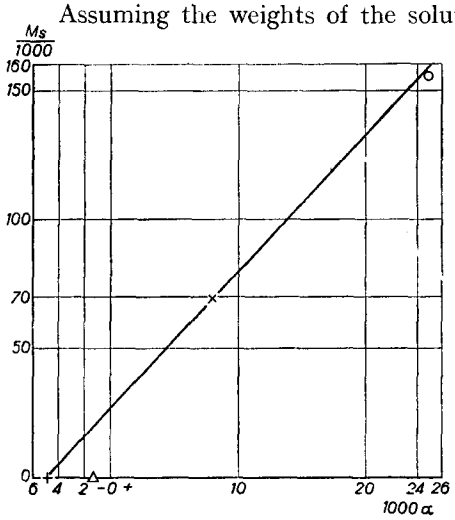

Fig. 3. Variation of α with Ms at 20° C. Aqueous solutions of:
+ sodium chloride
× ,, albuminate
○ ,, globulinate
△ sucrose

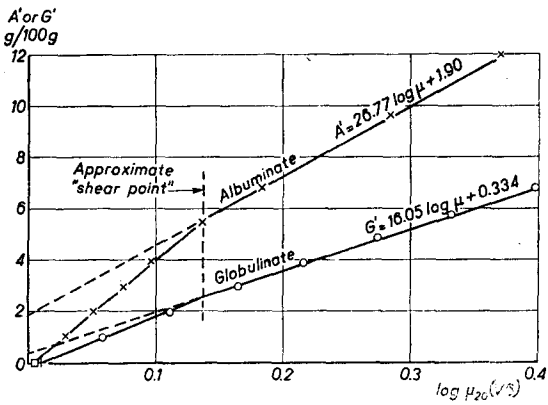

Fig. 4. Variation of $\log \mu_{20}$ with concentration:
× sodium albuminate (V6)
○ ,, globulinate (V6)
+ ,, chloride (0.895 %)
□ water

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155000 we may plot the values of α against M_S. This is done in Fig. III, which shows a very nearly linear relationship.

If α were strictly proportional to M_S in ionic solutions, then taking the weight of the Albumin solute molecule as 69000, we should calculate that of the mean Globulin solute molecule to be 159400.

For the non-electrolyte Sucrose, the value of — 0.00143 for α shows that the α/M_S relationship is quite different.

VIII. CONSIDERATION OF FIG. IV

Here log μ is plotted against the values of A′ and G′. Each separate fraction can be represented by two straight lines, although curvature of the Globulin function begins to be appreciable at high viscosities. The apparent discontinuity in the Albumin lines is very distinct and occurs approximately at log $\mu = 0.14$, the "shear point" in Fig. II.

Approximate equations for the log μ-concentration lines (log $\mu \geqslant 0.14$) are

$$A' = 26.77 \log \mu + 1.90 \tag{11}$$

and

$$G' = 16.05 \log \mu + 0.334 \tag{12}$$

These, of course, apply to viscometer V6 only.

The concentrations at the point of discontinuity, corresponding to the "shear point" in Fig. II are

$$A' \triangleq 5.65\,\%$$
$$G' \triangleq 2.58\,\%$$

i.e., roughly inversely as the molecular weights of Albumin and whole-Globulin.

IX. DISCUSSION

In section (5) we stated that temperature changes in the viscosity of aqueous Sucrose solutions can be represented by a rotation of the line (7) about μ_f. This statement requires some elaboration. If μ_f be taken as a fixed point (log $\mu_f \triangleq \bar{2}.64$; M′ = O) then the general form of equation (7) is

$$\log \mu = \bar{2}.64 + m.M' \tag{13}$$

the gradient m at any temperature being fixed by the viscosity of water at that temperature, and its molecular weight.

Now the expression (8) for M′ is a purely interpolational one in the case of a relatively small molecule such as Sucrose: it compensates for the absence of the $\frac{S}{M_S}$ term by the inclusion of a factor (1 + α S). The value of α, given as — 0.00143 for Sucrose at 20° C, becomes about — 0.00153 at 0° C, i.e., its rate of change is about $5 \cdot 10^{-6}$ per degree centigrade.

Hence to express temperature-changes in aqueous Sucrose solutions purely as a rotation of (7) about μ_f is not quite accurate if expression (8) be used for M′; but using the full expression (3) for M′ it can be shown that the viscosity-concentration-temperature system for aqueous Sucrose solutions may be geometrically represented by rotation of a line of the form (7) about a point whose mass is in the neighbourhood of zero and for which log μ lies between $\bar{2}.63$ and $\bar{2}.65$.

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Full discussion of this conception is beyond the scope of the present paper, but the outline is presented here for the consideration of other workers.

In section (7) we considered the variations in the parameter a with respect to the solute molecular weights in the three ionic solutions, namely Sodium Chloride, Albuminate and Globulinate. As a matter of interest we evaluated the weight of the mean Globulin solute molecule to be 159400 or about 2.31 times that of the assumed Albumin solute molecule. Although this is probably nearly correct, it is most improbable that it is exactly so; for there is ample evidence in the literature of viscosity that in general the anion and cation have quantitatively quite different effects on the viscosity of the solution; and while the effect of the protein ions may be overwhelming in comparison with that of the Sodium ions, the relative effect of the Chloride and Sodium ions in normal saline requires further consideration. There is, in fact, a discrepancy here; for, as we have mentioned, equation (7) requires M′ for normal saline to be 18.08, while the full moment-formula (3) gives M′ = 18.11. An explanation of this discrepancy, which may well become more pronounced at higher concentrations, may lie in the different quantitative effect of anion and cation.

Turning to section (6), Fig. II is suggestive of a method of calibrating viscometers for work on blood plasma and serum, in terms of a hypothetical "ideal" viscometer which would give results following the Sucrose line. This would be preferable to the method which the present writers have so far employed, that of comparing all viscometers with an arbitrarily-chosen existing one.

Finally, the primary purpose of the present investigations was, as stated in section (2), to obtain viscosity-concentration data for the separated protein fractions in terms of a viscometer whose "colloid characteristics" had been studied. The data thus provided should help to illuminate the more complex phenomena encountered in viscosimetric studies of native serum.

TABLE I

HUMAN SERUM ALBUMIN DISSOLVED IN DISTILLED WATER

Experimental		Interpolated					
A g/100 ml	ν_{V6}	A g/100 ml	ν_{V6}	ϱ *	μ_{V6}	log μ_{V6}	A′ g/100 g
15.21	2.795	15	2.745	1.0553	2.897	0.4620	14.22
10.54	1.934	12.5	2.250	1.0438	2.349	0.3709	11.98
8.79	1.691	10	1.857	1.0362	1.924	0.2842	9.654
7.33	1.523	7	1.488	1.0247	1.525	0.1832	6.829
6.28	1.409	5.5	1.342	1.0190	1.367	0.1358	5.400
5.33	1.310	4	1.232	1.0133	1.246	0.0955	3.950
4.10	1.235	3	1.174	1.0095	1.185	0.0738	2.970
3.18	1.177	2	1.119	1.0056	1.125	0.0511	1.988
2.29	1.133	1	1.065	1.0018	1.067	0.0282	0.9982
0.71	1.056	0	1.010	0.9980	1.008	0.0033	0
0.36	1.030						
0	1.010						

* $\varrho_A = 0.9980 + 0.00382$ A
All determinations at 20.0° C

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TABLE II

HUMAN SERUM GLOBULIN DISSOLVED IN NORMAL SALINE

Experimental		Interpolated					
G g/100 ml	ν_{V6}	G g/100 ml	ν_{V6}	ϱ *	μ_{V6}	$\log\mu_{V6}$	G′ g/100 g
8.41	3.041	8	2.845	1.0461	2.955	0.4705	7.649
6.28	2.152	7	2.404	1.0410	2.503	0.3984	6.725
4.06	1.621	6	2.072	1.0359	2.146	0.3316	5.793
2.02	1.274	5	1.825	1.0308	1.881	0.2744	4.852
1.01	1.130	4	1.605	1.0257	1.646	0.2164	3.899
0	1.016	3	1.430	1.0206	1.459	0.1641	2.939
(saline)		2	1.270	1.0155	1.290	0.1106	1.971
		1	1.130	1.0104	1.142	0.0577	0.9902
		0 (saline)	1.016	1.0053	1.021	0.0090	0

* $\varrho_G = 1.0053 + 0.00510\ G$
All determinations at 20.0° C

TABLE III

S g/100 g	μ_{20}	$\log\mu_{20}$	$\frac{1800}{100-S}$	$M' = \left(\frac{1800}{100-S}\right)(1 + \alpha S)$
SUCROSE (BINGHAM & JACKSON)				$\alpha = -0.00143$
20	1.960	0.2923	22.50	21.86
40	6.200	0.7924	30.00	28.71
60	56.5	1.7520	45.00	41.14
ALBUMIN (Interpolated from Table I)				$\alpha = +0.008$
1		0.028		18.32
2		0.051		18.67
2.73		0.068		18.91
4		0.097		19.34
5.5		0.139		19.89
7		0.189		20.40
8		0.225		20.84
9		0.261		21.21
10		0.297		21.60
GLOBULIN (Interpolated from Table II)				$M' = \left(\frac{1810}{100-S}\right)(1 + \alpha S)$ $\alpha = +0.025$
0.5		0.034		18.46
1.0		0.058		18.74
1.25		0.072		18.91
1.5		0.085		19.05
2.0		0.112		19.40
2.5		0.140		19.73
3		0.168		20.08
4		0.223		20.77
5		0.284		21.43
6		0.346		22.18
7		0.420		22.90

Sucrose viscosity is expressed in terms of BINGHAM & JACKSON's viscometer;
Albumin and Globulin in terms of V6
M′ is calculated on a 30 cm slide-rule for Albumin and Globulin

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SUMMARY

1. The "effective" molecular weights of various aqueous solutions are tentatively discussed.
2. Approximately exponential variations of viscosity with concentration of separated proteins are given (at 20° C).
3. These variations are also given in terms of calculated "effective" molecular weights of the solutions.
4. An exponential variation of the viscosity of aqueous sucrose solutions is given in terms of calculated "effective" molecular weights of the solutions.
5. In the protein solutions, there is a fairly distinct viscosity at which the slope of the approximate logarithmic lines changes. We have called this the "shear point".
6. At viscosities above the "shear point" individual viscometers diverge from one another in their results for colloidal solutions of this type.
7. A method of comparing viscometers for use with such colloidal solutions, by reference to the "Sucrose line" is suggested.
8. A geometrical method of representing the temperature-viscosity-concentration system for aqueous solutions of sucrose is tentatively advanced.
9. The viscosimetric behaviour of solutions of the non-electrolyte, sucrose, is contrasted with that of solutions of the dissociable salts.

RÉSUMÉ

1. Les poids moléculaires "effectifs" de diverses solutions aqueuses sont discutés.
2. On a montré des variations, à peu près exponentielles, de la viscosité en fonction de la concentration de solutions de protéines pures à 20° C.
3. Ces variations sont également exprimées en fonction des poids moléculaires "effectifs" calculés des solutions.
4. La variation exponentielle de la viscosité de solutions aqueuses de saccharose est exprimée en fonction des poids moléculaires "effectifs" calculés de ces solutions.
5. Dans les solutions de protéines, il existe une viscosité bien définie, pour laquelle la pente de la droite logarithmique change. Les auteurs ont donné à cette viscosité le nom de "point de brisure" ("shear point").
6. Aux viscosités dépassant le "point de brisure", les différents viscosimètres fournissent des résultats divergents, pour les solutions colloïdales de protéines.
7. Une méthode est indiquée, permettant de comparer divers viscosimètres pour leur utilisation avec de telles solutions colloïdales, en les étalonnant avec la "droite du saccharose".
8. Une méthode géométrique est proposée pour représenter le système température-viscosité-concentration dans le cas de solutions aqueuses de saccharose.
9. La viscosité des solutions du non-électrolyte, le saccharose, est comparée à celle de solutions des sels dissociables.

ZUSAMMENFASSUNG

1. Die "effektiven" Molekulargewichte verschiedener wässeriger Lösungen werden vorläufig diskutiert.
2. Annähernd exponentiell mit der Konzentration separierter Eiweisskörper variierende Viskositätswerte werden gegeben (bei 20° C).
3. Diese Variationen werden ebenfalls ausgedrückt als berechnete, "effektive" Molekulargewichte der Lösungen, gegeben.
4. Eine exponentielle Variation der Viskosität wässriger Saccharoselösungen, die in den berechneten "effektiven" Molekulargewichten der Lösungen, ausgedrückt ist wird dargestellt.
5. Bei den Eiweisslösungen gibt es eine ziemlich deutliche Viskosität, bei welcher sich der Tangens der annähernd logarithmischen Linien ändert. Wir haben diesen Punkt "Scherpunkt" genannt.
6. Bei Viskositäten über dem "Scherpunkt" weichen individuelle Viskosimeter voneinander in ihren Ergebnissen für kolloidale Lösungen dieses Typs ab.
7. Eine Methode um Viskosimeter bei der Benutzung solcher kolloidaler Lösungen vergleichsweise durch Beziehung auf die "Saccharosekurve" einzustellen, wird vorgeschlagen.
8. Eine geometrische Methode, um das Temperatur-Viskosität-Konzentrationssystem für wässrige Saccharoselösungen darzustellen, wird versuchsweise vorgeschlagen.
9. Das viskosimetrische Verhalten von Lösungen des Nicht-elektrolyten, der Saccharose, wird dem Verhalten von Lösungen dissoziierender Salze gegenübergestellt.

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