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OVERDRUK UIT COLLOID SCIENCE II

HOOFDSTUK VII

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[illegible] ed. Elsevier Publ. Comp.
Amsterdam, Houston, London, Brussels
(1949) pag. 184-[illegible]

VII. SOLS OF MACROMOLECULAR COLLOIDS WITH ELECTROLYTIC NATURE

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§ 1. INTRODUCTION AND SURVEY OF THE SUBSTANCES TO BE TREATED IN THIS AND IN THE FOLLOWING CHAPTERS

a. The electric charge as special characteristic of a group of colloids

The most striking difference between the sols from Volume I and the sols which have been dealt with in Ch. VI, p. 153, is that in the former the colloid particles carry an electric charge but in the latter they do not. In this chapter systems will now be dealt with which in a certain sense form a bridge between Volume II and Volume I, since on the one hand their colloidal nature is due to the macromolecular character of the substances but on the other hand by the possession of electric charges they show similarities with systems in Volume I.

Before we proceed to discuss in detail the various aspects which the electric charge of the macromolecules involves, a general observation may first be made.

If the electrolyte character of a macromolecule is to be demonstrable, this must be dissolved in a polar solvent, for example water. Through the presence of this polar solvent, even apart from the charge of the particles, the interaction between macromolecule and solvent will be greater as a consequence of the stronger polar interactions (hydrogen bonds, etc.) than in non-polar media and it is certainly no accident that it is just the charged systems in a polar medium dealt with here, which have given the collective name "hydrophilic (or more generally lyophilic) colloids" to the whole group of colloids treated in this second Volume.

In this and in the succeeding chapters we shall deal with the various properties of the sols of macromolecular colloids in so far as they depend on the charge of the particles.

In the first place the charge itself demands our attention. It will appear that in most cases it is not produced by adsorption as in the systems of Volume I but is due to the dissociation of groups firmly attached to the macromolecule, such as COOH etc. The charge thus becomes dependent on the degree of dissociation and consequently on the pH. Nevertheless so-called indifferent electrolytes can also influence the charge and even make it reverse in sign (see Ch. IX, p. 259).

One of the most direct consequences of the presence of charge is the movement of the particles in an electric field. The electrophoresis of these macromolecules both in solution and in the adsorbed form will certainly be an important aid in the investigation.

The osmotic pressure, of the greatest importance with the uncharged macromolecules for the determination of the molecular weight, is that also in principle for the charge-carrying macromolecules, but in this group the osmotic phenomenon is largely complicated by the occurrence of the DONNAN equilibrium which can frequently even quantitatively predominate over the osmotic action of the large molecules themselves. Since the DONNAN equilibrium is so closely connected with all double layer phenomena, it has been discussed extensively in Volume I. This discussion will not be repeated here.

The influence of the charge on the viscosity is a phenomenon very typical of this group of macromolecules. It is clear that in the presence of charges the skein will not assume its statistically most probable form, but will have a more rarified form through the interaction of the charged spots (mutual repulsion) or a more compact form if positive and negative charges are present simultaneously. As a result the viscosity, sensitive reagent for the skein form, is one of the most important aids in the investigation of systems with charged macromolecules.

Also the stability of the solutions of this group of charged macromolecules, characterised by the circumstances in which the solubility is just transgressed, has on account of the strong polar interactions a much more varied aspect than in the case of uncharged macromolecules. It appears that the charge can act both so as to raise and also so as to lower the solubility. The chapter on complex relations has this to thank for its particular significance.

b. Shape and size of the kinetic units

In the previous subparagraph it has already been mentioned that the shape of the skeins is influenced by the electric charges and especially by their mutual interaction. If there are only charged spots of one sign on the macromolecule then the skein will be relatively rarified. If charged spots of both signs are present then the skein will just be more compact than corresponds to the most probable state. Since this remodelling of the skein depends on the interaction of the charged spots, it is dependent not only on the number of charged spots but also on the extension of the ion atmosphere around each charged spot. The shape of the skein will therefore also depend on the concentration and nature of the indifferent electrolytes in the solution since these determine the thickness of the ion atmosphere[1].

Furthermore it appears that among the charged macromolecules there also occurs a group of substances the solutions of which have a relatively low viscosity and the viscosity of which does not depend greatly on the electrolyte concentration. One must indeed assume for this and other reasons that one is not here dealing with skein shape (possibly modified) but that in these "corpuscular proteins" the macromolecule is evidently rigid and — as is shown from the X-ray picture — folded in a very regular way. To what forces this formation is due is not yet known in full detail. In all probability cystine S—S linkages, salt bridges and hydrogen bonds play a part here.

The transition from the corpuscular type to the free skein shape is known from denaturation of proteins. It is quite in keeping with the above argument that this denaturation can be brought about by, among other things, extreme acid or alkaline

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[1] c.f. two very recent communications on this subject given independently by W. KUHN and by J. J. HERMANS and J. TH. G. OVERBEEK, presented at the meeting on large molecules held at Liège (Belgium) in April 1948. To be published in *Bull. soc. chim. Belges,* 57 (1948).

media whereby all charged spots on the protein have the same sign and thus the expanding forces are as strong as possible, so strong that the forces binding the structure together can no longer resist them and the corpuscular structure ceases to exist.

In the following chapters attention will in the main be directed to the charged macromolecules of the statistical skein type not because the corpuscular proteins would be less important, quite the contrary, but because various excellent monographs on proteins[1] have appeared recently and they deal with this group of substances much more extensively than would be possible here.

c. Division of the colloids to be treated, according to the nature and sign of the charge of the ionogenic groups

The macromolecules with electrolyte character can be divided into three categories, to wit:

A. Colloids with acid character which carry only acid groups such as $—COO^-$, $—OSO_3^-$, $—OPO_3H^-$.

B. Colloids with basic character which carry only basic groups such as $—NH_3^+$, $—NH—C(NH_2)_2^+$.

C. Colloids with amphoteric character, which carry both sorts of groups.

The dissociation of the various groups can be more or less strong according to the pH (and the electrolyte content) of the medium. Thus for instance the carboxyl colloids of group A in strongly acid medium are practically uncharged.

The amphoteric colloids will at very low pH behave practically as "ionised colloids with basic character", at high pH just as "acid colloids[2]", while in the intermediate region positive and negative charges occur together and just equilibrate one another at the isoelectric point (I. E. P.).

With regard to the above division it may be remarked that types A and C both occur naturally and have been prepared synthetically. Pure representatives of group B, the basic colloids, are not known.

However we find substances which approximate closely to group B in the so-called basic proteins. Clupein, for example, consists to an extent of $^2/_3$ of arginine and contains no dibasic aminoacids. Along the whole chain there are thus 2 positive charged spots for each 3 peptide units and only one single COOH group at the end of the chain. In acid medium where the COOH group is not dissociated, cluplein behaves as a purely basic colloid while in less acid or alkaline medium also the positive character still greatly predominates.

If now we ask ourselves definitely what substances belong to the above groups, we are led automatically to a second kind of division, namely into:

Natural colloids: example gum arabic.

Modified natural colloids: examples, cellulose xanthogenate, pectate.

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[1] CARL SCHMIDT, *The chemistry of the aminoacids and proteins*, THOMAS, Baltimore 1938.
E. J. COHN and J. T. EDSALL, *Proteins, amino acids, and peptides*, REINHOLD, New York 1943.
M. L. ANSON and J. T. EDSALL, *Advances in protein chemistry*, I, II, III, Academic Press, New York 1944, 1945, 1947.

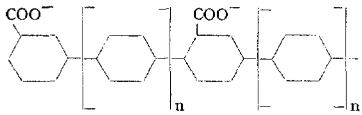
[2] This is however only correct to a first approximation with most of the proteins. In positive casein sols, for example, — casein contains phosphate groups — in the strongly acid region, where the dissociation of the carboxyl groups is entirely suppressed, negatively charged spots which originate in the phosphate groups are also present as well as the positive charge. Similarly in many proteins which contain arginine the total charge is also built up of negative and positive contributions in alkaline media on account of the strongly basic character of the guanidino group.

Wholly synthetic products: example polyacrylic acid.

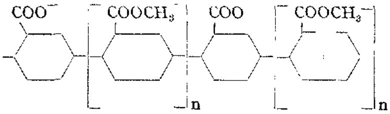
We shall occupy ourselves in the following pages mainly with the natural and a few modified natural colloids, whereby, as we have already mentioned, the corpuscular proteins will be practically left out of consideration.

Since there are many among the natural substances in question the structure of which is not yet well or not generally known, and yet on the other hand it is desirable for a good understanding to have a schematic picture of the various substances involved in the investigation, we add here in the form of a list, schematic, highly simplified structural formulae of some important substances.

A. Colloids with COO^- as the carrier of the charge.

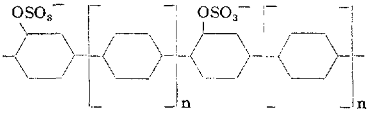


glycogen	n = ∞
soluble starch	n = very large
oxidised starch	n = large
gum arabic	n = about 6
semen lini mucilage	n = small
alginate	n = 0
pectate	n = 0



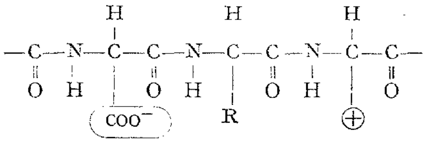
pectin	n = ∞
pectinate	n > 0
pectate	n = 0

B. Colloids with OSO_3^- as the carrier of the charge



agar	n = about 20
carrageen	n = 0

C. Amphoteric colloids with COO^- as the carrier of the negative charge



proteins

D. Amphoteric colloids with phosphate as negative group.

Thymus nucleic acid[1]. Scheme, see p. 188.

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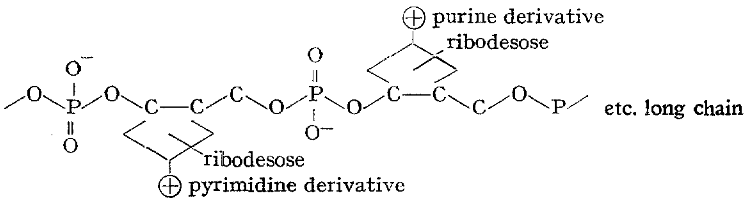
[1] See FREY-WYSSLING, *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*, Borntraeger, Berlin (1938), p. 170.

In the scheme positive charges are drawn in the side chains to emphasise the amphoteric character*. The positive charges can be produced by dissociation of NH_2 groups which are permanently attached to rings of aromatic character. Consequently their basic character is very weak and dissociation only takes place in definitely acid medium (50% dissociation occurs in cytosine only at pH 4.2, in adenine at pH 3.7 and in guanine at pH 2.3). The I.E.P. lies very low (I.E.P. < 2). *All this results in nucleic acid behaving in the* pH *range* 6—8 *as a purely acid colloid in which phosphate groups are exclusively the carriers of the negative charge.*

At still higher values of the pH the negative charge increases still further through dissociation of aromatic OH groups in the side-chains (uracil and guanine: 50% ionisation at pH about 10) and at yet higher pH of OH groups in the sugar units (50% ionisation at pH > 13).

* For the dissociation behaviour of nucleic acid, nucleotides and nucleosides see: P. A. LEVENE and L. W. BASS, *Nucleic Acids*, Chemical Catalog Comp. New York (1931), p. 212, 280—286.

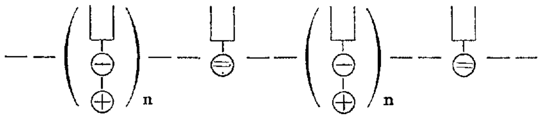


Yeast nucleic acid is probably similarly constructed. The sugar component is here ribose. It is a short chain consisting of only 4 ribose phosphate groups.

E. Association colloids with phosphate groups.

Further there is a group of substances which do not belong to the macromolecules but whose solutions behave as colloidal solutions and which will be used a few times as an example of colloids with phosphate groups as the carrier of the charge.

The substances in question are phosphatides such as lecithine, kephaline, etc.



The groups indicated by (⊖) and (⊜) are phosphate groups, the (⊕) charge comes from choline in lecithine or colamine in kephaline), (⊜) symbolises the phosphate group of the phosphatidic acid which is almost always present in the phosphatides and which can dissociate off one or two H˙ ions[1] according to the value of the pH. In the completely pure state the phosphatides would consist only of the symbols in brackets.

§ 2. NATURE OF THE ELECTRIC CHARGE OF MACROMOLECULAR COLLOIDS

a. Origin of the charge

How the charge of hydrophobic colloids comes into being has been discussed in detail in Volume I of this book. In this discussion the idea came to the fore that the carriers of the charge on the particles are in this case usually adsorbed ions. The degree of adsorption and with it the magnitude of the charge depends on the electrolyte concentration in the intermicellar liquid, in the first place on the concentration of the potential-determining ions and secondarily also on the concentration of the other (indifferent)[2] electrolytes.

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[1] The scheme given is not intended to suggest a picture of the way in which the components are united into associated groups. These associations of groups are probably not linear but double sheeted planes. Compare on this point Chapter 14, *Association Colloids* and Palmer, K. J. and Schmitt, F. O., *J. Cellular Comp. Physiol.*, 17 (1941) 385.

[2] In this and in the following chapters the term "indifferent electrolytes" will be frequently used for all those electrolytes that are not potential determining, or that do not give ions ($H+$ or $OH-$) which can react directly with the charged groups. The term "indifferent electrolyte" is preferred over the older term "neutral salt".

With the macromolecular colloids a different view on the origin of the electric charge appears however to be more appropriate. As already mentioned in § 1 c macromolecules can contain in their structure groups such as the carboxyl group, the sulphonic acid group, the amino group, etc., which by electrolytic dissociation can be the origin of the charge of these colloids.

Thus the proteins have carboxyl, amino, guanidino, imidazol groups, substances such as gum arabic and pectinic acid have carboxyl groups, agar ester sulphate groups, nucleates phosphate groups as carriers of the electric charge.

Many points of similarity can be found with the electrochemical behaviour of hydrophobic surfaces. In particular the dissociation of the above mentioned groups is greatly influenced by the pH, so that the H^+ ions (and the OH^- ions) can be considered as potential-determining ions.

The influence of indifferent electrolytes is also qualitatively the same as in hydrophobic systems. In both cases addition of the indifferent electrolyte increases the charge.

But if one wants to look for a quantitative explanation for the way in which the charge of macromolecular electrolytes depends on the composition of the medium, one should rather associate it with the theories of electrolytic dissociation such as also hold for small molecules than with the theory of the double layer.

b. The influence of the pH and the composition of the solution on the charge of the macromolecule

From an electrometric titration with acid or alkali one can determine the charge of a macromolecular electrolyte as a function of the pH. It is true one encounters in the interpretation of the measurements all the difficulties[1] which are connected

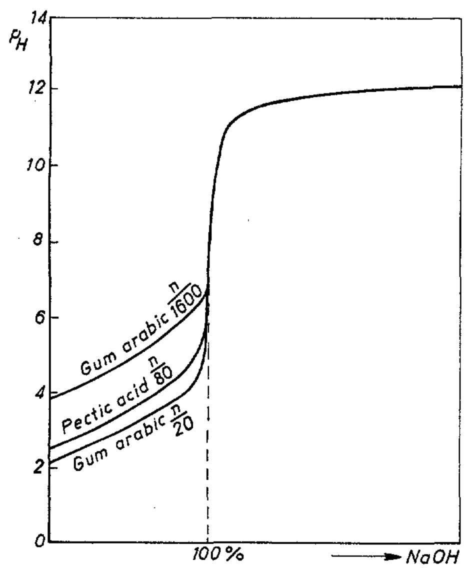

Fig. 1. Titration curves of gum arabic and pectic acid.

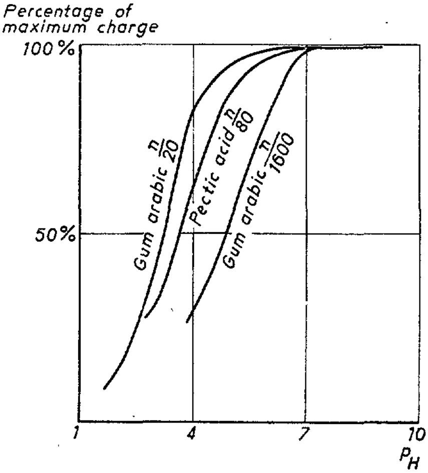

Fig. 2. Relation between charge and pH of gum arabic and of pectic acid deduced from the titration curves.

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[1] See Volume I, Ch. X, Donnan *equilibria and sol-concentration effect.*

with the activity of a single ion (here the H^+ ion) not being unambiguously defined but nevertheless the application of the simple hypothesis, that the activity coefficients of the small ions are not influenced by the presence of the colloidal particles, has permitted a useful interpretation of the titration curves in various cases.

In the simplest cases we have to do with a macromolecule which contains a number of identical dissociable groups.

We give the titration curves of gum arabic[1] and pectinic acid[2] on page 189.

By means of the relation

$$\text{Charge} = \text{added NaOH} + \text{conc. } H^+ - \text{conc. } OH^-$$

in which C_{H^+} and C_{OH^-} are calculated from the pH assuming that the activity coefficients are 1 (or a known constant < 1 *in a medium containing* a salt), one can readily deduce curves, from the titration curves, which give the connection between charge and pH (see Fig. 2).

If the carboxyl groups are all linked in the same way in the macromolecule and are situated so far apart that their interaction can be neglected, the titration curves ought to be identical with those of a monobasic weak acid. This appears however not to be the case. If one determines the dissociation exponent pK at various points of the curve

$$\text{pK} = \text{pH} - \log \frac{[A^-]}{[HA]}$$

in which $[A^-] = [H^+] + [Na^+] - [OH^-]$ and $[HA]$ = gross concentration of colloid $-[A^-]$ then pK is found to rise with increasing degree of neutralisation, in pectin for example from 2.7 at the beginning of the titration to 4.0 close to the equivalence point.

One could interpret this by assuming that strong acid groups first react, then weak acids and ascribe this difference to a difference in binding of the carboxylic groups.

An explanation more in accord with our knowledge of the structure of these substances starts from the assumption that the intrinsic dissociation constants of all the acid groups is the same, but that dissociation is more difficult the higher the charge of the macromolecule. The negative charges already present make, by a simple electrostatic interaction, a further dissociation more difficult. As the interaction between two charges in an electrolytic medium is weakened by an increase of the ionic strength, it is to be expected that the change in pK becomes less and less important, the higher the ionic strength. A quantitative relation between pK, the charge of the large molecule and the ionic strength has been given very recently independently by KUHN and by OVERBEEK[3].

In the titration of proteins which through the occurrence of different kinds of dissociating groups are themselves more complicated than the cases sketched above,

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[1] A. W. THOMAS and H. A. MURRAY, *J. Phys. Chem.*, 32 (1928) 676; D. BRIGGS, *J. Phys. Chem.*, 38 (1934) 867.

[2] J. BONNER, *Proc. Acad. Sci. Amsterdam*, 38 (1935) 346.

[3] W. KUHN and coworkers, J. TH. G. OVERBEEK, *Meeting on large molecules held at Liège (Belgium)* in April 1948. To be published in *Bull. soc. chim. Belges*, 57 (1948)

the determination of dissociation constants has been carried out with more care and on a greater scale.

With certain modifications due to the globular character of the proteins, in contradistinction to the more open skein structure of the acids mentioned above, the same interpretation can be used here[1].

As an example, in Fig. 3 we give the charge — pH curve[2] deduced from a titration curve of carboxyhemoglobin.

This curve clearly exhibits a number of different steps which obviously originate in different types of charge-carrying groups. Other proteins also show a similar variation of charge with pH.

It has been possible to analyse these titration curves and attribute the various steps to the carboxyl groups of glutamic acid and aspartic acid (to about pH = 5), the imidazol group of the histidine (pH=5—9) NH_2 groups of lysine (pH=9—11) and the guanidino group of arginine (pH ∾ 12) and perhaps also the phenolic OH of tyrosine at very high values of the pH.

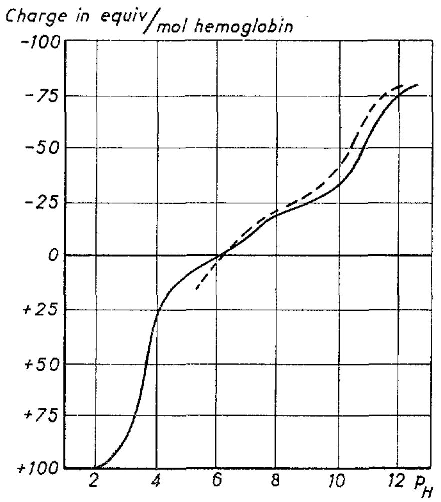

Fig. 3. Relation between charge and pH of carboxyhemoglobin.
——— practically salt-free.
- - - - - - in 1 M. NaCl.

For various proteins these data deduced from the titrations agree well with other analytical data such as amino acid composition, amino nitrogen content etc.[3].

On addition of salts the titration curves show a steeper course as can be seen in Fig. 3 where the full line holds for a salt-poor medium and the dotted line for a medium which is 1 M in NaCl. This steeper course is readily explained if one remembers that in the salt-rich medium the interaction between the various charge - carrying groups becomes smaller through the compression of the ion atmospheres and consequently therefore the dissociation proceeds more easily at the same pH.

The dissociation behaviour of the phosphatides discussed on page 188 is also interesting. Since choline is a strong base and phosphoric acid a strong acid, the I.E.P. of pure lecithin lies very close to the neutral point, and in a broad pH range around the I.E.P. the lecithin is present almost exclusively as amphoion (with net charge =0). (Fig. 4a). In this pH range the + and — charge compensate one another almost completely (Fig. 4b full curve).

A small admixture of phosphatidic acid in lecithin (as supposed in the scheme on p. 188) must then result in the full curve in Fig. 4b being shifted somewhat down-

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[1] K. Linderstrøm-Lang, *Compt. rend. trav. lab. Carlsberg,* 15 (1924) 7.
R. K. Cannan, A. Kibrick, and A. H. Palmer, *Ann. New York Acad. Sci.,* 41 (1941) 243.

[2] E. J. Cohn, A. A. Green, M. H. Blanchard, *J. Am. Chem. Soc.,* 59 (1937) 509.

[3] See for example β-lactoglobulin, R. K. Cannan, A. H. Palmer, A. C. Kibrick, *J. Biol. Chem.,* 142 (1942) 803, and E. Brand, L. J. Saidel, W. H. Goldwater, B. Kassel, F. J. Ryan, *J. Am. Chem. Soc.,* 67 (1945) 1524.

wards as a whole as a result of which the I.E.P. is displaced very rapidly to lower pH's. Since the phosphate group of the phosphatidic acid possesses two hydrogen atoms which can be dissociated off, it may be anticipated that with this shift downwards an additional S-shaped course in the curve (as indicated in the dotted curve) will appear at the same time in the neighbourhood of the pK value corresponding to the second dissociation constant of the phosphate group.

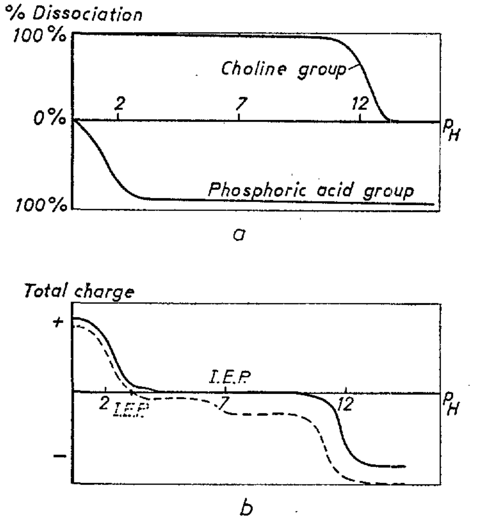

Fig. 4a. The dissociation of the phosphate group and of the choline group occurring in lecithin.
Fig. 4b. ——— The charge of theoretical lecithin as a function of the pH
- - - - - - The charge of lecithin, containing some phosphatidic acid

Lecithin preparations produced in practice (without very rigorous precautions) do indeed possess I.E.P. which are very much lower than the theoretical I.E.P. (p. 294 Ch. IX § 21). As a result of the facts mentioned above they behave in the neighbourhood of the neutral point as colloids with acidic character, in which the phosphate group is the carrier of the negative charge.

Similar considerations hold with regard to kephalin, only as a result of the weaker basic character of colamine (compared with choline) the I.E.P. here lies at a somewhat lower pH in the pure substance than is the case with pure lecithin. Here also admixture of a small amount of phosphatidic acid will result in a considerable lowering of the I. E. P.

It is also worth while pointing out that with macromolecular electrolytes the charge bears always a statistical character. Thus in the case of hemoglobin in which the isoelectric point is situated in the dissociation region of the imidazol groups (33 per molecule) one can calculate that at the isoelectric point ($pH = 6.4$) only about 20 percent of all the molecules are really isoelectrical at any given instant and that molecules with a charge of $+ 3$ or $- 3$ units are certainly not rare. Table I gives an idea of the charge distribution for the various molecules assuming that of the 33 imidazol residues 13 have a dissociation exponent of 5.7 and 20 a dissociation exponent of 7.5, which fits as closely as possible with the titration curve of Fig. 3.

For a more detailed discussion of the topic dealt with above reference may be made to the book of COHN and EDSALL[1].

Summarising one can say that the charge of macromolecular electrolytes is above all governed by the acid (or base) strength of the dissociating groups and by the pH of the medium, while the salt concentration also exerts a secondary influence.

In the part of this chapter in which the viscosity is treated and in Chapter IX (on reversal of charge), we shall see that besides this dissociation of the specific groups

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[1] E. J. COHN, J. T. EDSALL, *Proteins, amino acids and peptides*, Reinhold, New York 1943. See especially Ch. 20, *Proteins as acids and bases*.

TABLE 1

CHARGE DISTRIBUTION OVER THE VARIOUS MOLECULES OF HEMOGLOBIN AT THE ISOELECTRIC POINT pH = 6.4

Charged imidazol residues	Uncharged imidazol residues	Charge on the molecule	% molecules which carry the charge given in column 3
25	8	+ 4	1.26
24	9	+ 3	3.90
23	10	+ 2	9.42
22	11	+ 1	16.98
21	12	0	22.40
20	13	— 1	21.20
19	14	— 2	14.30
18	15	— 3	6.99
17	16	— 4	2.65
16	17	— 5	0.71

adsorption of other ions must also play a part in the establishment of the charge. Although this effect may not always be evident in the amphoteric proteins [1], the rôle of adsorption is brought out more clearly in the reversal of charge phenomena in the case of macromolecules with exclusively negative groups. In fact by suppression of the dissociation we should at most be able to obtain discharge while in reality we observe that the charge from being negative can become positive. Here we have to do with a direct analogue of reversal of charge in hydrophobic colloids, which could also not be understood from the theory of the diffuse double layer but where in addition specific adsorption of ions (or charged complexes) had to be brought in for the explanation.

§ 3. THE ELECTRIC CHARGE OF MACROMOLECULES AS DETERMINED BY ELECTROPHORESIS

a. The interpretation of the electrophoresis of macromolecules

In principle one can also establish the charge of a macromolecule from electrophoresis measurements. This method has even definite advantages over the analytical method dealt with in the previous §. Indeed in the titration one only determines that part of the charge which is based on binding of H^+ or OH^- ions. In electrophoresis one also takes into account charge of a different origin, that is to say, produced by adsorption of other than H^+ or OH^- ions. Furthermore it is frequently difficult with the titration method to fix the charge-zero while this can be done very easily with electrophoresis.

On the other hand however the quantitative interpretation of the electrophoresis still raises various difficulties, whereby the conclusions will again become less certain. For a detailed discussion of electrophoresis, also as concerns the methodics, reference may be made to Chapter V of Volume I. We shall however deal briefly here with the interpretation of electrophoresis.

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[1] On this point see however § 3b, the comparison of electrophoresis and titration.

To a first approximation one can calculate the ζ potential from the electrophoretic velocity (*E.V.*) with the aid of SMOLUCHOWSKI's relation

$$\zeta = \frac{4\,\pi\,\eta}{\varepsilon}\,.\,v \tag{1}$$

or with the refinements given by HENRY[1] and others.

This relation holds for compact particles whose dimensions are large compared with the thickness of the double layer. For smaller particles various corrections must be applied of which especially the one for the relaxation of the ion cloud still contains difficulties[2].

Next one must calculate the charge from the ζ potential and this brings two new difficulties with it. As is well known the ζ potential is the potential on the "slipping-plane" between particles and liquid. In general a layer of liquid of one or more molecules thick will stick hydrodynamically to the particle and quite a portion of the ions from the outer coating of the double-layer may be present in this layer. As a result of this the charge calculated from the ζ potential is always lower than the charge of the particle itself and this lowering can be important especially at higher electrolyte concentrations. A second difficulty is formed by the fact that calculation of the charge from ζ calls for accurate knowledge of the shape and size of the particle and this is not always easy to obtain. Determinations of the molecular weight and the velocity of diffusion leave two interpretations open if the diffusion is slower than corresponds to a compact sphere. Either one assumes that the particle is spherical but enlarged by hydration or that there is no hydration but the particle has an elongated form. And the two hypotheses lead to different values of the charge for the same value of ζ[3].

As a general conclusion one may state that, provided the ζ potential, and thus the electrophoretic velocity, is small ($\zeta < 25\ mV$, $v < 1\ \mu\ cm/Vsec$) the charge of the particles is directly proportional to ζ and therefore also to v. The exact indication of the proportionality factor (which also depends on the electrolyte concentrations of the system) still presents difficulties.

The above statements hold for the electrophoresis of compact particles. These considerations cannot be applied to a charged skein molecule without further discussion. Apart from the motion of the particle with respect to the surrounding liquid, the motion of the liquid through the meshes of the skein molecule has certainly to be taken into account. No theory has yet been given for the electrophoresis of clewed molecules. The only thing which one may expect with high probability here is that, again for the region of small velocities charge and electrophoretic velocity are proportional to one another and that electrophoretic stand-still thus signifies that the macromolecule carries no charge. But in this case we are even worse informed regarding the proportionality factors between charge and electrophoretic velocity than in the case of compact particles.

A much used expedient in the electrophoretic investigation of macromolecules is the determination of the *E.V.* not of free molecules but of rather coarse particles

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[1] D. C. HENRY, *Proc. Roy. Soc. London,* 133 (1931) 106.

[2] See Chapter V, part I and J. TH. G. OVERBEEK. *On the interpretation of the electrophoretic velocity, in Advances in Colloid Science III,* Interscience New York, in the press.

[3] See H. A. ABRAMSON, L. S. MOYER, and M. H. GORIN, *Electrophoresis of proteins,* New York 1942, p. 151 *et seq.*

(quartz, carbon, oil drops etc.) which are covered to saturation with macromolecules on their surface. The *E.V.* of these coarse particles can then be determined very readily by microelectrophoretic methods.

Since now we are dealing with very large particles, SMOLUCHOWSKI's electrophoresis equation (1) can be applied with confidence and the ζ potential is therefore calculable. From ζ the charge per cm^2 can then be calculated if the electrolyte concentration is known but to find the charge per macromolecule from this, the density of covering of the surface must be known and that is in general not known. Thus with this method also we do not get any further than to a relative measure for the charge of the macromolecules in which an influence of the absorbing particle on the electrochemical properties of the absorbed substance can possibly also occur as a complication.

b. Some examples

In the first place we give some comparisons between the electrophoretic velocity of corpuscular proteins in solution and of the same proteins absorbed on various particles under the same conditions as regards pH and salt concentration. It appears that in various cases these two *E.V.* are identical (serum albumin, Fig. 5, pseudoglobulin, Fig. 6) and that in a case such as ovalbumin (Fig. 7) where a definite difference exists, this difference is nevertheless small and can practically be described as a shift in the direction of denaturation which results in the *E.V.* of the absorbed ovalbumin resembling more that of the denatured protein.

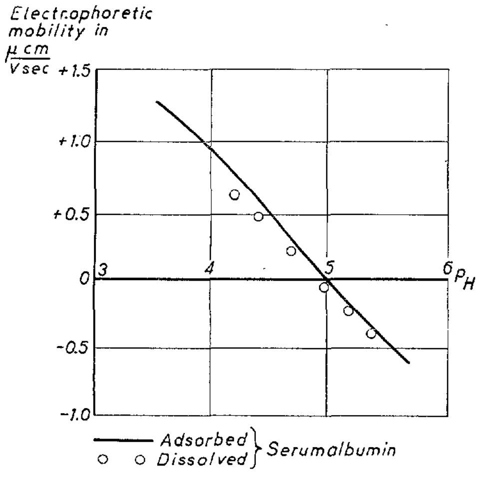

Fig. 5. Electrophoretic velocity of dissolved and adsorbed serum albumin.

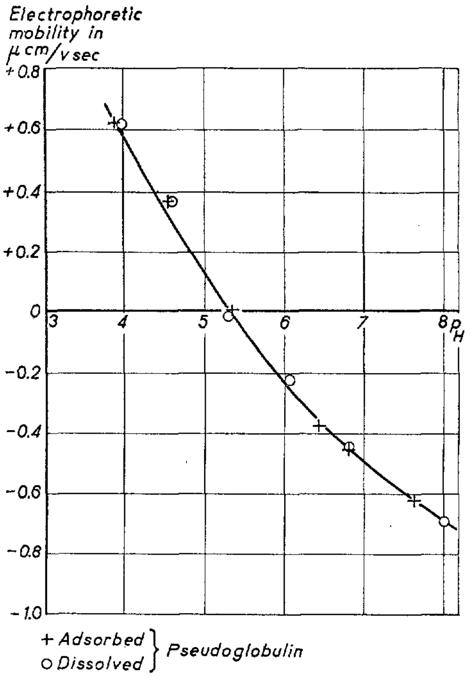

Fig. 6. Electrophoretic velocity of dissolved and adsorbed pseudoglobulin.

Although fundamentally this identity or great similarity is unexpected and not at all explained, one can nevertheless conclude from it that one obtains valuable information by electrophoretic investigations on adsorbed macromolecules even on the behaviour of these substances in solution, which is of great importance since the measurements with the adsorption method are so much simpler.

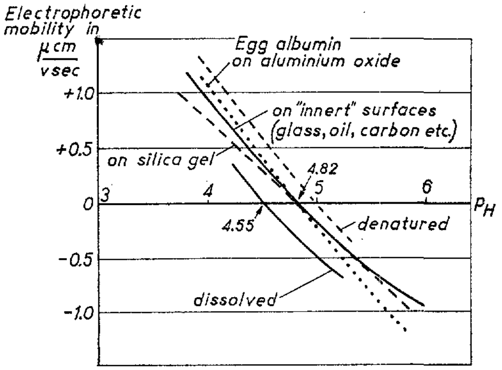

Fig. 7. Electrophoretic velocity of ovalbumin, in the dissolved state, adsorbed on particles of acidic (silica gel), basic (Al_2O_3) and inert (oil, carbon etc.) nature and in the denatured state (surface denatured).

Electrophoretic measurements by this adsorption method on many biocolloids of the skein type will be dealt with in Chapter IX (p. 259). Unfortunately a comparison with the free, non-adsorbed, substances is here entirely missing.

Next, it is worth while comparing the data from titration curves with those from electrophoresis. Here again the best investigations have been made on corpuscular proteins. A beautiful example is to be found in the work of CANNAN, KIBRICK and PALMER[1] on the titration and that of LONGSWORTH[2] on the electrophoresis of ovalbumin.

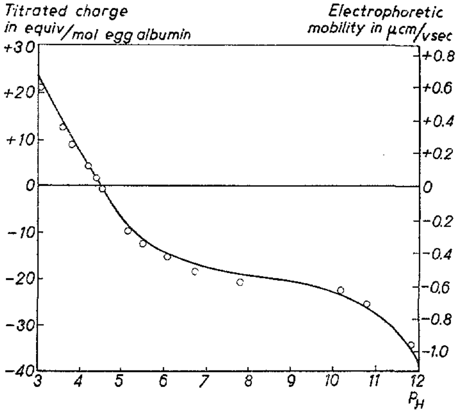

Fig. 8. Comparison of the electrophoretic velocity and the charge as deduced from titration.
o o o o o measured electrophoretic velocity
———— titrated charge
The I.E.P. of the titrated charge has been arbitrarily made to coincide with the electrophoretic I.E.P. The ordinate scales were so chosen that the electrophoretic velocities and the titration approximately coincide.

Electrophoresis measurements are indicated in Fig. 8 by circles. The titration curve is made to fit as well as possible with the electrophoresis measurements by assuming the same *I.E.P.* for the titration as was found in electrophoresis and by suitably choosing the scale of the titration. It is seen that a good proportionality then exists between *E.V.* and titrated charge. Making use of the molecular weight of ovalbumin (45 000) one can then calculate that the following empirical relation exists between the charge Q of one molecule of ovalbumin in terms of the electron charge and the *E.V.* (v) in μ *cm/Vsec*:

$$Q = 36\ v$$

Application of HENRY's electrophoresis formula[3] treated in Volume I leads however to the relation:

$$Q = 22\ v$$

while a somewhat refined calculation of ABRAMSON, MOYER, and GORIN[4] also leads to a similar value. The electrophoretic

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[1] R. K. CANNAN, A. KIBRICK, and A. H. PALMER, *Ann. New York Acad. Sci.*, 41 (1941) 243.
[2] L. G. LONGSWORTH, *ibid.*, p. 267.
[3] For details see the article by LONGSWORTH quoted above.
[4] H. A. ABRAMSON, L. S. MOYER, and M. H. GORIN, *Electrophoresis of proteins*, New York 1942, p. 152 *et seq.*

velocities, which play a part here, are still so small that one can indeed neglect the relaxation correction.

Thus in the best investigated case in this line there is an important difference between the electrophoretic charge and the titrated charge and of such a kind that the titrated charge is appreciably larger.

An explanation which is satisfactory in all respects has not yet been given for this. Attempts in this direction have been made by various workers[1], in which the suggestion is mainly that of an attachment of other ions than H^+ and OH^- (for example Cl^- ions) to the protein. Qualitatively another good explanation of this anomaly can be given by taking into account an adsorbed water-layer (see Volume I, Ch. V). Indeed at the fairly high electrolyte concentrations used in this case (0.1 n) a by no means negligible portion of the counter ions may already have been taken up in this layer.

Another pretty example[2] of the application of electrophoretic velocities is the comparison of gelatin with "desaminised gelatin". It can clearly be seen in Fig. 9 that desamination amounts to the removal of positive charged spots, whereby the whole electrophoresis curve shifts downwards.

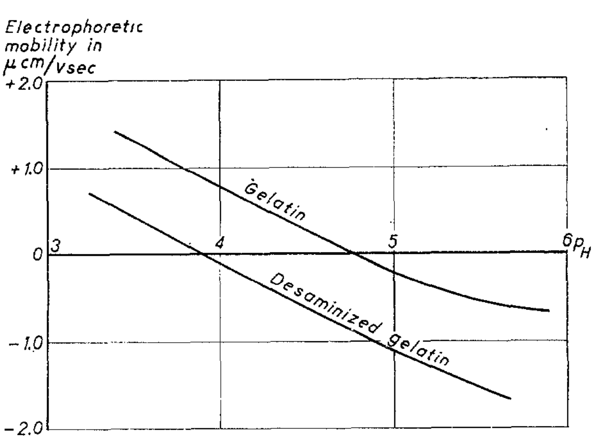

Fig. 9. Electrophoretic velocity of normal and desaminised gelatin.

§ 4. FORMER CONCEPTIONS ON THE NATURE OF THE KINETIC UNITS IN HYDROPHILIC SOLS OF THE HIGH VISCOUS TYPE

Kruyt, Bungenberg de Jong and coworkers[3], developed some twenty years ago a theory of hydrophilic colloids, which may be characterised briefly as follows: Their sols must be regarded as two-phase systems, in the same sense as this is convenient in discussing hydrophobic sols. The kinetic units of their sols are not different from those in lyophobic sols as regards the occurrence of a capillary electric charge (characterised by a ζ potential) at the boundary of the particles. They only differ in having a strong solvation, which acts as an extra stability factor. This solvation may be regarded either as a thick shell of hydration around the solid particle, or as a less thick shell of hydration water surrounding the swollen particle, but always with the characteristic feature, that a sharp periphery of the hydration shell is absent.

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[1] H. A. Abramson, L. S. Moyer, and M. H. Gorin, *l.c.*, p. 158. L. J. Longsworth, *l.c.* J. Steinhardt, *Ann. New York Acad. Sci.*, 41 (1941) 287.

[2] H. A. Abramson, *J. Gen. Physiol.*, 15 (1932) 575.

[3] H. R. Kruyt and H. G. Bungenberg de Jong, *Kolloidchem. Beih.*, 28 (1928) 1 and other publications with coworkers in the *Kolloidchem. Beihefte* under the title *Zur Kenntnis der lyophilen Kolloide.*

Only such a “diffuse solvation shell” could account for the solvation being a stability factor.

“Hydrophobic sols” have only one stability factor: the capillary electric charge, “hydrophilic sols” however have two stability factors: capillary electric charge and hydration. Flocculation of the latter will only occur if both factors are sufficiently suppressed. This condition can be reached in steps along two ways: see Fig. 10.

A. by first removing the charge and afterwards the hydration, as by adding $MgSO_4$ in increasing quantities to an agar sol. In small concentrations the capillary electric charge is suppressed, in much higher concentrations dehydration sets in, the colloid is “salted out”.

B. by first dehydrating and then removing the charge, as can be realised with sufficiently diluted agar sols, with alcohol and afterwards adding a little of an indifferent salt.

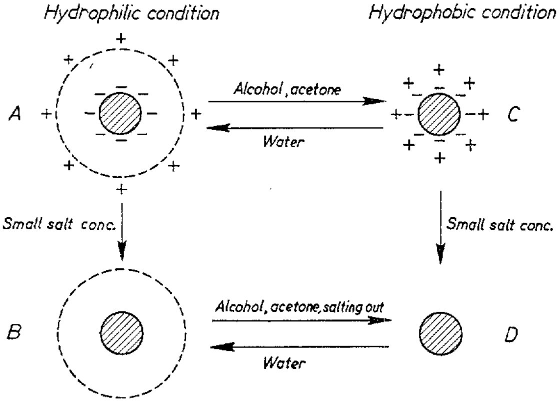

Fig. 10. Change of state of the kinetic units of hydrophilic colloids according to earlier ideas.
A. In pure water the kinetic unit is characterised by the presence of a double layer and a diffuse hydration (here represented as an external shell, but can also be taken up for a great part inside the particle). B. After addition of a small amount of salt: the double layer (not shown) has receded inside the hydration shell. C. and D. are similar to A and B as regards charge but do not possess the diffuse hydration shell. A possesses two, B and C one and D no stability factors.

This stability theory, which was also extended to amphoteric colloids — at first proved a useful guide in experimental work (e.g., also in the first stages of the investigations on coacervation, see p. 243, Chapter VIII, § 3). But gradually facts were met with which are not compatible with it. Its usefulness became less and less and nowadays we may say it has only historical value.

Indeed at the present time there seems no longer to be any real need for a “stability” theory of hydrophilic sols, as we now regard the colloid substance as truly dissolved, and its electrical properties as caused by ionisation of ionogenic groups in the dissolved macromolecules. Flocculations or coacervations are now regarded as transgressions of solubility. Many of the facts, the above stability theory seemed to explain have received other explanations. See for instance for the opalescent sols with lyophobic character obtained by adding alcohol to the sol Chapter VIII § 1c (p. 234), and for the flocculation or coacervation with alcohol + indifferent salt Chapter X § 3f (p. 396).

The viscosimetric data, upon which for a greater part the now discarded stability theory was based, remain, and they need a reinterpretation from the macromolecular point of view.

Before doing so, we give in the next subparagraph first a survey of these data (and their bearing to the former conceptions).

§ 5. CHANGES IN $(\eta_s - \eta_o)/\eta_o$ AT CONSTANT COLLOID CONCENTRATION, CAUSED BY ALTERING THE COMPOSITION OF THE SOLVENT

In all theories which try to connect viscosity of sols with the state or structure of its kinetic units, this connection is always made through relative viscosity η_s/η_o or relative viscosity increase $\frac{\eta_s - \eta_o}{\eta_o}$ now frequently symbolized by η_{spec}, in which η_s is the viscosity of the sol and η_o the viscosity of the dispersion medium (in older conceptions of hydrophilic colloids) or of the solvent (modern conceptions).

Kruyt, Bungenberg de Jong and coworkers based their stability theory on the changes that the relative viscosity of hydrophilic sols shows if at constant colloid concentration (and constant temperature) the composition of the "dispersion medium" is systematically altered. In using the term relative viscosity it will be clear, that it has here a different meaning from elsewhere in physical chemistry.

Instead of comparing the measured viscosities with that of a single constant calibration liquid, η_s/η_o means here the values to be calculated with reference to the viscosity of the dispersion medium (at the same temperature), which medium each time has a different composition. Thus below in Fig. 12 the influence of increasing concentrations of alcohol on $\frac{\eta_s - \eta_o}{\eta_o}$ of a dilute agar sol is given. This figure is obtained by calculation from the two sets of viscosity determinations represented in Fig. 11, *viz.*, from the viscosities of a dilute agar sol containing increasing amounts of alcohol (upper curve), and from the viscosities of the corresponding alcohol water mixtures (lower curve). In these two sets of determinations water is taken as calibration liquid. By division of the corresponding viscosity values a series of relative viscosities in the sense as above indicated is obtained, and by subtracting unity from them, the values of $\frac{\eta_s - \eta_o}{\eta_o}$ used as ordinates in Fig. 12 are obtained.

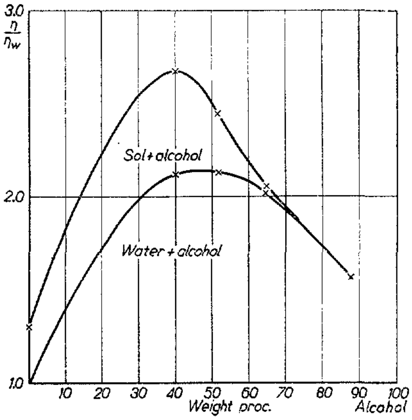

Fig. 11. Viscosity measurements. (45° C) required for the calculation of the curve of Fig. 12.
Lower curve: alcohol-water mixtures.
Upper curve: alcohol-water mixtures which contain 0.134 g agar per 100 ml.
Ordinates: Relative viscosities with water at 45° as calibration liquid.
Abscissae: Alcohol content in weight percents.

Al further viscosity changes to be discussed below refer to relative viscosities in the above sense; we keep further to a constant low colloid concentration and a constant temperature and only vary the composition of the dispersion medium.

The now following survey falls into two sections, which relate to obviously different causes for the changes in relative viscosity. We have labelled them provisionally "*Solvation*" and "*Electric Charge*", thus following more or less the interpretations suggested in the stability-theory of § 4 (p. 197). A reinterpretation from the macromolecular point of view will be postponed till later (p. 209 § 6).

A: "*Solvation*".

For an interpretation of viscosity data of sols obeying POISEUILLE's law KRUYT and BUNGENBERG DE JONG took EINSTEIN's equation

$$\eta_s = \eta_o\,(1 + 2.5\,\varphi) \quad \text{or} \quad \frac{\eta_s - \eta_o}{\eta_o} = 2.5\,\varphi$$

as a starting point. In this formula φ is the ratio of the volume of the dispersed phase and the volume of the whole sol.

Determinations performed with dilute sols (the formula indeed has been deduced for values of φ not exceeding a few per cent (by volume) of many colloids (e.g., agar, gum arabic, amylum solubile, gelatin and others) have shown that frequently the value of φ as calculated from the above formula with the factor 2.5, appears to be many times (e.g., in some cases more than 100 ×) larger than could be expected from the volume of the dispersed substance in the dry state. Now the formula is valid for rigid particles of spherical shape only. With other shapes of particles we must write $(\eta_s - \eta_o)/\eta_o = k\,\varphi$ (SMOLUCHOWSKI[1]), where $k > 2.5$.

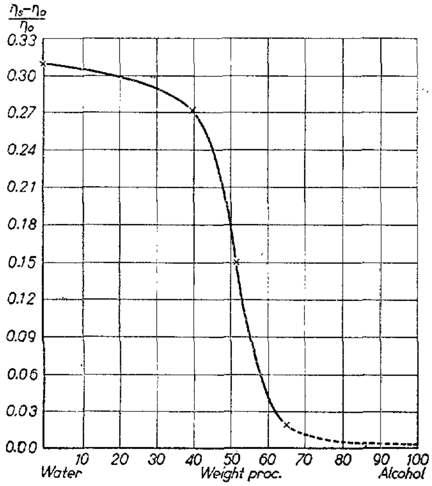

Fig. 12. Influence of alcohol on $(\eta_s - \eta_o)/\eta_o$ of a 0.134% agar sol (45° C).

The ordinates are obtained by dividing the values η_s/η_w of the upper curve in Fig. 11 by those of the lower curve (at the same alcohol content) and then subtracting one from the values obtained for η_s/η_o (relative viscosities in the sense described in the text).

Dotted part of the curve: probable further course of the curve, assuming that the latter at 100% alcohol ends at the value 0.002 which follows from EINSTEIN's formula (with factor 2.5) for dry agar (density about 1.5).

In looking for an interpretation of the much too great values of φ it is consequently uncertain whether this should be attributed to the non-spherical shape, or to a swelling (solvation) of the particles.

So long as only one dispersion medium is used, there is no possibility of distinguishing between these two cases for a given temperature and given concentration of the dispersed substance.

If the too high values for φ originated from the first cause (rigid non-spherical particles), according to the EINSTEIN formula, determinations performed with various dispersion media (variation of η_o), provided both temperature and colloid concentration were kept constant, would always lead to the same value of $(\eta_s - \eta_o)/\eta_o$.

Experimentally this appears not be the case. Here then we find a starting point

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[1] M. VON SMOLUCHOWSKI, *Kolloid-Z.*, 18 (1916) 190.

for experimental work. By systematically changing the dispersion medium, results may be obtained which will show us changes in solvation (or in solvation + amount of occlusion liquid).

For a number of negatively charged hydrophilic sols curves of the type represented in Fig. 12 are obtained if alcohol or acetone is gradually substituted for water (the diagram reproduced refers to an agar sol in water-alcohol mixtures). It is seen that in the concentration range 40—60% alcohol there is a strong decrease of $(\eta_s - \eta_o)/\eta_o$. At the same time the stability changes in a conspicuous way. Whereas the sol in aqueous surroundings appears stable against the addition of salts such as KCl or $BaCl_2$, this is no longer the case after the descent of the curve. It is now exceedingly sensitive to the addition of electrolytes, minute quantities of which will bring about flocculation or coacervation. It has thus obtained the character of a lyophobic sol (see stability theory p. 198, § 4).

The decrease of $(\eta_s - \eta_o)/\eta_o$ from the point of view of EINSTEIN's equation must be interpreted as a process of *desolvation* (the much too high values of φ ascribed to solvation, being reduced to values nearer the calculated ones).

Besides the type mentioned there is a second type of curve[1] which is obtained upon addition of tannin, (sols of agar, amylum, gelatin and in the case of proteins also upon addition of lower phenols). Some instances have been represented in Fig. 13a (agar sol at 40° C and 50° C upon addition of tannin) and Fig. 13b (gelatin sol upon addition of resorcinol).

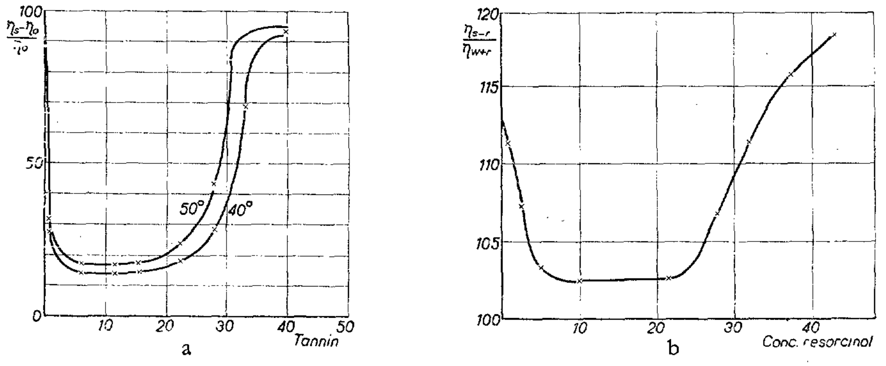

Fig. 13. Influence of tannin on a dilute agar sol (a, 40° and 50° C) and of resorcinol on a dilute isoelectric gelatin sol (b, 42° C).

Ordinates: $(\eta_s - \eta_o)/\eta_o$ (in % of the initial value), or η_s/η_o (η_o is in this case always the viscosity of the corresponding tannin - water or resorcinol-water mixture).

Abscissae: gr. tannin or resorcinol per 100 ml.

The curves drop fairly steeply at relatively small concentrations, rise afterwards in the region of large concentrations. With gelatin the curve rises above the initial value. The clearly noticeable lowering of the pH as a consequence of the very high resorcinol concentration may contribute to this effect.

The effect produced by tannin and polyphenols is fairly strongly dependent on the temperature; it decreases as the temperature rises. This is also evident from the relative positions of the two curves in a.

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[1] H. G. BUNGENBERG DE JONG, *Rec. trav. chim.*, 42 (1923) 437; 43 (1924) 36; 46 (1927) 727; 84 (1929) 494.

In this case we first have a strong decrease of $(\eta_s - \eta_o)/\eta_o$ for relatively small concentrations of the added substance and afterwards an increase at much higher concentration.

As appears also from the changes in stability we must conclude that a desolvation process takes place at the lower concentrations, whereas at a higher concentration the solvation increases again considerably.

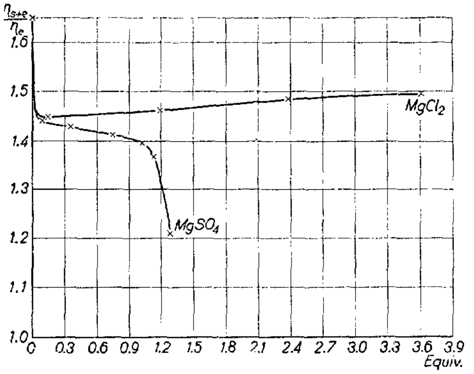

Fig. 14. Influence of $MgCl_2$ and $MgSO_4$ on the relative viscosity of a 0.14% agar sol (50° C).

Ordinates: relative viscosities (relative to the corresponding salt solution without agar).

Abscissae: salt concentration in equiv. per l.

After a considerable fall at the small concentrations, which is characteristic of all electrolytes, the relative viscosity changes but little for $MgCl_2$ (increases), but with $MgSO_4$ a sharp drop begins at about 1 N. The further course of the $MgSO_4$ curve downwards could not be measured on account of salting-out. One may expect a course in this case such as that for alcohol at high concentrations in Fig. 12.

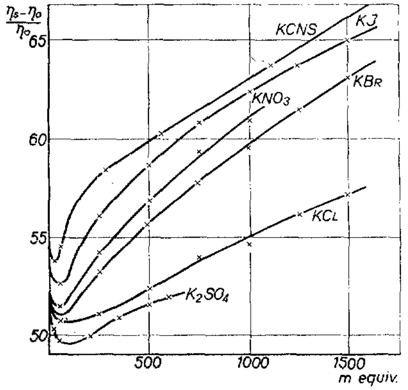

Fig. 15. Influence of a number of K salts on $(\eta_s - \eta_o)/\eta_o$ in a 0.5% sol of amylum solubile (MERCK) at 25° C.

Ordinates: $(\eta_s - \eta_o)/\eta_o$ (in % of the initial value), in which η_o is throughout the viscosity of the corresponding salt solution at the same temperature.

Abscissae: salt concentration in milli equiv. per l.

The curves begin at $(\eta_s - \eta_o)/\eta_o = 100$ and drop very rapidly in the first 10 m. eq. per l.

The text discusses the significance of the relative positions of the curves in the region of higher concentrations (10—1500 m. eq. per l) which is that of the so-called lyotropic series of anions.

Not only non-electrolytes (alcohol, acetone, tannin, resorcinol etc.) but also electrolytes may alter the "degree of solvation". An example of curves obtained in this way has been given in Fig. 14, representing the effect of $MgSO_4$ and $MgCl_2$ on the relative viscosity of a diluted agar sol.

After a decrease at very small electrolyte concentrations, the meaning of which will be discussed in B (p. 203), further addition of $MgCl_2$ changes the specific viscosity very little, $MgSO_4$ on the other hand causes a second decrease at higher concentration. Now $MgCl_2$, even in the highest concentrations does not flocculate the agar sol; salting out with $MgSO_4$, however, occurs exactly at the point where the curve could not be measured further downwards. Hence from the point of view of the EINSTEIN equation: this second decrease in specific viscosity is due to a desolvation process. $MgCl_2$, instead, seems to increase the solvation slightly.

With other sols electrolytes may give similar effects. After a strong decrease at very low concentrations, to be discussed below, at higher concentrations $(\eta_s - \eta_o)/\eta_o$ may increase, which must be interpreted as increase of solvation. Fig. 15 gives an example, referring to amylum solubile[1] and a number of potassium salts. The interpretation, that these changes in spec. viscosity are really related to changes in solvation seems fortified by the order of the curves, which is that of the so-called lyotropic series of the anions: CNS > J > NO_3 > Br > Cl > SO_4

B: "*Electric Charge*" *(Electroviscous Effect)*

In Figs. 14 and 15 we found a peculiar descent of $(\eta_s - \eta_o)/\eta_o$ at very low electrolyte concentrations, which descent can hardly be interpreted as a variation of the degree of solvation. Indeed, if the effect of various types of electrolytes is measured, there is no indication of the appearance of a lyotropic order. SCHULZE-HARDY's rule on the other hand, comes conspicuously to the front: the curves are arranged in narrow bundles, for each of which the valency of one of the ions is characteristic. See Fig. 16, which, for the agar sol[2], shows the results obtained within the concentration range 0—4 m.eq. p. l. It appears, that the fourteen investigated salts give four bundles of curves. The upper bundle comprises KCl, NaCl, LiCl, NH_4Cl, KCNS, K_2SO_4 and $K_4Fe(CN)_6$, that are salts having in common a *monovalent cation.*

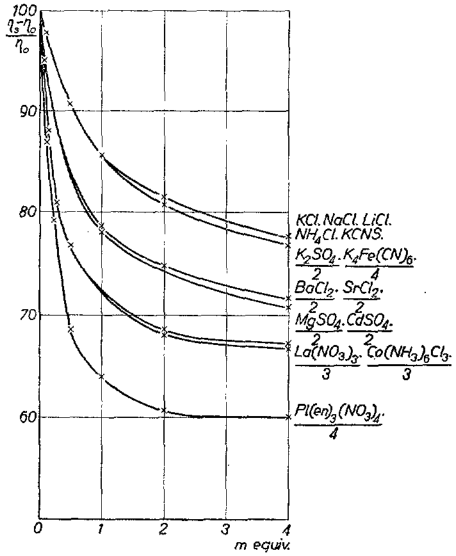

Fig. 16. Influence of salts on $(\eta_s - \eta_o)/\eta_o$ of a 0.14% agar sol (50° C) in the region of small concentrations (Neutralisation of the electroviscous effect).

Ordinates: $(\eta_s - \eta_o)/\eta_o$ in % of this value without added salt.

Abscissae: salt concentration in m. eq. p. l.

The salts arrange themselves in four bundles each characterised by the valency of the cation.

With the complex Pt salt opalescence appears along the lower portion of the curve (see § 10).

The second bundle unites salts having a *divalent cation,* viz., $BaCl_2$, $SrCl_2$, $MgSO_4$, $CdSO_4$. The third bundle contains salts with a *trivalent cation* viz., $La(NO_3)_3$ and $Co(NH_3)_6Cl_3$, whereas the fourth bundle, characterised by a *tetravalent cation,* is represented here only by a complex platinum salt $[Pt(en)_3](NO_3)_4$ where (en) stands for ethylenediamine.

The results obtained strongly recall the influence of indifferent salts on a negatively charged "lyophobic" sol, where also valency of the cations is of primary importance in lowering the capillary electric potential (see Volume I). As in the electric field agar moves to the anode, the kinetic units in

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[1] H. R. KRUYT and H. J. EDELMAN, *Kolloidchem. Beihefte,* 36 (1932) 350.

[2] H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloidchem. Beihefte,* 37 (1928) 1. Preliminary publication *Z. phys. Chem.,* 100 (1921) 250.

the agar sol bear indeed a negative charge. Further it was shown that added salts really decrease the electrophoretic velocity of agar.

Thus it seemed quite natural to correlate the sharp fall in viscosity by added salt with the decrease of the charge on the kinetic units, the higher relative viscosity of the original agar sol being caused by the electric charge of the kinetic units.

For this increasing effect of the charge on the viscosity the term "*Electroviscous effect*" was proposed.

If the conclusion drawn is correct, a similar behaviour of other negative "hydrophilic" sols as to the influence of indifferent salts on the relative viscosity was to be expected. Indeed the electroviscous effect has been found to exist in all cases investigated, viz., gum arabic[1], soluble starch[2], mucilage of semen lini[3] and of carrageen[3], sodium thymus nucleate[4] and sodium yeast nucleate[5]. Pecularities shown by some of these examples will be discussed later p. 223 § 10 and p. 227 § 11.

It may be expected that electroviscous effects are not restricted to the colloids of acidic nature, summed up in § 1c (p. 186), but must also occur in such pronounced ampholytic colloids as proteins. Here at a pH higher than the *I.E.P.* a behaviour must occur quite similar to that of the agar sol. At pH values lower than the *I.E.P.* indifferent salts must also depress the relative viscosity but as the charge is here positive, the separate curve bundles should each be characterised by the different valency of the anions of the added salts.

In the older literature depressing effects of indifferent salts on the relative viscosity of protein sols had already been published and various explanations of this phenomenon had been given. LOEB[6], experimenting with gelatin sols, could show that the valency of the cation is of primary importance for sols at a pH higher than the *I.E.P.*, and the valency of the anion for sols at a pH lower than the *I.E.P.* In the first case the gelatin sol is negatively charged, in the latter positively charged. Thus instead of the explanation LOEB gave based on the DONNAN equilibrium, these depression effects seemed to be much simpler interpretated as electroviscous effects.

KRUYT and LIER[7] using an exact technique of viscosity measurement could obtain quite normal electroviscous effects with casein sols.

The results with the negative sol (Fig. 17 a) did not differ from those with the agar sol[8]. Three bundles are obtained, each characterised by the valency of the cation. In the positive sol (Fig. 17b) only four salts were investigated, but their curves lay just in the order to be expected, the valency of the anion being of primary importance.

The only complication occurring here is that the spreading of the curves for

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[1] H. R. KRUYT and H. J. C. TENDELOO, *Kolloidchem. Beihefte*, 29 (1929) 396.

[2] H. G. BUNGENBERG DE JONG, *Rec. trav. chim.* 43 (1924) 189.

[3] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 29 (1929) 436.

[4] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 31 (1930) 89.

[5] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 49 (1930) 658.

[6] J. LOEB, *Proteins and the theory of colloidal behaviour*, Mc Graw-Hill Publishing Co. Ltd., London, 1922.

[7] H. R. KRUYT and H. LIER, *Kolloidchem. Beihefte*, 28 (1929) 407.

[8] The curve for NH_4Cl lies quite abnormally to the left of that for $Co(NH_3)_6Cl_3$, but the pH change (10.5 → 9.7) which this salt of a weak base (NH_4OH) brings about in the original sol accounts for this abnormal behaviour. The other salts used did not alter pH in the concentrations used. That NH_4Cl, behaves as other salts of the type 1 — 1 in the agar sol (Fig. 16) is due to the fact that small pH changes in the neighbourhood of pH 6 — 5, have not yet any detectable influence on the dissociation state of the acidic ionogenic groups of agar, which are ester sulphate groups.

the tri- and tetravalent anion is very small, to which peculiarity we shall return later in § 10.

The primary importance of the anion valency can be seen to apply also for positively charged ichthyocoll sols [1] from the relative positions in Fig. 18 of the curves obtained with KCl (1—1), K_2SO_4 (1—2) and $K_3Fe(CN)_6$ (1—3). Fig. 18c shows moreover, that in these positive sols the cation influence is of no importance, the curves for $CaCl_2$ (2—1) and $Co(NH_3)_6Cl$ (3—1), nearly coinciding with the curve for KCl (1—1).

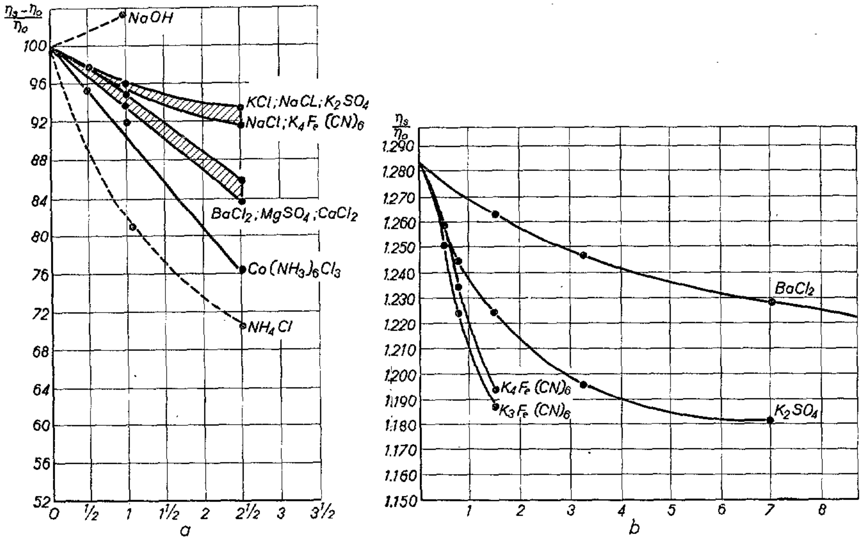

Fig. 17. Influence of salts on the relative viscosity of a negatively (a) and a positively charged (b) 1% casein sol (50° C.).
a: at pH 10.5 the curves arrange themselves in bundles characterised by the valency of the cation.
b: at pH 2.75 on the other hand the valency of the anion is determinative for the relative sequence of the curves.
In a the curve for NH_4Cl is discrepant; which results from the displacement of the pH to lower values. NaOH addition does in fact increase the relative viscosity. Flocculation occurs at the lower end of the curves for $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (see § 10).

For the influence of salts on η_s/η_o of positively charged clupein sols [2] see later in § 12.

In 1916 SMOLUCHOWSKI [3] gave an extension of EINSTEIN's formula for the case when the (undeformable) particles bear a capillary electric charge:

$$\frac{\eta_s - \eta_o}{\eta_o} = k\varphi \left[1 + \frac{1}{\eta_o \varkappa r^2} \left(\frac{D\zeta}{2\pi} \right)^2 \right]$$

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[1] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 50 (1931) 238.

[2] H. G. BUNGENBERG DE JONG, W. A. L. DEKKER and P. VAN DER LINDE, *Rec. trav.. chim.* 54 (1935) 1.

[3] M. VON SMOLUCHOWSKI, *Kolloid-Z.*, 18 (1916) 190, c.f. also W. KRASNY-ERGEN, *Kolloid-Z.* 74 (1936) 172. F. BOOTH, *Nature*, 161 (1948) 83.

From the formula it can be seen that a positive as well as a negative charge on the particles increases the relative viscosity. For the additional term

$$\frac{1}{\eta_0 \varkappa r^2}\left(\frac{D\zeta}{2\pi}\right)^2$$

called by SMOLUCHOWSKI the "quasiviscous effect", is intrinsically positive, the capillary electric potential ζ occurring in it in the quadratic form.

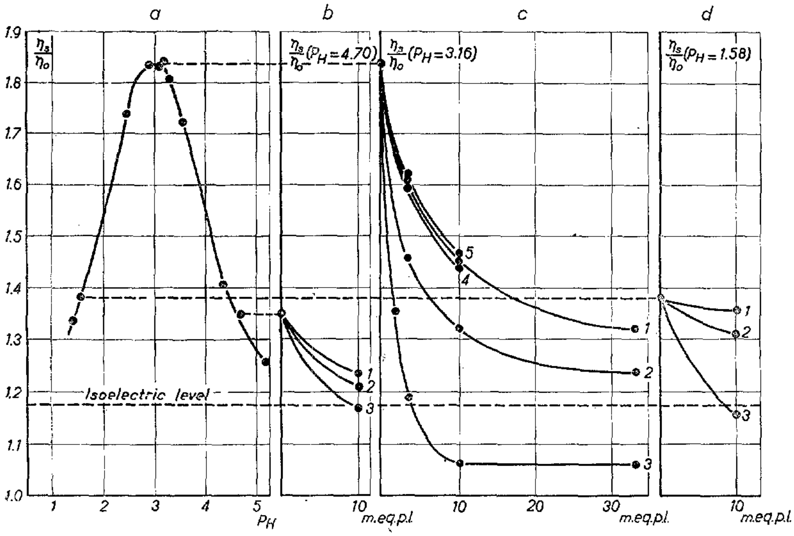

Fig. 18. Influence of salts on the relative viscosity of positive 0.42% ichthyocoll sols (37° C).
a: Part of the pH-viscosity curve at pH values lower than the I.E.P. (see Fig. 20 curve A).
b, c & d: influence of salts in which a point is chosen respectively on the rising branch, at the maximum and on the falling branch of the curve in a as working point.
1 = KCl, 2 = K_2SO_4, 3 = $K_3Fe(CN)_6$, 4 = $CaCl_2$, 5 = $Co(NH_3)_6Cl_3$.
The $K_3Fe(CN)_6$ curves *fall to below the value of* η_s/η_0 *corresponding* to the I.E.P. (lowest dotted horizontal line). This is associated in c with the occurrence of highly cloudy systems, which on microscopic examination is found to be based on coacervation. (On this point see also § 10).

This theoretical deduction of SMOLUCHOWSKI seems to give at least a qualitative interpretation of the experimental results discussed above.

If we assume, that the kinetic units are undeformable, which in the small concentrations used in Fig. 16, 17 and 18 do not alter their volume, then in the above formula r the radius of the particles and φ, the total volume of the particles, expressed as a fraction of the volume of the sol, will remain constant. The only variables which may alter the relative viscosity, are then $\varkappa$, the conductivity of the dispersion medium and ζ the electrocapillary potential.

The increase of $\varkappa$ by added salts to a sol relatively free from electrolyte impurities will in itself already cause the "quasiviscous" term or — as KRUYT and BUNGENBERG DE JONG preferred to call it — the *electroviscous* term to become very small. Thus the decrease in relative viscosity by added salts within the first 100 m. eq. p. l. strictly speaking does not yet mean a discharging effect, but only that the charge can no longer manifest itself in the relative viscosity at salt concentrations higher than 0.1 N.

The fact however, that distinct bundles of curves appear, each characterised by the valency of only one of the ions of the added salts (cations in the case of negative sols and anions in the case of positive sols), and the relative position of these bundles (the higher the valency of the ion, the lower the position of the bundle), does indicate a discharging effect of the added salts.

Summarising we may say that viscosimetry seemed to give an insight into the nature of the sol particles of hydrophilic sols: Added salts in low concentrations reveal the existence of a double layer, resembling very nearly that which is present on the particles of hydrophobic sols. At concentrations higher than 0.1 N, the electroviscous effect now being practically eliminated, large changes in relative viscosity mean changes in solvation only. These conclusions were the basis of the stability theory discussed in § 4 (see p. 198).

In the early stages of this simplistic stability theory, the removal of the electroviscous effect in soluble starch sols was studied[1]. These results seemed to draw the analogy between "hydrophobic" and "hydrophylic" sols as regards the nature of the electric charge a step further still.

In the first named sols a reversal of charge may in many cases be obtained by salts with polyvalent oppositely charged ions (see Volume I). We then meet with a zône of flocculation around the point of charge reversal („Unregelmäszige Reihen" i.e., "irregular Series"). If this reversal of charge should occur also in hydrophilic sols, no flocculation zône is to be expected according to the above named stability theory. For the hydration shell in itself would assure stability though a capillary electric charge were absent.

Assuming that such a reversal of charge would occur in such low salt concentrations, that by the smallness of $\varkappa$ electroviscous effects may still manifest themselves, then the presence of such a reversal of charge will show itself in a peculiar form of the corresponding viscosity curve.

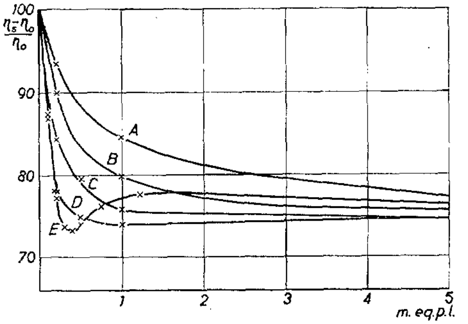

Fig. 19. Influence of salts on $(\eta_s - \eta_o)/\eta_o$ of a 1.97% sol of amylum solubile (20° C) in the region of small concentrations.
Ordinates and abscissae: as in Fig. 16. A = KCl, B = $BaCl_2$, C = $Co(NH_3)_6Cl_3$ and D = $[Pt(en)_3](NO_3)_4$ fit completely with the behaviour in Fig. 16. E = hexol nitrate (composition, see note 1 on page 208) gives a curve with a minimum. At this minimum reversal of charge of the sol takes place from negative to positive. Flocculation does not take place with any of the salts, even not around the minimum of the hexol nitrate curve.

For as the "electroviscous term" (formula on page 205) contains ζ in the quadratic form, this term can be either zero (if $\zeta = 0$) or have a positive value (ζ being either negative or positive).

The curve for the relative viscosity must therefore show a minimum, which

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[1] H. G. Bungenberg de Jong, *Rec. trav. chim.*, 43 (1924) 189.

corresponds to the concentration at which reversal of charge occurs. At higher concentrations the relative viscosity must thus increase, but because of the simultaneously increasing $\varkappa$ it must soon reach a maximum and then decrease further.

This state of affairs has been shown to exist in the sol of soluble starch using hexol nitrate (a complex cobalt salt with hexavalent cation[1]).

Fig. 19 shows the results obtained. The curves for the salts with mono- di- tri and tetravalent cations, have the same relative positions and shapes as with the agar sol (Fig. 16). Hexol nitrate — in accordance with the still higher valency of the cation — initially lowers the relative viscosity still more than the complex Platinum salt, and shows further the above predicted curve form.

By electrophoresis it could indeed be shown that reversal of charge takes place. Further flocculation nowhere occurs (as also neither with the other salts). But as was to be expected, with an appropriate amount of added alcohol maximum flocculation around the minimum of the hexol nitrate curve could be obtained.

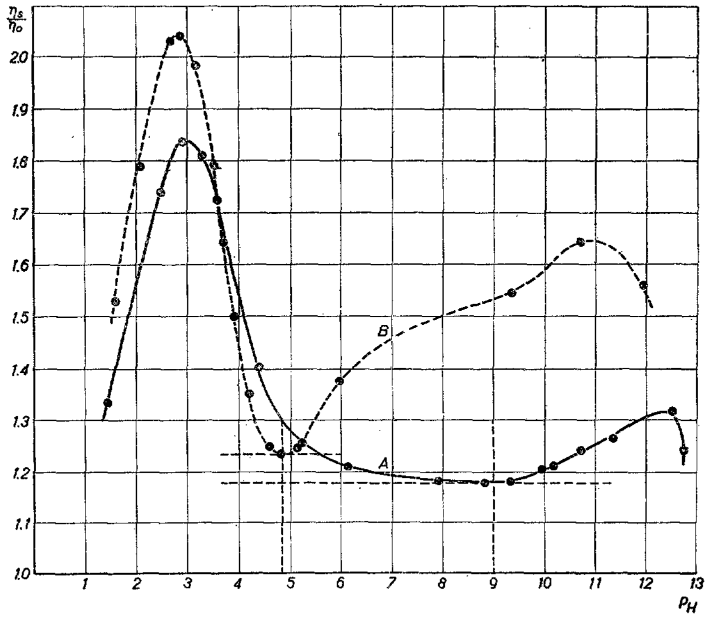

Fig. 20. pH-viscosity curves of a 0.42% ichthyocollsol (37° C) and of a 0.67% gelatin sol (42° C). The relative viscosity is a minimum at the I.E.P. (gelatin pH 4.8; ichthyocoll pH 9) and rises on either side (fairly rapidly with gelatin, much less steeply with ichthyocoll). The viscosity falls again at sufficiently low and sufficiently high pH so that a maximum is encountered on either side of the I.E.P.

Though the underlying mechanism of the reversal of charge with proteins by changing the pH is quite different, the minimum of the well known viscosity-pH curve is comparable to the minimum discussed for soluble starch with hexol nitrate. This minimum, occurring at the *I.E.P.*, is here quite easily detectable, because of the still low values of $\varkappa$.

The shape of the viscosity pH curve (see Fig. 20: gelatin and ichthyocoll[2]) may than be interpreted as follows: Starting from the *I.E.P.* i.e., $\zeta = 0$, a pH change originates a positive or negative charge. By the smallness of $\varkappa$ in this range electroviscous effects can manifest themselves. Thus viscosity is increased. By

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[1] Hexol nitrate = $[\mathrm{Co}\{(\mathrm{OH})_2\mathrm{Co}(\mathrm{H_2N{-}CH_2{-}CH_2{-}NH_2})_2\}_3](\mathrm{NO_3})_6$.

[2] H. G. Bungenberg de Jong and N. F. de Vries, *Rec. trav. chim.*, 50 (1931) 238.

still adding more HCl or more NaOH, $\varkappa$ becomes so great, that electroviscous effects must be gradually suppressed. The viscosity-pH curve must therefore show two maxima. See also Fig. 37 on p. 229, referring to clupein.

The different forms of the curves for gelatin and ichthyocoll in the region of the *I.E.P.*'s, which is shown in a completely analogous way in the titration curve may be explained by the supposition that the histidine content of gelatin is much larger than that of ichthyocoll. As mentioned in § 2b, the dissociation of the imidazol groups varies strongly just in this region of pH.

§ 6. REINTERPRETATION FROM THE MACROMOLECULAR POINT OF VIEW OF THE CHANGES OF $(\eta_s - \eta_o)/\eta_o$ CAUSED BY ALTERING THE COMPOSITION OF THE SOLVENT (AT CONSTANT COLLOID CONCENTRATION)

As a result of further investigations, especially those studying various properties of colloids as functions of the sol concentration BUNGENBERG DE JONG, KRUYT and LENS[1] came to an interpretation of $\frac{\eta_s - \eta_o}{\eta_o}$ changes, which differs considerably from the original ideas and can now be valued as a step half way to the modern interpretations. We shall not enter into detail as to the experimental facts upon which this change in ideas was based. Let it suffice to say that the sol particle was considered as a very loose structured material system of colloid substance, interpenetrated by for the greater part very loosely bound water. It was supposed that in taking up this large amount of intramicellar hydration water, still enough energy becomes available to expand the loosely structured particle very considerably until opposing elastic forces set an end to it.

All changes in $\frac{\eta_s - \eta_o}{\eta_o}$ were now attributed to changes in the expansion equilibrium. In "desolvation processes" the loosely built structure contracts, and the circumscribed volume of the particle decreasing, $\frac{\eta_s - \eta_o}{\eta_o}$ diminishes.

But also the electroviscous effect can be seen from the same point of view. For if ionogenic groups situated at the periphery of the particle dissociate, establishing a diffuse double layer, the ionised groups attached to the structure will by mutual repulsion help to expand the particle. Thus more water can be taken in till equilibrium with the opposing elastic forces is reached at a now greater volume of the expanded particle.

By adding an indifferent salt, the diffuse double layer is compressed, with the result that the repulsion between the ionised groups is diminished so that the additional expansion factor of electrical nature is greatly lessened. The swollen particle must thus expell part of the water it contains until a new equilibrium is reached at a lower total volume. The electroviscous effect and its removal by added salts are thus seen in the first place as volume changes of the expanded particles.

The picture of the sol particles as expanded very loosely built structures, has

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[1] H. G. BUNGENBERG DE JONG, H. R. KRUYT, and J. LENS, *Kolloidchem. Beihefte*, 36 (1932) 429; 37 (1933) 395.

many advantages. Still it possesses a grave difficulty, which disappears however completely in introducing the randomly kinked macromolecule as its intrinsic structure.

This difficulty may be formulated in the form of the following controversy.

A. Because of the rapid decrease of hydration forces with distance a hydration layer can hardly be thicker than a few molecules of water. It is therefore very improbable that the large amount of water taken up by the dry particle is firmly bound by hydration forces. The remaining quantity must therefore rather be considered as "free" water, which is only present between the expanded structure as occlusion water.

B. The dry particle brought into water takes up a definite amount of water, shown by the definite $(\eta_s - \eta_o)/\eta_o$ values. This amount can be shifted reversibly to other definite values by changing the composition of the medium. To explain these definite amounts of water taken in there seemed no other escape than the assumption that an equilibrium is set up between expanding forces and opposed contracting forces — hydration forces versus elastic forces of the expanded particle structure. In this picture therefore we were forced to assume that all the water taken in is hydration water somehow (small amount of firmly bound water + very large amount of "diffuse hydration" water). As the residual hydration forces which bind the diffuse hydration water can only be very small, it followed that the internal structure of the kinetic unit must be very loose, enabling a great expansion before opposing elastic forces set an end to it.

In introducing the concept of the randomly kinked macromolecule as the intrinsic structure of the sol particle the above mentioned controversy no longer exists. If the dry macromolecule is brought into a medium that acts as a solvent, it will expand to the randomly kinked form described in Chapter IV, occluding a relatively large amount of solvent. The "expansion" takes place by itself, the "expanded state" being the most probable form (state of maximum entropy). Thus no work has to be done on elastic forces proper and no special forces to do this work are needed. The solvent taken in is thus not bound by solvation forces but can be described as free occluded solvent, a very small part being excluded which serves for true solvation of for instance polar groups of the macromolecule.

We have now to consider the case that the macromolecule carries ionised groups along its length. Then instead of assuming its most probable form, the macromolecule will assume as a result of the repulsive forces between the ionised groups a less probable and more voluminous form. If now pH is changed so that electrolytic dissociation of the ionogenic groups is suppressed, the volume of the macromolecule will decrease to its normal value.

A decrease of this volume will however also occur at constant pH on adding a small concentration of indifferent salts, which will diminish the mutual repulsion of the ionised groups. Here the valency of the oppositely charged ions of the added salt will be of primary importance in screening off the ionised groups.

Thus in principle the *electroviscous effect* finds a simple explanation. All other facts discussed in the preceeding section may also be explained in an analogous way.

Thus in introducing the randomly kinked macromolecule or macromolecular ion as the kinetic unit in the hydrophilic sols, (indeed all the preceding examples of colloids do belong to this class and not to the corpuscular type, which is restricted to native proteins), volume changes of the kinetic units as postulated by BUNGENBERG DE JONG, KRUYT and LENS do certainly occur, but they need not in the ideal case to give exclusively the basis for an interpretation of the $(\eta_s - \eta_o)/\eta_o$ values experimentally found.

Indeed this volume effect enters here only as a correction factor, in the case in which the macromolecular skein is relatively dense, so that the occluded solvent acts for a part as hydrodynamically immobilized. Taking the ideal case, that the occluded solvent is not immobilized, theoretically the specific viscosity increase is given by the formula (see Chapter IV p. 108) $\frac{\eta_s - \eta_o}{\eta_o} \backsim N^2 . A^3 . G$ in which A is the length of a chain element, N the number of chain elements constituting the macro- molecule and G the number of macromolecules per unit of volume.

In this equation the factor NA^2 forms a measure for the density of the skein, the factor NA representing the resistance caused by the different chain elements. (See Ch. IV). If by introduction of ionised groups the density of the skein is diminished, the factor NA^2 should be replaced by a larger value. Consequently the specific viscosity is increased above the theoretical value[1].

Now in reviewing in short the changes in $(\eta_s - \eta_o)/\eta_o$ represented in the preceding figures, we must conclude that minor changes in the true hydration of polar groups situated along the macromolecule, may give an explanation of the relatively small increases in $(\eta_s - \eta_o)/\eta_o$, caused by various indifferent salts (Fig. 15 and $MgCl_2$ in Fig. 14, p. 202) in the range of higher concentration (an enforced hydration probably diminishing the flexibility of the macromolecule, cf. p. 109, 110).

The sharp fall in $(\eta_s - \eta_o)/\eta_o$ occurring with $MgSO_4$ in Fig. 14 means then, that the solvent is changed by adding this salt in a direction in which the ideal solvability, first existing, is lost. In this "salting out" medium, the macromolecule can no longer expand to the ideal statistical form, but the higher the salt concentration, the more loops of the macromolecule in the interior of the skein mutually adhere, followed soon by adhering of loops between adjacent macromolecules: the colloid substance is "salted out".

In the same way the large decrease of $(\eta_s - \eta_o)/\eta_o$ by addition of alcohol is explained (Fig. 12, p. 200). In higher alcohol concentrations the macromolecular substance is not soluble, the opalescent systems obtained with small colloid concentrations really being systems of quite other nature than originally supposed (see Chapter VIII, § 1c p. 234: "Apparent single systems of composite nature").

Further, still another factor enters here, namely the influence of alcohol in diminishing the dissociation of the ionogenic groups, this influence aiding also in the direction of interlinking loops in the interior of the macromolecule and between adjacent macromolecules (see Chapter X § 3g, p. 400).

The results obtained with tannin and simple phenols (Fig. 13, p. 201) must also be interpreted as a departure from ideal solvability. This occurs in relatively low concentrations. The renewed increase of $\frac{\eta_s - \eta_o}{\eta_o}$ in higher concentrations is simply

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[1] See footnote 1 on page 185.

explained by the fact that the colloids considered are soluble in these higher concentrations of tannin or resorcinol.

The macromolecular skein can in these solvents re-expand and thus $\frac{\eta_s - \eta_o}{\eta_o}$ increases to the order of magnitude, comparable with that occurring originally in only watery medium.

The changes of $(\eta_s - \eta_o)/\eta_o$ occurring with indifferent salts (Fig. 16, 17, 18 and 19, p. 203-207) in small concentrations (the removal of the electroviscous effect) have already been discussed above (the decreased flexibility of the chain molecule caused by the presence of the ionised groups, is increased by the added salts).

It will not be difficult to understand also the occurrence of viscosity minima at reversal of charge points (Fig. 20 and hexol nitrate in Fig. 19) from analogous points of view. We shall however consider this point in more detail in § 9.

§ 7. INFLUENCE OF TEMPERATURE ON $(\eta_s - \eta_o)/\eta_o$

As discussed in Chapter IV, p. 108 theoretically $(\eta_s - \eta_o)/\eta_o$ should be independent of temperature. A small influence of the temperature should be ascribed to changes of the flexibility of the macromolecule. As both the direct influence of the temperature (more intense Brownian motion, less pronounced preference for configurations that are energetically favourable) and the indirect influence via the hydration (higher temperature, less hydration, greater flexibility) tend to diminish the number of monomeric groups in the chain element a small decrease of $(\eta_s - \eta_o)/\eta_o$ with increasing temperature must be expected. That in this respect a typical hydrophilic colloid such as agar (which according to its composition as the salt of a polyose-sulphuric acid[1], can hardly be otherwise seen as a long chain macromolecular ion) behaves according to the expectation, is seen from the following figures[2] relating to three agar sols. Each sol was successively measured in the order 50° → 45° → 40° → 45° → 50° C.

TABLE 2

temp. ° C.	$\frac{\eta_s}{\eta_o}$ ($^2/_7$ % sol)	$\frac{\eta_s}{\eta_o}$ ($^1/_7$ % sol)	$\frac{\eta_s}{\eta_o}$ ($^1/_{14}$ % sol)
50	2.364	1.652	1.322
45	2.393	1.662	1.325
40	2.419	1.674	1.330
45	2.393	1.663	1.326
50	2.362	1.652	1.321

From the figures may be concluded that in the short time of measurement no irreversible changes have occurred, so that the figures may be used to calculate the change of $(\eta_s - \eta_o)/\eta_o$ by increasing the temperature from 40° to 50° C.

We then calculate a decrease of 3.95% (2/7% sol); 3.26% (1/7% sol) and 2.49% (1/14% sol). Obviously this percentage decrease is still a concentration function and linear extrapolation to zero agar concentration then gives a decrease of approximately 2%.

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[1] NEUBERG and OHLE, *Biochem. Z.*, 125 (1921) 311.

[2] H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloidchem. Beihefte*, 28 (1928) 1.

§ 8. THE ELECTROVISCOUS EFFECT AS A DISTURBING FACTOR IN THE DETERMINATION OF THE LIMITING VALUE OF $(\eta_s - \eta_o)/\eta_o C$ AT COLLOID CONCENTRATION ZERO

It often has been claimed that it would be characteristic for hydrophilic colloids, that the viscosity concentration curve will deviate even at small concentrations in the positive direction from linearity. So for instance from the **Einstein** formula the viscosity concentration curve should be linear up to values of about $\eta_s/\eta_o = 1.05$ and at higher concentrations the curve should bend upwards (see Fig. 21).

In investigating the agar sol, **Kruyt** and **Bungenberg de Jong**[1], using an accurate method of viscosity measurement, found that even up to values of $\eta_s/\eta_o = 2.4$ only slight deviations from a linear

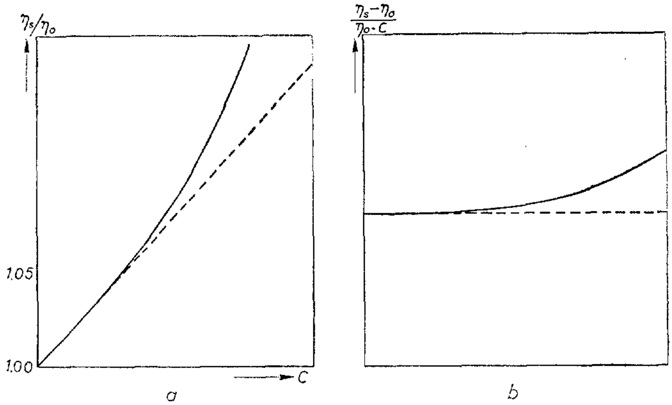

Fig. 21. Schemes to illustrate the relation to be expected between viscosity and sol concentration in the simplest case (see text).
a: the relative viscosity starts with a linear portion but at higher concentrations deviations in the positive sense occur.
b: The curve for $(\eta_s - \eta_o)/\eta_o \cdot C$ will, proceeding toward smaller concentrations, approaches a horizontal level asymptotically.

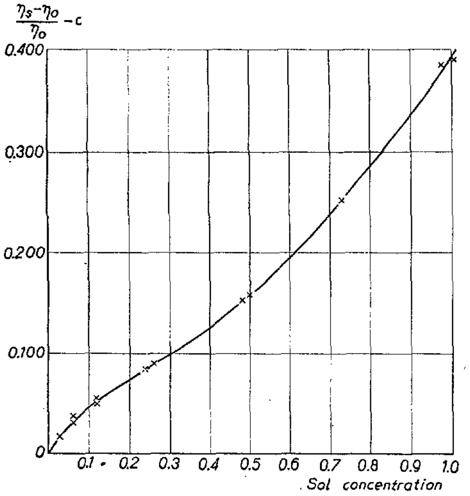

Fig. 22. Viscosity and sol concentration in agar (50° C).
Abscissae: Sol concentration expressed in terms of that of the highest concentrated sol (= 0.279% agar) as unit.
Ordinates: If the experimentally found values of the relative viscosity η_s/η_o are plotted against C, there results a relation between them which is visually near enough linear.

The curve is however S-shaped. To bring out this S-shaped character more clearly in a small scale figure the corresponding values of C (in the above mentioned sense) are each time subtracted from the values $(\eta_s - \eta_o)/\eta_o$.

Compare this figure with Fig. 21a.

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[1] H. R. Kruyt and H. G. Bungenberg de Jong, *Kolloidchem. Beihefte*, 28 (1928) 1.

function occur. These deviations had however a quite different character, the curve obtained being of the third degree, having an inflexion point at about $\eta_s/\eta_o = 1.5$

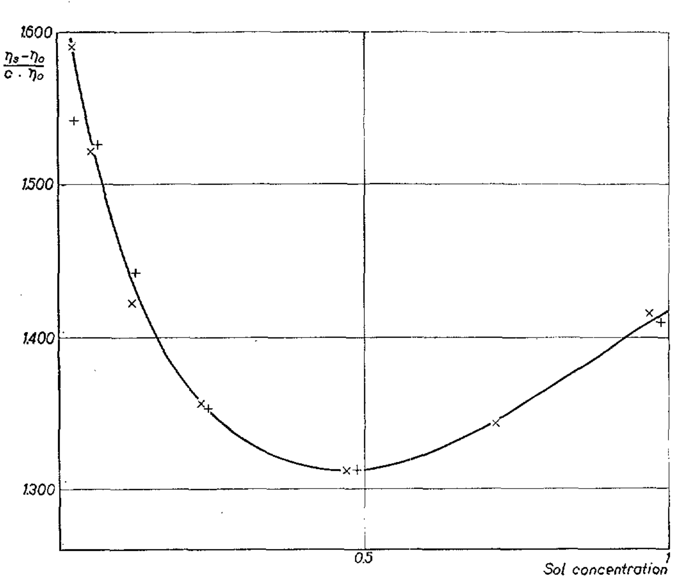

Fig. 23. The expression $(\eta_s - \eta_o)/\eta_o C$ as a function of the agar concentration.
Abscissae: agar concentration expressed in the same units as in Fig. 22.
Ordinates: The values of $(\eta_s - \eta_o)/\eta_o$ obtained from the original experimental figures, divided by the sol concentration C (in the same units as along the abscissa axis).
Compare this figure with Fig. 21b. Instead of approaching a horizontal level asymptotically as the sol concentration is lowered, the curve exhibits a minimum and seems to approach the ordinate axis asymptotically. A graphical determination of the limiting value of $(\eta_s - \eta_o)/\eta_o C$ for $C = 0$ is consequently impossible.

The deviations from linearity are so slight, that they will hardly be visible if the original figures of the measurements are used in a small scale figure. To enlarge them we have used in Fig 22 the values $[(\eta_s - \eta_o)/\eta_o] \cdot C$ as ordinates. Already without introducing artifical ordinate units the deviation from expectations is plainly visible if we take $\frac{\eta_s - \eta_o}{\eta_o C}$ as ordinates, i.e., the increase in relative viscosity divided by the colloid concentration. This has been done in Fig. 23 (Fig. 21b is the analogous curve corresponding to Fig. 21a).

Thus we meet here with the experimental fact that $\frac{\eta_s - \eta_o}{\eta_o C}$ does not decrease to reach an end-value asymptotically on decreasing the colloid concentration (as in Fig. 21b), but increases.

The shape of the curve Fig. 23 even suggests that at still smaller colloid concentrations $(\eta_s - \eta_o)/\eta_o C$ increases further, thus leaving it quite doubtful if extra-polation to zero colloid concentration would give a well-defined value for the intrinsic viscosity.

Curve forms as in Fig. 23, or at least the left descending branch of it, have been found in all sols that were investigated: agar, gum arabic[1], soluble starch[1], sodium thymus nucleate[2], sodium yeast nucleate [3] and gelatin[4].

It has been shown, that the "abnormal" curve form is suppressed if we investigate the viscosity concentration function at a sufficient, but still relatively small constant concentration of an added salt.

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[1] H. R. Kruyt and K. C. Winkler, *Kolloidchem. Beihefte*, 32 (1931) 374.
[2] H. G. Bungenberg de Jong and Ong Sian Gwan, *Kolloidchem. Beihefte*, 31 (1930) 89.
[3] H. G. Bungenberg de Jong and N. F. de Vries, *Rec. trav. chim.* 49 (1930) 658.
[4] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloidchem. Beihefte*, 36 (1932) 429.

As an example Fig. 24 gives the results for the thymus nucleate sol, the upper curve relating to sols without added salt, the lower to sols containing 5 m. eq. p. l. KCl.

This latter curve corresponding to the type of Fig. 21b is thus quite normal. The change of "abnormal" to "normal" curve forms is thus obtained in the region of salt concentrations in which the electroviscous effect is suppressed for the greater part.

The abnormal curve form for the blank can thus be explained by assuming that the electroviscous effect, being large at small colloid concentration, decreases by the mere increase of the colloid concentration. In a later investigation by Kruyt, Bungenberg de Jong, and Lens[1] on the viscosity of gum arabic as a function of sol concentration, the viscosity concentration function for sols without and with added electrolytes was studied once more. It was found, that in the presence of sufficient salt a very simple relation[2] exists:

$$\log \frac{\eta_s - \eta_o}{\eta_o C} = k_1 C + k_2$$

the logarithm of the specific viscosity increase being a linear function of the colloid concentration (see Fig. 25). This simple relation holds for the whole concentration range studied (up to 30% gum arabic), and it applied also perfectly for the viscosity of solutions of various high polymers without electrolyte character.

Gum arabic sols without added salt gave in the range 0—10% colloid concentration a strong deviation (Fig. 25) whereas at higher colloid concentrations the above formula applies here also. Compare also Fig. 26 which refers to only small colloid concentrations (0.028—2.558%) without added salt and in the presence of small KCl concentrations.

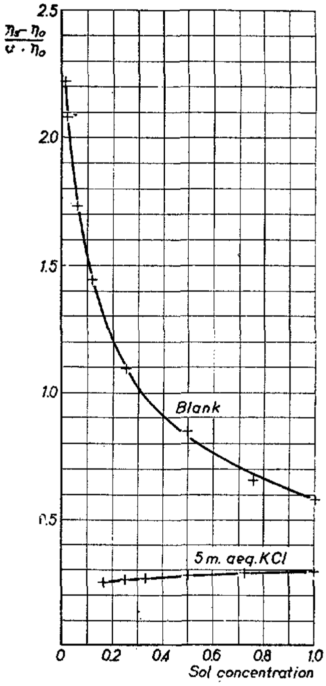

Fig. 24. $(\eta_s - \eta_o)/\eta_o$ C as a function of the concentration of the Na thymus nucleate sol (42° C). Abscissae: nucleate concentration expressed in that of the highest investigated concentration (= 0.164%) as unit.
Upper curve: experimental results of the sols diluted with distilled water.
Lower curve: similar experimental results for which care is taken that 5 m. eq. p. l. KCl is always present in the sols (see text.)

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[1] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloid Beihefte*, 36 (1932) 429.

[2] This relation may be compared with the very analogous relation

$$\frac{\eta_s - \eta_o}{\eta_o C} = k_2 + k_1 C$$

which describes the behaviour of dilute solutions of uncharged macromolecules very well. Cf. R. H. Ewart, in: *Advances in Colloid Science II*, 197, Interscience, New York 1946.

The logarithmic equation mentioned above however is valid up to very high concentrations (at least 30%) whereas the linear equation which has a better theoretical foundation is valid only up to concentrations of a few per cent.

In this first concentration range (up to 10% gum arabic) we have therefore once more the same abnormality as already discussed above occurring in agar (Fig. 23), thymus nucleate (Fig. 24) and other sols. It is caused by the presence of the electroviscous effect at low and the self-suppressing of it at high colloid concentrations.

Indeed as regards the influence of indifferent salt on less and more concentrated gum arabic sols, the fall in relative viscosity becomes smaller the higher the colloid concentration[1]. Compare the curve bundles in Fig. 27.

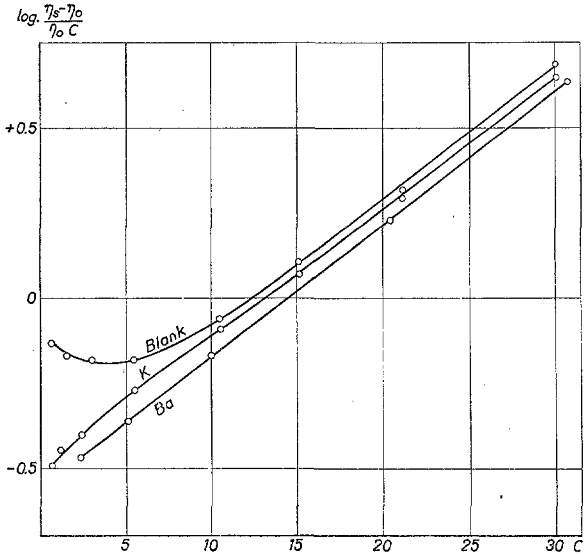

Fig. 25. log $(\eta_s - \eta_0)/\eta_0\, C$ as a function of the sol concentration in gum arabic (42° C).
Abscissae: gum arabic concentration expressed in gram per 100 ml.
Upper curve: experimental results of the sols diluted with distilled water.
The curves below; ditto at constant presence of 10 m. eq. p. l KCl or $BaCl_2$.
The abnormal shape of the blank curve has given place to a simple linear course, which offers a chance of extrapolation to the sol concentration = O. The slight deviations from the linear course of the KCl curve at the smaller concentrations are possibly derived from experimental errors. The downward bend is not present in Fig. 26.

Thus it seems, that already crowding together of the sol particles into a smaller volume (i. e., increase of colloid concentrations) removes part of the large electroviscous effect existing at low colloid concentrations. This crowding together has thus in principle the same result as the addition of an indifferent salt at constant colloid concentration.

Though for these high sol concentrations the simple formulation $(\eta_s - \eta_0)/\eta_0 \backsim N^2 . A^3 . G$ will no longer hold, it may be supposed that among the factors determining the actual (high) viscosities, the flexibility of the macromolecule will still play a similar rôle.

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[1] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloidchem. Beihefte,* 37 (1933) 395.

For an explanation of the "self suppression" of the electroviscous effect we may then suppose that the macromolecular electrolyte itself acts as indifferent electrolyte and thus diminishes the interaction between its own charged spots thus leading to a denser coil and a smaller electroviscous effect.

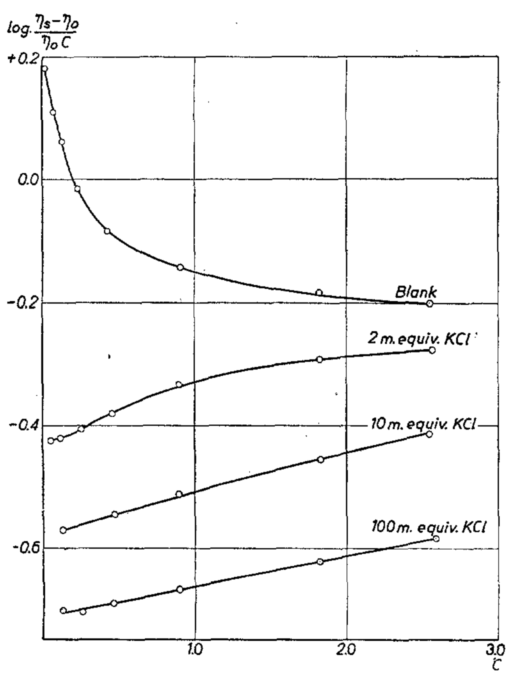

Fig. 26. log $(\eta_s - \eta_0)/\eta_0\, C$ as a function of the sol concentration with gum arabic (25° C). Abscissae: gum arabic concentration expressed in gram per 100 ml.
Top curve: without added salt. On account of the shape of the curve an extrapolation to the sol concentration = O is very uncertain.
Below: The abnormal course of the blank curve disappears on addition of sufficient KCl, as a result of which the above mentioned extrapolation does appear to be permissible.
(at 2 m. eq. p. 1 KCl the course of the curve is not yet quite linear).

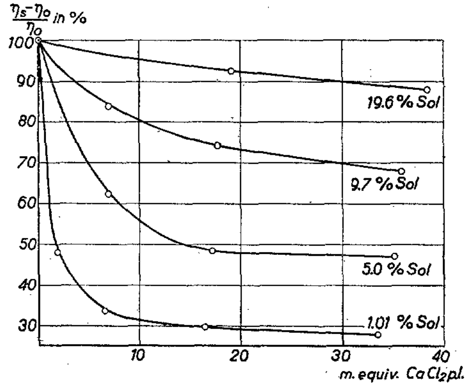

Fig. 27. Neutralisation of the electroviscous effect by $CaCl_2$ for a number of gum arabic sols of increasing concentrations.
The sol concentrations are expressed in grams per 100 ml.

§ 9. REVERSAL OF CHARGE AND ELECTROVISCOUS EFFECT

The occurrence of a viscosity minimum in the viscosity-pH curve of gelatin (see p. 208 Fig. 20) can be simply explained from the macromolecular point of view from changes in the flexibility of the macromolecule, for both a positive and a negative charge will diminish this flexibility and thus $(\eta_s - \eta_0)/\eta_0$ will then be greater than in the uncharged condition.

However we must thereby take into consideration that according to modern points of view an amphoteric protein is not really uncharged at the I. E. P., but contains an equal amount of positively and negatively ionised groups.

If ionised groups of opposite sign are thus present side by side on the macro-

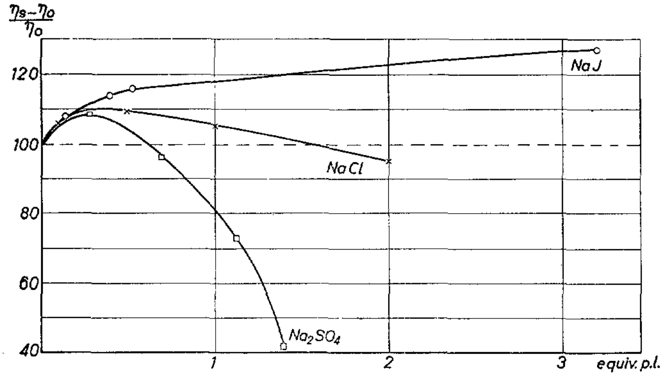

Fig. 28. Influence of NaI, NaCl and Na_2SO_4 on $(\eta_s - \eta_o)/\eta_o$ of a 0.8% isoelectric gelatin sol (50° C). In the region of small concentrations the curves of all three salts rise above the level of the isoelectric gelatin (the value of $(\eta_s - \eta_o)/\eta_o$ corresponding to this is called 100%). At higher concentrations the curves sort themselves out into the lyotropic sequence of the anions.

molecule, they will by their mutual attraction make the skein contract below the "most probable" volume. This must have as a result that $(\eta_s - \eta_o)/\eta_o$ of the isoelectric gelatin is smaller than in the really uncharged state. Added indifferent salts will by their screening effect lessen the mutual attraction of the oppositely charged groups, and thus make the skein expand again to a volume nearer to the "most probable" one. It can thus be foreseen that added salts will increase $(\eta_s - \eta_o)/\eta_o$.

The increasing influence of indifferent salts in small concentrations on the relative viscosity of isoelectric gelatin sols found by HOLLEMAN, BUNGENBERG DE JONG, and MODDERMAN[1] can be explained in this way. Fig. 28 shows the influence of Na_2SO_4, NaCl and NaI up to relatively high concentrations.

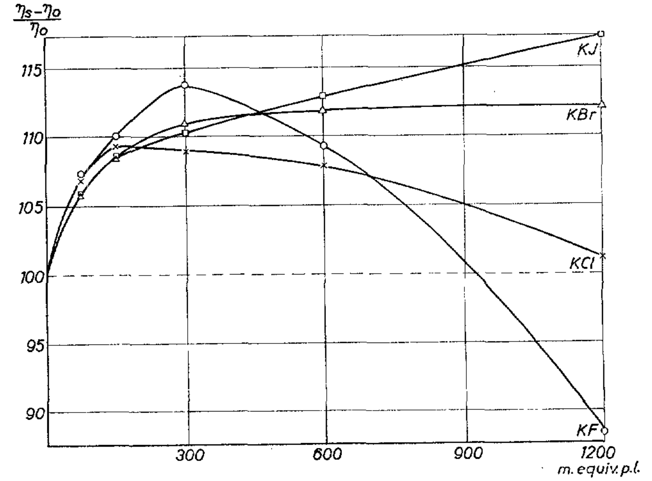

Fig. 29. Influence of some K salts on $(\eta_s - \eta_o)/\eta_o$ of a 2.5% isoelectric gelatin sol (50° C). As in Fig. 28: at smaller concentrations rise of all the curves above the level 100%, at higher concentrations arrangement in the sequence of the lyotropic series of the monovalent anions. For the course of the KF curve, see note. 1 on page 219.

Fig. 29 shows the same for KI, KBr, KCl and KF. In both figures so-called lyotropic influences of the anions are clearly seen, these causing the curves to bundle

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[1] L. W. J. HOLLEMAN, H. G. BUNGENBERG DE JONG, and R. S. TJADEN MODDERMAN, *Kolloidchem. Beihefte*, 38 (1933) 439.

The explanation given in this publication was not yet based on the macromolecular point of view, but already used the idea of expansion and contraction of the loosely built colloid particles.

out at higher concentrations. But we must direct our attention to the effect all salts have in common in small concentrations[1], this effect being an increase of the relative viscosity above the level for the isoelectric gelatin sol without added salts. The above given reasoning will thus explain this general effect of salts, but it foresees also the experimental fact that in this range of small concentrations not all salts will increase $(\eta_s - \eta_o)/\eta_o$ exactly in the same way.

Compare Fig. 30 which shows the influence of KCl, KNO_3 and KI of the type 1 — 1, further of K_2SO_4 of the type 1 — 2, of $K_3Fe(CN)_6$ of the type 1 — 3 and $K_4Fe(CN)_6$ of the type 1 — 4. By inspecting the figure, the most important factor determining the steepness of the initial increase in relative viscosity (in the concentration range 0 — 150 m. eq. p. l) is without doubt the valency of the anion.

KCl, KNO_3 and KI[2], salts of the type 1 — 1, together form a bundle giving the lowest increase in relative viscosity. K_2SO_4 (type 1 — 2) mounts somewhat higher (compare at 150 m. eq. p. l), whereas $K_3Fe(CN)_6$ (type 1 — 3) gives a still more rapidly mounting curve which is far surpassed by $K_4Fe(CN)_6$ (type 1—4).

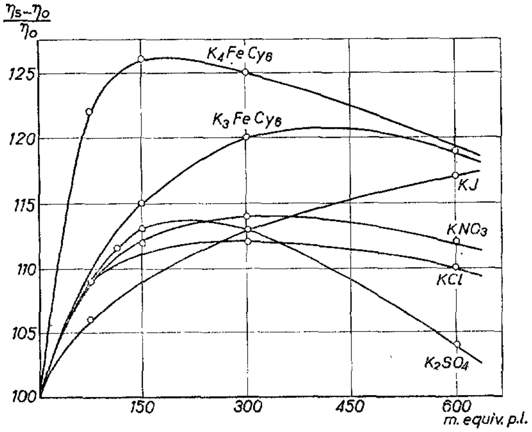

Fig. 30. Influence of some salts of the types 1—1, 1—2, 1—3, 1—4 on $(\eta_s - \eta_o)/\eta_o$ of a 0.8% isoelectric gelatin sol (50° C).
All the salts produce a rise at small concentrations for *which the valency of the anion is of significance.* Compare the sequence of the curves at 150 m. eq.: from bottom to top there follow first the three salts of the type 1—1, then successively 1—2, 1—3 and 1—4 (see further text).

This experimentally found importance of the ion valency can be quite easily foreseen from the general explanation given above.

We must then first state, that the above reasoning will apply in its purest form only for such a salt, of which the cation will screen off the negative ionised groups of the macromolecule to exactly the same extent as its anion screens off the positively ionised groups of the macromolecule.

Such an ideal salt will not alter the isoelectric condition, and we shall assume that salts of the type 1 — 1 come near to this ideal salt[3]. With salts of the type 1 — 2, 1 — 3 and 1 — 4, the polyvalent anion will in the given order screen off the positively charged groups of the macromolecule to an increasingly greater extent than the monovalent cation screens off the negative ionised groups.

Thus also an unbalancing of the original electrical compensation characteristic of the isoelectric state, will be the result, which will increase in the order 1—2, 1—3, 1—4. Of course this unbalancing will in itself diminish the efficiency of the mutual

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[1] The irregular course of the KF curve in Fig. 29 in the range of smaller concentrations is probably caused by an increase of the pH, KF being the salt of the weak acid HF.

[2] The very low value for KI at 75 m. eq. p. l seems suspect and may be due to an experimental error.

[3] The differences between KCl, KNO_3 KI in Fig. 30 show already, that besides the valency other factors of specific nature play a rôle. They will not be considered here. See Chapter IX, p. 259.

attraction of the oppositely charged ionised groups of the macromolecule and the sequence of the curves 1—4 > 1—3 > 1—2 > 1—1 in Fig. 30 may be explained by this.

Reversal of charge with hexol nitrate of negatively charged macromolecular sols — first met with in the case of amylum solubile (see p. 207 Fig. 19) — has been shown to occur very generally. In general they do not lend themselves to a viscosimetric analysis because, in contrast to amylum solubile, flocculation or coacervation phenomena in most cases accompany the reversal of charge (see p. 223 § 10).

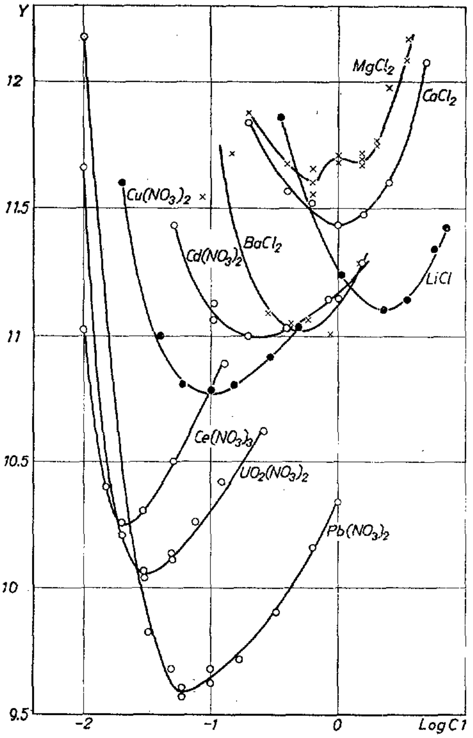

Fig. 31. Influence of a number of salts on $(\eta_s - \eta_o)/\eta_o$ of a 1% Na arabinate sol (25° C). Ordinates: Y = quantity which apart from a small correcting factor (which is however constant) corresponds to $(\eta_s - \eta_o)/\eta_o$ expressed in % of the corresponding value for the sol without added salt. Abscissae: logarithm of the salt concentration, the latter expressed in eq. per l (thus — 2 = 0.01 N, — 1 = 0.1 N, 0 = 1N).
With all the salts a minimum curve is obtained which lies very close to those concentrations at which reversal of charge occurs (compare with Fig. 32 and 33. See further text).

Also lower valent cations share this property of bringing about reversal of charge, but the required salt concentrations are then increasingly higher the lower the valency of the cation (see p. 276 Chapter IX § 2).

If such a colloid and such salts are chosen, that form favourable combinations for viscosimetric investigation, — favourable here meaning that no flocculation or coacervation accompanies the reversal of charge — then it may be expected that, similar to the combination amylum solubile + hexol nitrate minima in the $(\eta_s - \eta_o)/\eta_o$ curves must also occur.

The viscosimetric detection of these minima for salts, which bring about reversal of charge at not very low concentrations, will however not be very easy. For at these higher salt concentrations (where activity coefficients are already low) it may be expected that the branch ascending from the minimum at the side of the higher salt concentrations will be still much less steep than for hexol nitrate in Fig. 19.

The ordinary technique of viscosimetry, which gives figures accurate to a few 0.1%, will at most be able to detect the existence of the predicted minima in the viscosity curves, but will not suffice to study them in more detail. Holleman and Bungenberg de Jong[1]

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[1] L. W. J. Holleman and H. G. Bungenberg de Jong, *Kolloidchem. Beihefte*, 46 (1937) 113.

used for that purpose a technique permitting the measurement of the time of flow with a reproducibility of a few 0.01 %. With the sodium arabinate sol they could detect the existence of these minima in the relative viscosity, using one trivalent, seven divalent and one monovalent cations.

The results are given in Fig. 31, in which y represents $\frac{\eta_s - \eta_0}{\eta_0}$ in per cent of the blank. This quantity is given as a function of the logarithm of the salt concentration, (the use of log *C* is here of advantage to make the minimum character of the viscosity curves for all salts plainly visible in one and the same figure).

The reversal of charge concentrations were also determined electrophoretically on the movements of suspended SiO_2 particles in the arabinate sol, see Fig. 32.

Comparing the curve minima with the reversal of charge, a direct correlation is plainly visible, see Fig. 33. The order in which the minima follow from left to right is the same as that of the reversal of charge concentrations, but in general the latter lie somewhat (mean 0.2 units) to the right.

This investigation has thus shown that indeed minima in the relative viscosity occur as predicted for salts bringing about reversal of charge, though the systematic non-coincidence of minimum and reversal of charge experimentally found, still needs an explanation[1].

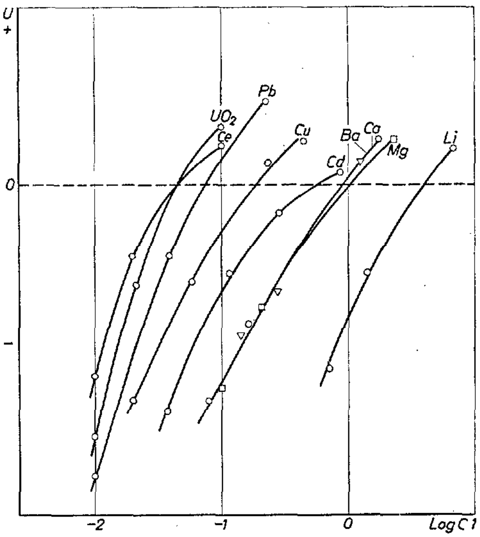

Fig. 32. Reversal of charge of 1% Na arabinate with the salts of Fig. 31.
Ordinates: Electrophoretic velocities measured on suspended SiO_2 particles, expressed in arbitrarily chosen units.
Abscissae: log of the salt concentration (expressed in equiv. per l).

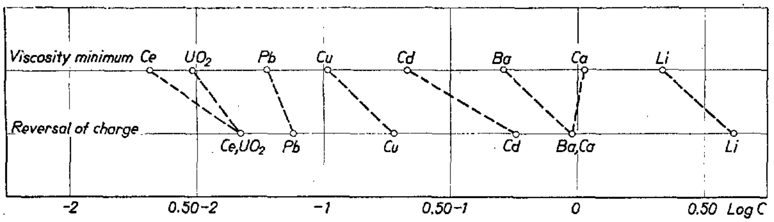

Fig. 33. *Relative positions of the viscosity minima of Fig. 31 and of the reversal of charge concentrations of Fig. 32.*
Abscissae: logarithms of the salt concentrations (expressed in equiv. per l).
The reversal of charge concentrations lie in general somewhat higher (average 0.2 logarithmic units).

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[1] For instance it may be asked if the reversal of charge of the adsorbed arabinate film on SiO_2 does coincide with that of the arabinate particles free in solution, or lies systematically too high. Other possible causes are discussed in the original publication.

Of more importance are here the different levels, at which for every salt used the relative viscosity reaches its minimum value.

If at the reversal of charge point the macromolecule was really uncharged, we would expect that the minima with all salts investigated would lie on the same horizontal level in Fig. 31.

As discussed above (p. 218) for the *I.E.P.* of gelatin, we can however here also assume, that the reversal of charge state does not represent a state in which all charges on the macromolecule are absent, but one in which positive and negative charges compensate one another. Local positive charges can for instance arise from polyvalent cations fixed on the negative monovalent ionised carboxyl groups of the arabinate molecule. For monovalent cations this will not suffice, but an extra fixation on other polar groupings (e.g., OH groups) must be assumed to create local positive charges on the macromolecule.

In principle the different levels at which the minima are situated in Fig. 31 are then no longer inexplicable.

For we must assume here as above for the *I.E.P.* of gelatin that the simultaneously present positive and negative charges on the macromolecule will cause a "contraction" of the macromolecular skein, and this contraction can be of different grades, it being e.g., greater if the salt concentration at the viscosity minimum is lower (a smaller general screening effect of ions present then opposes this "contraction").

Inspection of Fig. 31 reveals that these minima are not distributed at random over the figure. On the contrary a distinct correlation between y *min* and log *C* (of the minimum or of the reversal of charge) exists. Low values of y *min* are found with $Ce^{\cdot\cdot\cdot}$, $UO_2^{\cdot\cdot}$ and $Pb^{\cdot\cdot}$ showing low values for log C, whereas high values of y *min* are found with $Mg^{\cdot\cdot}$, $Ca^{\cdot\cdot}$ and $Li^{\cdot}$, having high values of log *C*.

We shall see later that reversal of charge is directly connected with fixation of cations on the ionised groups of the colloid and that as regards this fixation specific properties of the cations play a great rôle. Here no longer valency of the cation is the only important quality, but others such as volume, polarisability and polarising power of the cation come into play. See Ch. IX, § 2, p. 276.

Now in Fig. 31 such specific features also occur, the valency of the cation not being the only factor which determines the $\frac{\eta_s - \eta_0}{\eta_0}$ value at the minimum.

The divalent cations of Pb and Cu, belonging to the B subgroups of the periodic system act in this respect much more strongly than the divalent cations of Ba, Mg and Ca, belonging to the A subgroups of the periodic system, and even stronger than the trivalent cation Ce.

The specific actions of course disturb the expected correlation between height of the viscosity minimum and reversal of charge concentrations.

If we consider cations belonging to a natural family, thus the series Ba, Ca, Mg or the series Pb, Cu, Cd, then we find that indeed with a higher reversal of charge concentration there corresponds a higher $\frac{\eta_s - \eta_0}{\eta_0}$ value in the minimum.

We may conclude that the different $(\eta_s - \eta_0)/\eta_0$ values, which occur in Fig. 31 at the viscosity minima must be connected with different grades of contraction of the macromolecular skeins, caused by electrical attraction forces, set up by localised attachment of cations on the macromolecule, especially on its negatively charged ionised groups.

Such attractions may also exist between adjacent macromolecular skeins. Thus it is probable that at and around the viscosity minima associations of macromolecules exist.

With the cations studied here the arabinate sol still remains perfectly "stable", but with 4 and 6 valent cations flocculation or coacervation occurs. These higher valent cations have lower or even very low (true) reversal of charge concentrations (see p. 259 Chapter IX § 1 and 2), that means:

a. that the attachment of cations is here much stronger,
b. that the general screening effect of ions present is now much less.

Both factors will thus be favourable for producing still stronger contractions in the macromolecular skeins and much more intense mutual interactions between adjacent macromolecules. Obviously with these 4 and 6 valent cations the latter reach such a magnitude that actual transgressions of solubility occur.

§ 10. TRANSGRESSIONS OF SOLUBILITY ACCOMPANYING THE SUPPRESSION OF THE ELECTROVISCOUS EFFECT

The behaviour of soluble starch sol towards reversal of charge with hexol nitrate, without flocculation accompanying it, is rather an exceptional case (p. 208).

As a rule negative colloids of acidic nature are flocculated or coacervated by small amounts of hexol nitrate, rhodochrome chloride and $Pt(en)_3(NO_3)_4$, that are salts with 6, 5 or 4 valent complex cations (see p. 270, Table 2)

In the simplistic stability theory discussed in § 4, this "instability" occurring as a result of "discharging" at very low concentrations, does not fit in. One would have to assume, that simultaneously with the reduction of ζ a dehydration occurs, the reason why being obscure. For at these low concentrations (e.g., of a few m. eq. p. l or less) there is no reason to explain this "dehydration" as a "salting out effect", comparable for instance with the action of $(NH_4)_2SO_4$ on protein sols or of $MgSO_4$ on agar sol (see Fig. 14), which occurs as a rule at relatively high concentrations.

That these flocculations (or coacervations) with high valent cations are of a quite different nature is clearly shown in the case of gum arabic. Its sols, as Pohl[1] has already shown, cannot be salted out even by the highest concentrations of salts commonly used for this purpose. Nevertheless a 1% sol is coacervated by hexol nitrate in concentrations higher than 5 m. eq. p. l, and more diluted sols at proportionally lower concentrations, the latter fact already indicating that the expression "concentration" is here of a doubtful use (for fuller information see p. 262, Chap. IX § 1b).

Still another example may be quoted to illustrate the quite different nature of salting out and of flocculations with hexol nitrate and other salts with polyvalent ions in small concentrations: amylum solubile sols are readily salted out e.g., with certain sulphates, whereas its sols do not flocculate with the named 6, 5, or 4 valent complex cations.

Salting out must be considered as being caused by changing the solvent in such a direction, that the macromolecule becomes difficultly soluble along larger parts of

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[1] Pohl, *Z. physiol. Chem.*, 14 (1890) 155.

its length, irrespective of the presence or absence of ionised groups. The flocculations (coacervations) with the named polyvalent ions on the other hand are primarily connected only with fixations of these ions on the ionised groups of the macromolecule and one very important factor is thereby the frequency of occurrence of the ionised groups along the macromolecule.

If this frequency is very small, as in soluble starch, even the highest valent cation will not suffice to cause flocculation or coacervation.

In gum arabic the frequency is much greater and it allows of coacervation with salts of the types 6—1, 5—1, 4—1, yet not with lower valent cations.

Other colloids have still greater frequency of occurrence of the ionised groups and here also transgression of solubility is possible with 3—1 or even with 2—1. (For fuller information on the above statements see Chapter IX in particular § 1 d, p. 269 and Chapter X in particular § 3 d, p. 392).

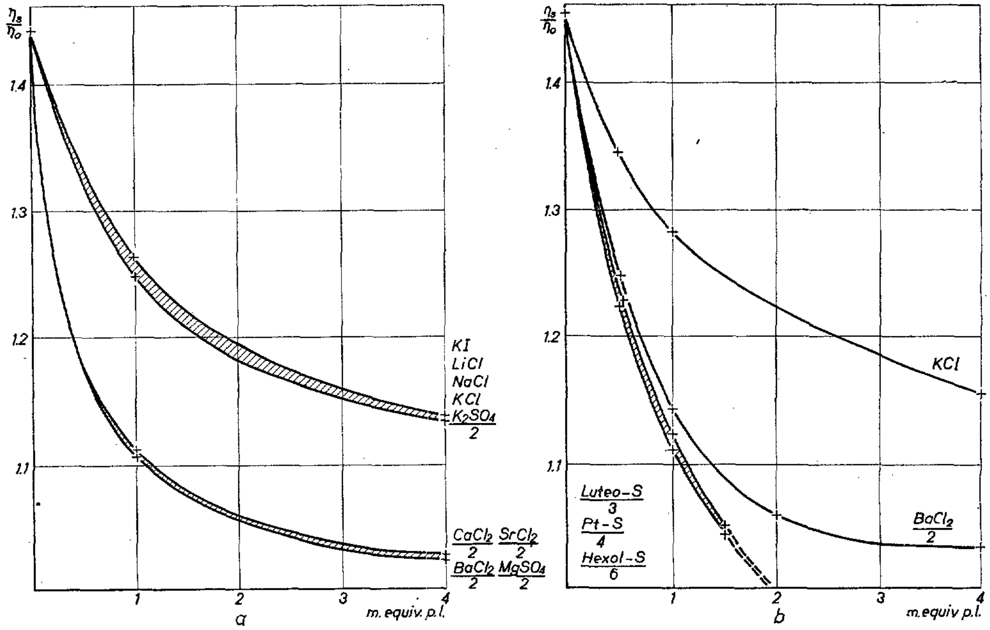

Fig. 34. Influence of salts in small concentrations on the relative viscosity of 0.1% Na thymus nucleate. (42° C).
Ordinates: relative viscosity.
Abscissae: salt concentration in m. eq. p. l.
a: The given salts divide into two groups, characterized by the valency of the cation. K_2SO_4, although of the type 1—2, acts as strongly as the four given salts of the type 1—1. In the same way $MgSO_4$ (2—2) fits with the three salts of the type 2—1.
b: The normal grouping of the curves according to the valency of the cation is restricted to 1—1, 2—1 and 3—1 but 3—1, 4—1 and 6—1 are practically equivalent and with these flocculation occurs at the bottom of the curves.
Luteo-S abbreviation for $Co(NH_3)_6Cl_3$ (type 3—1).
Pt-S ,, ,, $[Pt(en)_3](NO_3)_4$ (type 4—1).
Hexol-S ,, $[Co\{(OH)_2\ Co(en)_2\}_3](NO_3)_6$ (type 6—1).
At very small concentrations $BaCl_2$ also fits closely with 3—1, 4—1 and 6—1.

An interesting feature in the course of the viscosity curves for such cases in which flocculation (or coacervation) accompanies the depression of the electroviscous effect, is the crowding together of these curves. As examples in Fig. 34 we give the influence of some indifferent salts on the relative viscosity of the sodium thymus nucleate sol[1] and in Fig. 35 the same for the carrageen sol[2].

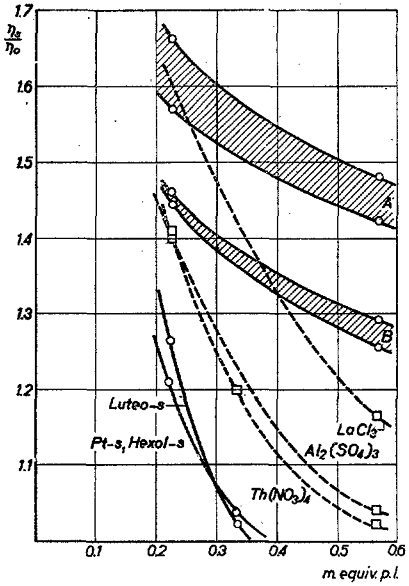

Fig. 35. Influence of salts in very small concentrations of the relative viscosity of 1/90% carrageen sol (42° C). Ordinates, abscissae, luteo-s, Pt-s and hexol-s as in Fig. 34.
The curves start at $\eta_s/\eta_o = 2.3$ for the salt concentration zero; no measurements have however been made in the concentration range 0—0.22 m. eq. per l.
In the shaded strip A lie the curves for: LiCl, NaCl, KCNS, KCl, K_2SO_4, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ thus salts with monovalent cations (types 1—1, 1—2, 1—3, 1—4).
In the shaded strip B lie the curves for: $Ba(CNS)_2$, $Ba(NO_3)_2$, $BaCl_2$, $Mg(NO_3)_2$, $MgCl_2$, $MgSO_4$, $CaCl_2$ and $SrCl_2$, thus salts with divalent cations (types 2—1 and 2—2).
Luteo—s (3—1), Pt—s (4—1) and Hexol—s (6—1) act practically equally strongly and give flocculation at the lower end of the curves.
The three dotted curves run quite discrepantly which is attributable to disturbances, connected with the strong tendency to hydrolysis of the salts in question.

In the first named sol, we see in Fig. 34b the normal sequence 1 — 1 . . . 2 — 1 . . . 3 — 1 . . ., but 3 — 1, 4 — 1 and 6 — 1 give approximately the same curve and all three flocculate the nucleate sol. The curves here fall to extremely low values of $\frac{\eta_s - \eta_o}{\eta_o}$

In principle the same is shown by the carrageen sol, though here the bundles corresponding to the salts with monovalent or divalent cations are very broad. Here also 3 — 1 . . . 4 — 1 . . . and 6 — 1 form one bundle, ending in flocculation and very low values of relative viscosity.

This carrageen sol is interesting in further respects. It belongs to the extremely high viscous type, the 1/90% sol showing a relative viscosity of 2.3. The very great electroviscous effects may partly be caused by the small sol concentration (see § 8), partly be connected with the abnormal viscosity character, this sol not following POISEUILLE's law, the viscosity varying in the domain of the low shearing stresses commonly used in the OSTWALD viscometer.

For an explanation of the crowding together or even practical coincidence of the curves for polyvalent ions, we must first state that this recalls very much the

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[1] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 31 (1930) 89.
[2] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 29 (1929) 436.

so-called *equivalent flocculation* known in lyophobic sols (see Volume I). Electro-equivalent amounts of added 6, 4 or 3 valent cations in Fig. 34 and 35 lower the relative viscosity to the same extent, and lead to transgression of the solubility of the macromolecular colloid. These facts are much in favour of the above postulated fixation of the cations on the ionised groups of the macromolecular colloid, as the cause of flocculations and of reversal of charge phenomena.

In general flocculation (or coacervation) sets in already before the reversal of charge point is reached. We shall later see an example in which equivalent amounts of 6 and 3 valent ions are indeed fixed at the reversal of charge point (see p. 265 Fig. 4).

As it may be assumed that the fixation of these polyvalent ions is very strong, the concentrations of the cations free in the solvent in equilibrium with the fixed cations may be very small at that gross concentration in which the viscosity curves descend in Fig. 34 and 35. In this case, though the amounts of free cations may still be very different for 6, 4 and 3 valent cations, nevertheless practically every cation added to the sol may be fixed on the colloid. So that the curves may practically coincide and flocculation may occur at the same apparent concentration.

With lower valent cations, the concentrations of the ions free in the solvent, that correspond with a certain occupation of the ionised groups with cations, are so great, that they can no longer be neglected. Coincidence of the curves for salts of the type 2—1 over the whole length with the curves for 6—1, 4—1 and 3—1 will then no longer occur. It is only at very low concentrations (where fixation of cations is relatively much stronger) that the number of the cations free in the solvent may become small compared with the number fixed on the colloid. This explains the fact that in Fig. 34 at low concentrations the $BaCl_2$ curve comes very near to the bundle containing the curves for 6—1, 4—1 and 3—1.

With KCl, the conditions are still more unfavourable, so that the KCl and $BaCl_2$ curve do directly take different courses.

Reviewing the action of salts on negatively charged colloids, we may discern three cases:

A. *Simple screening effect* by the cation of the added salt, this effect giving a typical spreading of curves in the suppression of the electroviscous effect according to the valency of the cation. With increasing salt concentration sooner or later, depending on the nature of the cation and the nature of the colloid, this effect emerges into B.

B. *Fixation of cations on the ionised groups.* This effect gives crowding together of curves in the suppression of the electroviscous effect, is often accompanied by transgression of solubility, and can further lead to reversal of charge phenomena.

C. *Salting out effet*, for which the presence of ionised groups on the macromolecule is not a *conditio sine qua non* as in A and B. Relatively large concentrations are needed (see the bend in the $MgSO_4$ curve in Fig. 14 on p. 202) and the position of both cation and anion in particular of the latter, in the lyotropic series is here of primary importance.

For the action of salts on positively charged colloids the same division into three cases applies, for A and B now the valency and specific[1] properties of the anion being of primary importance. As for C (salting out) as well the action of the anion preponderates over the action of the cation, it may sometimes be difficult to discern if a flocculation or coacervation of a positive sol, e.g., with monovalent anions, is the result of the actions B or C.

Discrimination is then often possible by determining the relative intensities of flocculation with other monovalent anions.

Indications for C are then, that the order of increasing flocculation is found: CNS — I — Br — Cl — (F), the concentration needed being as a rule not very small. Indications for B, that the order is just the reverse (F) — Cl — Br — I — CNS, the concentrations needed being here sometimes relatively small (See p. 299, Chapter IX § 3b and p. 407, Chapter X § 3j).

§ 11. VISCOUS BEHAVIOUR OF SHORT CHAIN-MACROMOLECULAR "COLLOIDS"

Sols of long chain macromolecular colloids may be of the low viscous type if the kinetic units in their sols consist of densely built corpuscules — the long chain molecule being folded up — as is assumed to hold for many native proteins.

If the kinetic unit is not built up in that way, the macromolecular colloids belong to the high viscous type, their long chain molecules forming statistical skeins in solution.

From the formula $(\eta_s - \eta_o)/\eta_o \backsim N^2A^3G$ it may be seen that in a "homologous polymeric series" viscosity will increase with increasing molecular weight, (which is proportional to NA). The earliest members of such a series however must necessarily give low viscous sols. Such short chain molecules are for instance present in *Na-yeast nucleate* (which is generally formulated as a tetranucleotide, consisting of only four mononucleotides linked together by ester phosphate linkages. *Na-thymus nucleate* is constituted on similar lines (abstraction made from certain details, which are not important here) but here not only four nucleotides but a very great number are linked together by ester phosphate linkages (see formula on p. 188 § 1c).

Thus it is interesting to compare the viscous behaviour of yeast nucleate and thymus nucleate.

We have already seen in § 10, that the latter is indeed a colloid of the very high viscous type (compare the relatively high values of η_s/η_o at very small sol concentration), this being in agreement with the high polymeric nature of its molecules.

The large decrease in η_s/η_o by suppressing the electroviscous effect, is also to be expected, considering that in the backbone of the molecule — probably $(C\text{-}C\text{-}C\text{-}O\text{-}P\text{-}O)_n$ — the phosphorus atoms recurring at short distances are the centres of monovalent ionised phosphate groups. The macromolecule may then be symbolized by —⊖—⊖—⊖— etc., that is a long chain molecule in which negative charges are situated at short intervals. This of course will contribute to a markedly diminished flexibility of the macromolecule.

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[1] The normal crowding together but now of polyvalent anions, is to be seen in Fig. 17b (p. 205), with the positive casein sol for the curves obtained with $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$. They also end in flocculation.

It may even occur, that specific properties are of so great importance that an inversion of the normal sequence of the curves in the suppression of the electroviscous effect is the result. See Fig. 38 (p. 230) in which $K_3CH(SO_3)_3$ (type 1—3) decreases η_s/η_o more than $K_4Fe(CN)_6$ (type 1—4).

Thus a large electroviscous effect may be expected and consequently a large decrease in relative viscosity on adding indifferent salts (see p. 224 Fig. 34).

Now turning to Na-yeast nucleate, we meet with a "colloid" which only slightly increases the viscosity of the solvent. Compare Fig. 36, where we see that the 0.83% sol has only a relative viscosity of 1.052 (The 0.1% thymus nucleate however gives $\eta_s/\eta_0 = 1.45$).

For an accurate measurement of the influence of salts on the viscosity of yeast-nucleate it was necessary to use a technique which gave a reproducibility of a few 0.01% in the time measurements.

The results[1], see Fig. 36, gave a normal sequence of the curves for KCl, $BaCl_2$ and $Co(NH_3)_6Cl_3$ and we may interpret this decrease of the relative viscosity as a suppression of the electroviscous effect.

The yeast nucleate molecule is however relatively so small that its four mononucleotide residues possibly do not suffice to form a chain element. Thus a skein proper will not be present in solution. Nevertheless in principle the decrease in viscosity by added salt may be explained on similar lines from the increased flexibility of the (short) chain molecule. Without added salt the latter will by the mutual repulsion of the ionised phosphate groups assume a more or less stretched form. If this "electrical stiffening" is removed by added salts, which screen off the ionised phosphate groups, the chain molecule may assume a more bent form.

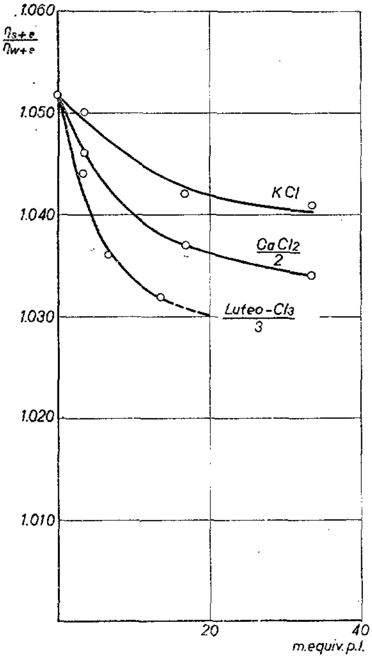

Fig. 36. Neutralisation of the electroviscous effect for a 0.83% sol of Na yeast nucleate (42° C). Beyond 13.3 m. eq. per l. Luteo—s (= $Co(NH_3)_6Cl_3$) flocculation occurs. This figure should be compared with Fig. 34 from which it appears that, notwithstanding the fact that yeast nucleate belongs to the low viscous, thymus nucleate to the very high viscous type of macromolecular colloids, the character of the bundle of curves is the same and also $Co(NH_3)_6Cl_3$ causes flocculation with both (and this occurs with both with $CaCl_2$ also at higher concentrations, see p. 270).

It is interesting that yeast nucleate also gives flocculation with $Co(NH_3)_6Cl_3$. Later (see p. 395) we shall see that as regards transgression of solubility by adding different cations the low polymeric yeast nucleate and the high polymeric thymus nucleate behave very similarly.

§ 12. LOW VISCOUS BEHAVIOUR AS A RESULT OF EXISTING "COMPLEX RELATIONS"

Investigations of the viscous behaviour of clupein sulphate sols have shown that these also give relatively low viscous sols, so that here also the measurement had to be

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[1] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 49 (1930) 658.

performed using a technique allowing reproducibility of time measurements to a few 0.01%.[1]

The viscosity-pH curve (Fig. 37) shows in principle the expected form, if one takes into consideration that the *I.E.P.* is situated at a high pH value, the only point which calls for explanation being the very low order of the η_s/η_o values which are obtained.

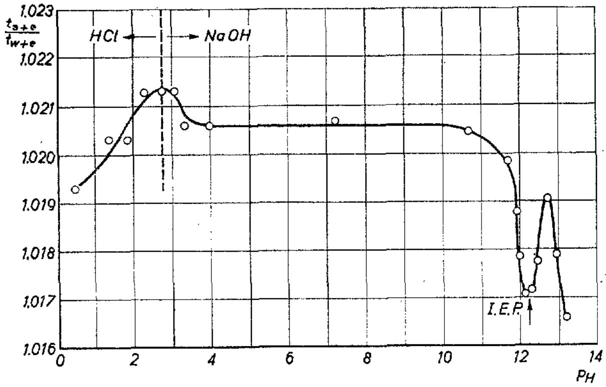

Fig. 37. pH-viscosity curve of a 0.292% clupein sulphate sol (25° C). (See text).
The ordinate gives the quotient of the times of flow in an OSTWALD viscometer for the sol (t_{s+e}) and a liquid of the same electrolyte composition but containing no clupein (t_{w+e}).
As the addition of the clupein has only a slight influence on the density, the ordinate gives practically the relative viscosity η_s/η_o.
The shape of the curve for this colloid of the low viscous type is in principle the same as those of proteins of the high viscous type (comp. Fig. 20). Minimum at the I.E.P. and fall of the curves at very high or very low pH values. As a consequence of the extreme position of the I.E.P. at already fairly high pH values, the maximum in the curve to the right of the I.E.P. is here very narrow, the maximum left of the I.E.P. on the other hand very broad.
The very flat course of the curve between pH = 10 and pH = 4 and the extra bump at pH = 3 can be directly explained from the amino acid composition of the clupein. Between pH = 10 and pH = 4 all guanidino groups are positively and the one carboxyl group negatively charged. The charge and thus the viscosity is constant in this range. In the neighbourhood of pH = 3 the dissociation of the carboxyl group is suppressed so that there the charge increases by one unit and the viscosity rises somewhat before it decreases again at lower values of the pH, due to high concentration of the ions present.
Turbidity (which is due to coacervation) occurs at the I.E.P.

There are sufficient indications that this low viscous character is not connected with its kinetic units being densely built corpuscules as in native proteins. There are for instance the absence of denaturation phenomena (by heat or otherwise) and the great ease with which coacervates of a distinctly liquid character can be obtained. Compare Ch. VIII § 3, p. 247 and Ch. X § 3j, p. 406.

The low viscous character could further be connected (as above in the case of Na-yeast nucleate) with the macromolecules being very short, consisting only of a few monomeric residues. This once more does not strictly apply for clupein, the latter though a protein of relative low molecular weight (a few thousands) still consists of more than 30 amino acid residues linked together by peptide groups.

We may expect that this comparatively small number of monomeric residues will not give very viscous solutions, but we hardly think it possible that the very low η_s/η_o value found experimentally can be explained wholly in this way.

Considering that $^2/_3$ of the amino acids consists of arginine, we might expect reasonable depressions of the relative viscosity on adding salts.

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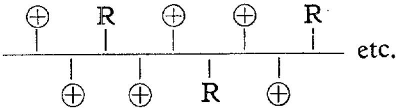
[1] H. G. BUNGENBERG DE JONG, W. A. L. DEKKER, and P. VAN DER LINDE, *Rec. trav. chim.* 54 (1935) 1.

For assuming a more or less regular distribution of the arginine side-chains along the macromolecule the latter may be symbolised by



$$\begin{array}{ccccccccc} \oplus & & R & & \oplus & & \oplus & & R \\ | & & | & & | & & | & & | \\ \hline & | & & | & & | & & | & \\ & \oplus & & \oplus & & R & & \oplus & \end{array}\ \text{etc.}$$

Then considerable electrical stiffening of the macromolecule may be expected (as was discussed in § 11, for thymus nucleate).

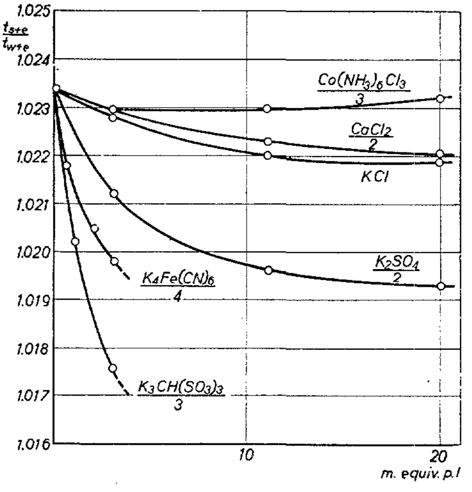


Fig. 38. Influence of small salt concentrations on the relative viscosity of a 0.32% clupein sulphate sol (pH 2.76, 25° C).
Some deviations, which are discussed in the text, here occur from a normal course of the curves in the suppression of the electroviscous effect of a positively charged sol.

Addition of salts will then increase the flexibility of the macromolecule, now decreasing considerably the number of monomeric residues constituting a chain element.

Investigation of the influence of salts on the relative viscosity (see Fig. 38) shows indeed a fall of η_s/η_0 with some salts, but this depression is certainly not very great.

The sequence KCl — K_2SO_4 is normal, which salts did not coacervate the clupein sol at the concentrations used.

The decrease in η_s/η_0 is greater with $K_3CH(SO_3)_3$ (1—3) and $K_4Fe(CN)_6$ (1—4) but the curves end here in coacervation. As discussed in § 10 such transgressions of solubility indicate "fixation" of ions on the ionised groups of the macromolecule. In such fixations the valency of the ions is no longer the only important factor, but specific factors of the ions concerned and of the nature of the ionised groups also enter. This gives a first explanation of the reversed order of the curves 1—3 and 1—4 in Fig. 38.

It is further important that K_2SO_4 at higher, but still relatively low, concentrations than used in Fig. 38 also coacervates the clupein sulphate sol, showing that such intimate relations between anions and positively charged ionised groups of clupein are already possible in the case of sulphate ions.

This gives us a clue for explaining the low viscous character of the clupein-sulphate sol. What we said above about the electrical stiffening of the macromolecule does not apply; this could only be the case if the sulphate ions were to be found at relatively great distances from the positively charged arginine side-chains. On the contrary we have to assume that already in the original sol an appreciable fraction of the sulphate anions take up positions very close to the positively charged ionised groups of the clupein (what in the previous paragraphs was called "fixation"), which necessarily will contribute to rolling up of the macromolecule into a relatively dense skein.

In this direction the divalence of the sulphate ions helps, as one SO_4 ion can then connect two monovalent positive ionised groups of the clupein molecule.

We might thus explain the low viscous character by the existence of so-called "complex relations" (see Chapter X: Complex Colloid Systems) between the positive ionised groups of the macromolecule and the dissociated sulphate ions[1].

In accordance with it is the fact, that clupein sulphate is only slightly soluble in water, addition of more clupein sulphate to its saturated solution giving two-phase systems, a liquid rich in clupein sulphate (coacervate) and a liquid poor in it (see p. 406 Chapter X § 3j).

A further detail in Fig. 38 also in accord with the supposition that complex relations are present in the original sol is the non-coincidence of the curves for 1—1, 2—1, 3—1, (compare Fig. 18c on p. 206, in which the curves for KCl, $CaCl_2$ and $Co(NH_3)_6Cl_3$ are very close together, thus showing the normal behaviour).

Considering in Fig. 38 the relative position of these curves and those for 1—2 and 1—3, we easily recognize the sequence of the so-called "continuous valency rule".

1—3 ... 1—2 ... 1—1 ... 2—1 ... 3—1

which, as will be discussed in Chapter X (p. 352, 388, 408, 415), is characteristic of systems in which complex relations are present. In the nomenclature of that chapter the clupein sulphate sol should be named a "Complex sol" (p. 336, 337).

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[1] In most other cases complex relations between colloids and oppositely charged ions arise only upon addition of electrolyte to the sol. In the case of the clupein sulphate, the complex relation is strong enough to be manifest in the pure sol.

VII. SOLS OF MACROMOLECULAR COLLOIDS WITH ELECTROLYTIC NATURE

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§ 1. INTRODUCTION AND SURVEY OF THE SUBSTANCES TO BE TREATED IN THIS AND IN THE FOLLOWING CHAPTERS

a. The electric charge as special characteristic of a group of colloids

The most striking difference between the sols from Volume I and the sols which have been dealt with in Ch. VI, p. 153, is that in the former the colloid particles carry an electric charge but in the latter they do not. In this chapter systems will now be dealt with which in a certain sense form a bridge between Volume II and Volume I, since on the one hand their colloidal nature is due to the macromolecular character of the substances but on the other hand by the possession of electric charges they show similarities with systems in Volume I.

Before we proceed to discuss in detail the various aspects which the electric charge of the macromolecules involves, a general observation may first be made.

If the electrolyte character of a macromolecule is to be demonstrable, this must be dissolved in a polar solvent, for example water. Through the presence of this polar solvent, even apart from the charge of the particles, the interaction between macromolecule and solvent will be greater as a consequence of the stronger polar interactions (hydrogen bonds, etc.) than in non-polar media and it is certainly no accident that it is just the charged systems in a polar medium dealt with here, which have given the collective name "hydrophilic (or more generally lyophilic) colloids" to the whole group of colloids treated in this second Volume.

In this and in the succeeding chapters we shall deal with the various properties of the sols of macromolecular colloids in so far as they depend on the charge of the particles.

In the first place the charge itself demands our attention. It will appear that in most cases it is not produced by adsorption as in the systems of Volume I but is due to the dissociation of groups firmly attached to the macromolecule, such as COOH etc. The charge thus becomes dependent on the degree of dissociation and consequently on the pH. Nevertheless so-called indifferent electrolytes can also influence the charge and even make it reverse in sign (see Ch. IX, p. 259).

One of the most direct consequences of the presence of charge is the movement of the particles in an electric field. The electrophoresis of these macromolecules both in solution and in the adsorbed form will certainly be an important aid in the investigation.

The osmotic pressure, of the greatest importance with the uncharged macromolecules for the determination of the molecular weight, is that also in principle for the charge-carrying macromolecules, but in this group the osmotic phenomenon is largely complicated by the occurrence of the DONNAN equilibrium which can frequently even quantitatively predominate over the osmotic action of the large molecules themselves. Since the DONNAN equilibrium is so closely connected with all double layer phenomena, it has been discussed extensively in Volume I. This discussion will not be repeated here.

The influence of the charge on the viscosity is a phenomenon very typical of this group of macromolecules. It is clear that in the presence of charges the skein will not assume its statistically most probable form, but will have a more rarified form through the interaction of the charged spots (mutual repulsion) or a more compact form if positive and negative charges are present simultaneously. As a result the viscosity, sensitive reagent for the skein form, is one of the most important aids in the investigation of systems with charged macromolecules.

Also the stability of the solutions of this group of charged macromolecules, characterised by the circumstances in which the solubility is just transgressed, has on account of the strong polar interactions a much more varied aspect than in the case of uncharged macromolecules. It appears that the charge can act both so as to raise and also so as to lower the solubility. The chapter on complex relations has this to thank for its particular significance.

b. Shape and size of the kinetic units

In the previous subparagraph it has already been mentioned that the shape of the skeins is influenced by the electric charges and especially by their mutual interaction. If there are only charged spots of one sign on the macromolecule then the skein will be relatively rarified. If charged spots of both signs are present then the skein will just be more compact than corresponds to the most probable state. Since this remodelling of the skein depends on the interaction of the charged spots, it is dependent not only on the number of charged spots but also on the extension of the ion atmosphere around each charged spot. The shape of the skein will therefore also depend on the concentration and nature of the indifferent electrolytes in the solution since these determine the thickness of the ion atmosphere[1].

Furthermore it appears that among the charged macromolecules there also occurs a group of substances the solutions of which have a relatively low viscosity and the viscosity of which does not depend greatly on the electrolyte concentration. One must indeed assume for this and other reasons that one is not here dealing with skein shape (possibly modified) but that in these "corpuscular proteins" the macromolecule is evidently rigid and — as is shown from the X-ray picture — folded in a very regular way. To what forces this formation is due is not yet known in full detail. In all probability cystine S—S linkages, salt bridges and hydrogen bonds play a part here.

The transition from the corpuscular type to the free skein shape is known from denaturation of proteins. It is quite in keeping with the above argument that this denaturation can be brought about by, among other things, extreme acid or alkaline

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[1] c.f. two very recent communications on this subject given independently by W. KUHN and by J. J. HERMANS and J. TH. G. OVERBEEK, presented at the meeting on large molecules held at Liège (Belgium) in April 1948. To be published in *Bull. soc. chim. Belges,* 57 (1948).

media whereby all charged spots on the protein have the same sign and thus the expanding forces are as strong as possible, so strong that the forces binding the structure together can no longer resist them and the corpuscular structure ceases to exist.

In the following chapters attention will in the main be directed to the charged macromolecules of the statistical skein type not because the corpuscular proteins would be less important, quite the contrary, but because various excellent monographs on proteins[1] have appeared recently and they deal with this group of substances much more extensively than would be possible here.

c. Division of the colloids to be treated, according to the nature and sign of the charge of the ionogenic groups

The macromolecules with electrolyte character can be divided into three categories, to wit:

A. Colloids with acid character which carry only acid groups such as $—COO^-$, $—OSO_3^-$, $—OPO_3H^-$.

B. Colloids with basic character which carry only basic groups such as $—NH_3^+$, $—NH—C(NH_2)_2^+$.

C. Colloids with amphoteric character, which carry both sorts of groups.

The dissociation of the various groups can be more or less strong according to the pH (and the electrolyte content) of the medium. Thus for instance the carboxyl colloids of group A in strongly acid medium are practically uncharged.

The amphoteric colloids will at very low pH behave practically as "ionised colloids with basic character", at high pH just as "acid colloids[2]", while in the intermediate region positive and negative charges occur together and just equilibrate one another at the isoelectric point (I. E. P.).

With regard to the above division it may be remarked that types A and C both occur naturally and have been prepared synthetically. Pure representatives of group B, the basic colloids, are not known.

However we find substances which approximate closely to group B in the so-called basic proteins. Clupein, for example, consists to an extent of $^2/_3$ of arginine and contains no dibasic aminoacids. Along the whole chain there are thus 2 positive charged spots for each 3 peptide units and only one single COOH group at the end of the chain. In acid medium where the COOH group is not dissociated, cluplein behaves as a purely basic colloid while in less acid or alkaline medium also the positive character still greatly predominates.

If now we ask ourselves definitely what substances belong to the above groups, we are led automatically to a second kind of division, namely into:

Natural colloids: example gum arabic.

Modified natural colloids: examples, cellulose xanthogenate, pectate.

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[1] Carl Schmidt, *The chemistry of the aminoacids and proteins*, Thomas, Baltimore 1938.
E. J. Cohn and J. T. Edsall, *Proteins, amino acids, and peptides*, Reinhold, New York 1943.
M. L. Anson and J. T. Edsall, *Advances in protein chemistry*, I, II, III, Academic Press, New York 1944, 1945, 1947.

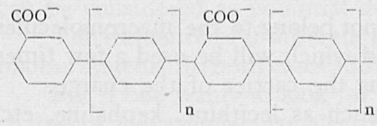
[2] This is however only correct to a first approximation with most of the proteins. In positive casein sols, for example, — casein contains phosphate groups — in the strongly acid region, where the dissociation of the carboxyl groups is entirely suppressed, negatively charged spots which originate in the phosphate groups are also present as well as the positive charge. Similarly in many proteins which contain arginine the total charge is also built up of negative and positive contributions in alkaline media on account of the strongly basic character of the guanidino group.

Wholly synthetic products: example polyacrylic acid.

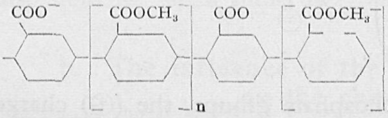
We shall occupy ourselves in the following pages mainly with the natural and a few modified natural colloids, whereby, as we have already mentioned, the corpuscular proteins will be practically left out of consideration.

Since there are many among the natural substances in question the structure of which is not yet well or not generally known, and yet on the other hand it is desirable for a good understanding to have a schematic picture of the various substances involved in the investigation, we add here in the form of a list, schematic, highly simplified structural formulae of some important substances.

A. Colloids with COO^- as the carrier of the charge.

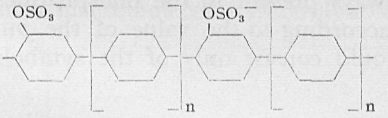


glycogen	$n = \infty$
soluble starch	n = very large
oxidised starch	n = large
gum arabic	n = about 6
semen lini mucilage	n = small
alginate	$n = 0$
pectate	$n = 0$



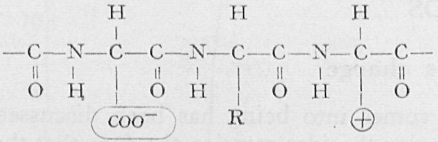
pectin	$n = \infty$
pectinate	$n > 0$
pectate	$n = 0$

B. Colloids with OSO_3^- as the carrier of the charge



agar	n = about 20
carrageen	$n = 0$

C. Amphoteric colloids with COO^- as the carrier of the negative charge



$$-\underset{\overset{\|}{O}}{C}-\underset{\overset{|}{H}}{N}-\overset{\overset{H}{|}}{\underset{\underset{COO^-}{|}}{C}}-\underset{\overset{\|}{O}}{C}-\underset{\overset{|}{H}}{N}-\overset{\overset{H}{|}}{\underset{\underset{R}{|}}{C}}-\underset{\overset{\|}{O}}{C}-\underset{\overset{|}{H}}{N}-\overset{\overset{H}{|}}{\underset{\underset{\oplus}{|}}{C}}-\underset{\overset{\|}{O}}{C}-$$

proteins

D. Amphoteric colloids with phosphate as negative group.

Thymus nucleic acid[1]. Scheme, see p. 188.

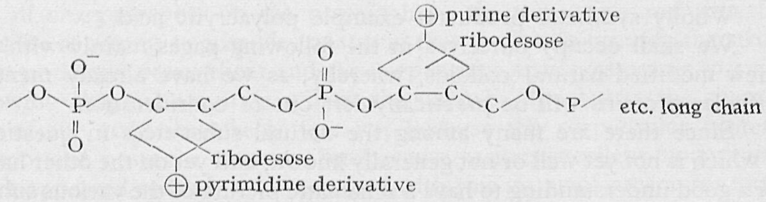
---

[1] See FREY-WYSSLING, *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*, Borntraeger, Berlin (1938), p. 170.

In the scheme positive charges are drawn in the side chains to emphasise the amphoteric character*. The positive charges can be produced by dissociation of NH_2 groups which are permanently attached to rings of aromatic character. Consequently their basic character is very weak and dissociation only takes place in definitely acid medium (50% dissociation occurs in cytosine only at pH 4.2, *in adenine at* pH 3.7 *and in guanine at* pH 2.3*). The I.E.P. lies very low* (I.E.P. < 2). *All this results in nucleic acid behaving in the* pH *range* 6—8 *as a purely acid colloid in which phosphate groups are exclusively the carriers of the negative charge.*

At still higher values of the pH the negative charge increases still further through dissociation of aromatic OH groups in the side-chains (uracil and guanine: 50% ionisation at pH about 10) and at yet higher pH of OH groups in the sugar units (50% ionisation at pH > 13).

* For the dissociation behaviour of nucleic acid, nucleotides and nucleosides see: P. A. LEVENE and L. W. BASS, *Nucleic Acids*, Chemical Catalog Comp. New York (1931), p. 212, 280—286.

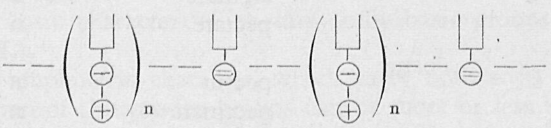


Yeast nucleic acid is probably similarly constructed. The sugar component is here ribose. It is a short chain consisting of only 4 ribose phosphate groups.

E. Association colloids with phosphate groups.

Further there is a group of substances which do not belong to the macromolecules but whose solutions behave as colloidal solutions and which will be used a few times as an example of colloids with phosphate groups as the carrier of the charge.

The substances in question are phosphatides such as lecithine, kephaline, etc.



The groups indicated by (⊖) and (⊜) are phosphate groups, the (⊕) charge comes from choline in lecithine or colamine in kephaline), (⊜) symbolises the phosphate group of the phosphatidic acid which is almost always present in the phosphatides and which can dissociate off one or two $H^{\cdot}$ ions[1] according to the value of the *p*H. In the completely pure state the phosphatides would consist only of the symbols in brackets.

§ 2. NATURE OF THE ELECTRIC CHARGE OF MACROMOLECULAR COLLOIDS

a. Origin of the charge

How the charge of hydrophobic colloids comes into being has been discussed in detail in Volume I of this book. In this discussion the idea came to the fore that the carriers of the charge on the particles are in this case usually adsorbed ions. The degree of adsorption and with it the magnitude of the charge depends on the electrolyte concentration in the intermicellar liquid, in the first place on the concentration of the potential-determining ions and secondarily also on the concentration of the other (indifferent)[2] electrolytes.

---

[1] The scheme given is not intended to suggest a picture of the way in which the components are united into associated groups. These associations of groups are probably not linear but double sheeted planes. Compare on this point Chapter 14, *Association Colloids* and PALMER, K. J. and SCHMITT, F. O., *J. Cellular Comp. Physiol.*, 17 (1941) 385.

[2] In this and in the following chapters the term "indifferent electrolytes" will be frequently used for all those electrolytes that are not potential determining, or that do not give ions (H^+ or OH^-) which can react directly with the charged groups. The term "indifferent electrolyte" is preferred over the older term "neutral salt".

With the macromolecular colloids a different view on the origin of the electric charge appears however to be more appropriate. As already mentioned in § 1 c macromolecules can contain in their structure groups such as the carboxyl group, the sulphonic acid group, the amino group, etc., which by electrolytic dissociation can be the origin of the charge of these colloids.

Thus the proteins have carboxyl, amino, guanidino, imidazol groups, substances such as gum arabic and pectinic acid have carboxyl groups, agar ester sulphate groups, nucleates phosphate groups as carriers of the electric charge.

Many points of similarity can be found with the electrochemical behaviour of hydrophobic surfaces. In particular the dissociation of the above mentioned groups is greatly influenced by the pH, so that the H^+ ions (and the OH^- ions) can be considered as potential-determining ions.

The influence of indifferent electrolytes is also qualitatively the same as in hydrophobic systems. In both cases addition of the indifferent electrolyte increases the charge.

But if one wants to look for a quantitative explanation for the way in which the charge of macromolecular electrolytes depends on the composition of the medium, one should rather associate it with the theories of electrolytic dissociation such as also hold for small molecules than with the theory of the double layer.

b. The influence of the pH and the composition of the solution on the charge of the macromolecule

From an electrometric titration with acid or alkali one can determine the charge of a macromolecular electrolyte as a function of the pH. It is true one encounters in the interpretation of the measurements all the difficulties[1] which are connected

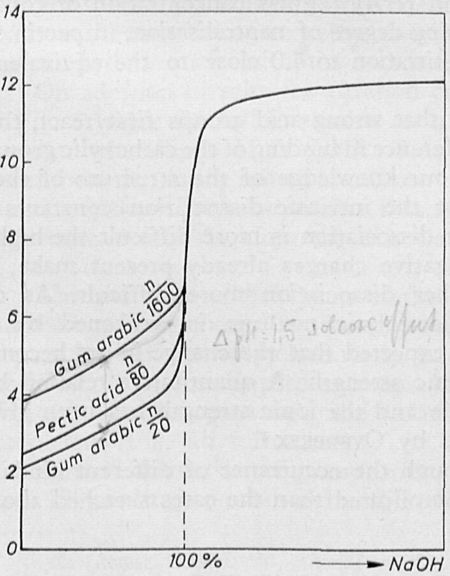


Fig. 1. Titration curves of gum arabic and pectic acid.

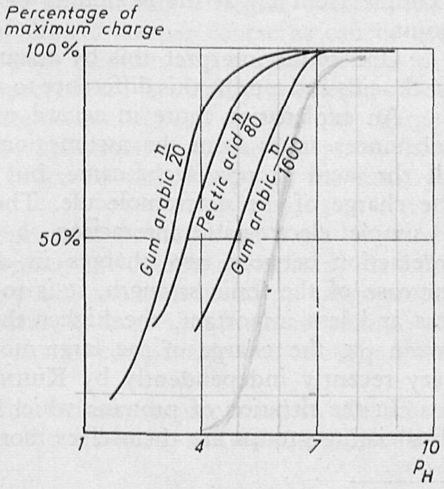


Fig. 2. Relation between charge and pH of gum arabic and of pectic acid deduced from the titration curves.

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[1] See Volume I, Ch. X, DONNAN *equilibria and sol-concentration effect.*

with the activity of a single ion (here the H^+ ion) not being unambiguously defined but nevertheless the application of the simple hypothesis, that the activity coefficients of the small ions are not influenced by the presence of the colloidal particles, has permitted a useful interpretation of the titration curves in various cases.

In the simplest cases we have to do with a macromolecule which contains a number of identical dissociable groups.

We give the titration curves of gum arabic[1] and pectinic acid[2] on page 189.

By means of the relation

$$\text{Charge} = \text{added NaOH} + \text{conc. } H^+ - \text{conc. } OH^-$$

in which C_{H^+} and C_{OH^-} are calculated from the pH assuming that the activity coefficients are 1 (or a known constant < 1 in a medium containing a salt), one can readily deduce curves, from the titration curves, which give the connection between charge and pH (see Fig. 2).

If the carboxyl groups are all linked in the same way in the macromolecule and are situated so far apart that their interaction can be neglected, the titration curves ought to be identical with those of a monobasic weak acid. This appears however not to be the case. If one determines the dissociation exponent pK at various points of the curve

$$pK = pH - \log \frac{[A^-]}{[HA]}$$

in which $[A^-] = [H^+] + [Na^+] - [OH^-]$ and $[HA]$ = gross concentration of colloid $-[A^-]$ then pK is found to rise with increasing degree of neutralisation, in pectin for example from 2.7 at the beginning of the titration to 4.0 close to the equivalence point.

One could interpret this by assuming that strong acid groups first react, then weak acids and ascribe this difference to a difference in binding of the carboxylic groups.

An explanation more in accord with our knowledge of the structure of these substances starts from the assumption that the intrinsic dissociation constants of all the acid groups is the same, but that dissociation is more difficult the higher the charge of the macromolecule. The negative charges already present make, by a simple electrostatic interaction, a further dissociation more difficult. As the interaction between two charges in an electrolytic medium is weakened by an increase of the ionic strength, it is to be expected that the change in pK becomes less and less important, the higher the ionic strength. A quantitative relation between pK, the charge of the large molecule and the ionic strength has been given very recently independently by KUHN and by OVERBEEK[3].

In the titration of proteins which through the occurrence of different kinds of dissociating groups are themselves more complicated than the cases sketched above,

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[1] A. W. THOMAS and H. A. MURRAY, *J. Phys. Chem.*, 32 (1928) 676; D. BRIGGS, *J. Phys. Chem.*, 38 (1934) 867.

[2] J. BONNER, *Proc. Acad. Sci. Amsterdam*, 38 (1935) 346.

[3] W. KUHN and coworkers, J. TH. G. OVERBEEK, *Meeting on large molecules held at Liège (Belgium)* in April 1948. To be published in *Bull. soc. chim. Belges*, 57 (1948)

the determination of dissociation constants has been carried out with more care and on a greater scale.

With certain modifications due to the globular character of the proteins, in contradistinction to the more open skein structure of the acids mentioned above, the same interpretation can be used here[1].

As an example, in Fig. 3 we give the charge — pH curve[2] deduced from a titration curve of carboxyhemoglobin.

This curve clearly exhibits a number of different steps which obviously originate in different types of charge-carrying groups. Other proteins also show a similar variation of charge with pH.

It has been possible to analyse these titration curves and attribute the various steps to the carboxyl groups of glutamic acid and aspartic acid (to about pH = 5), the imidazol group of the histidine (pH=5—9) NH_2 groups of lysine (pH=9—11) and the guanidino group of arginine (pH ∾ 12) and perhaps also the phenolic OH of tyrosine at very high values of the pH.

For various proteins these data deduced from the titrations agree well with other analytical data such as amino acid composition, amino nitrogen content etc.[3].

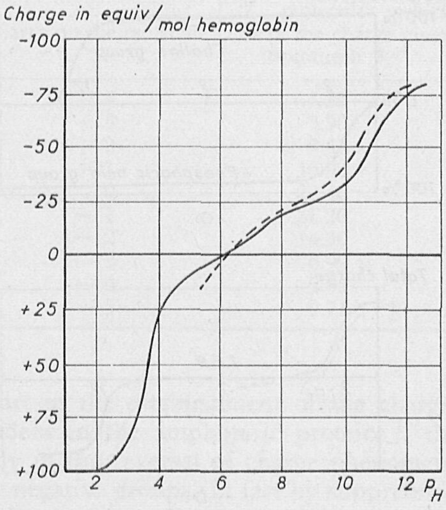

Fig. 3. Relation between charge and pH of carboxyhemoglobin.
———— practically salt-free.
- - - - - - in 1 M. NaCl.

On addition of salts the titration curves show a steeper course as can be seen in Fig. 3 where the full line holds for a salt-poor medium and the dotted line for a medium which is 1 M in NaCl. This steeper course is readily explained if one remembers that in the salt-rich medium the interaction between the various charge-carrying groups becomes smaller through the compression of the ion atmospheres and consequently therefore the dissociation proceeds more easily at the same pH.

The dissociation behaviour of the phosphatides discussed on page 188 is also interesting. Since choline is a strong base and phosphoric acid a strong acid, the I.E.P. of pure lecithin lies very close to the neutral point, and in a broad pH range around the I.E.P. the lecithin is present almost exclusively as amphoion (with net charge = 0). (Fig. 4a). In this pH range the + and — charge compensate one another almost completely (Fig. 4b full curve).

A small admixture of phosphatidic acid in lecithin (as supposed in the scheme on p. 188) must then result in the full curve in Fig. 4b being shifted somewhat down-

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[1] K. Linderstrøm-Lang, *Compt. rend. trav. lab. Carlsberg*, 15 (1924) 7.
R. K. Cannan, A. Kibrick, and A. H. Palmer, *Ann. New York Acad. Sci.*, 41 (1941) 243.

[2] E. J. Cohn, A. A. Green, M. H. Blanchard, *J. Am. Chem. Soc.*, 59 (1937) 509.

[3] See for example β-lactoglobulin, R. K. Cannan, A. H. Palmer, A. C. Kibrick, *J. Biol. Chem.*, 142 (1942) 803, and E. Brand, L. J. Saidel, W. H. Goldwater, B. Kassel, F. J. Ryan, *J. Am. Chem. Soc.*, 67 (1945) 1524.

wards as a whole as a result of which the I.E.P. is displaced very rapidly to lower pH's. Since the phosphate group of the phosphatidic acid possesses two hydrogen atoms which can be dissociated off, it may be anticipated that with this shift downwards an additional S-shaped course in the curve (as indicated in the dotted curve) will appear at the same time in the neighbourhood of the pK value corresponding to the second dissociation constant of the phosphate group.

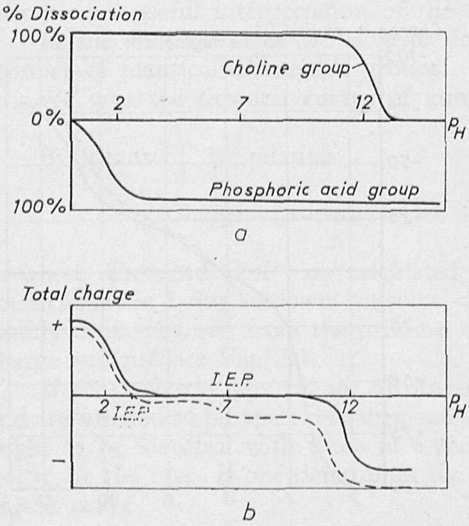

Fig. 4a. The dissociation of the phosphate group and of the choline group occurring in lecithin.
Fig. 4b. ——— The charge of theoretical lecithin as a function of the pH
- - - - - - The charge of lecithin, containing some phosphatidic acid

Lecithin preparations produced in practice (without very rigorous precautions) do indeed possess I.E.P. which are very much lower than the theoretical I.E.P. (p. 294 Ch. IX § 21). As a result of the facts mentioned above they behave in the neighbourhood of the neutral point as colloids with acidic character, in which the phosphate group is the carrier of the negative charge.

Similar considerations hold with regard to kephalin, only as a result of the weaker basic character of colamine (compared with choline) the I.E.P. here lies at a somewhat lower pH in the pure substance than is the case with pure lecithin. Here also admixture of a small amount of phosphatidic acid will result in a considerable lowering of the I. E. P.

It is also worth while pointing out that with macromolecular electrolytes the charge bears always a statistical character. Thus in the case of hemoglobin in which the isoelectric point is situated in the dissociation region of the imidazol groups (33 per molecule) one can calculate that at the isoelectric point ($pH = 6.4$) only about 20 percent of all the molecules are really isoelectrical at any given instant and that molecules with a charge of $+3$ or -3 units are certainly not rare. Table I gives an idea of the charge distribution for the various molecules assuming that of the 33 imidazol residues 13 have a dissociation exponent of 5.7 and 20 a dissociation exponent of 7.5, which fits as closely as possible with the titration curve of Fig. 3.

For a more detailed discussion of the topic dealt with above reference may be made to the book of COHN and EDSALL[1].

Summarising one can say that the charge of macromolecular electrolytes is above all governed by the acid (or base) strength of the dissociating groups and by the pH of the medium, while the salt concentration also exerts a secondary influence.

In the part of this chapter in which the viscosity is treated and in Chapter IX (on reversal of charge), we shall see that besides this dissociation of the specific groups

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[1] E. J. COHN, J. T. EDSALL, *Proteins, amino acids and peptides*, Reinhold, New York 1943. See especially Ch. 20, *Proteins as acids and bases.*

TABLE 1

CHARGE DISTRIBUTION OVER THE VARIOUS MOLECULES OF HEMOGLOBIN AT THE ISOELECTRIC POINT pH = 6.4

Charged imidazol residues	Uncharged imidazol residues	Charge on the molecule	% molecules which carry the charge given in column 3
25	8	+ 4	1.26
24	9	+ 3	3.90
23	10	+ 2	9.42
22	11	+ 1	16.98
21	12	0	22.40
20	13	− 1	21.20
19	14	− 2	14.30
18	15	− 3	6.99
17	16	− 4	2.65
16	17	− 5	0.71

adsorption of other ions must also play a part in the establishment of the charge. Although this effect may not always be evident in the amphoteric proteins [1], the rôle of adsorption is brought out more clearly in the reversal of charge phenomena in the case of macromolecules with exclusively negative groups. In fact by suppression of the dissociation we should at most be able to obtain discharge while in reality we observe that the charge from being negative can become positive. Here we have to do with a direct analogue of reversal of charge in hydrophobic colloids, which could also not be understood from the theory of the diffuse double layer but where in addition specific adsorption of ions (or charged complexes) had to be brought in for the explanation.

§ 3. THE ELECTRIC CHARGE OF MACROMOLECULES AS DETERMINED BY ELECTROPHORESIS

a. The interpretation of the electrophoresis of macromolecules

In principle one can also establish the charge of a macromolecule from electrophoresis measurements. This method has even definite advantages over the analytical method dealt with in the previous §. Indeed in the titration one only determines that part of the charge which is based on binding of H^+ or OH^- ions. In electrophoresis one also takes into account charge of a different origin, that is to say, produced by adsorption of other than H^+ or OH^- ions. Furthermore it is frequently difficult with the titration method to fix the charge-zero while this can be done very easily with electrophoresis.

On the other hand however the quantitative interpretation of the electrophoresis still raises various difficulties, whereby the conclusions will again become less certain. For a detailed discussion of electrophoresis, also as concerns the methodics, reference may be made to Chapter V of Volume I. We shall however deal briefly here with the interpretation of electrophoresis.

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[1] On this point see however § 3b, the comparison of electrophoresis and titration.

To a first approximation one can calculate the ζ potential from the electrophoretic velocity (*E.V.*) with the aid of SMOLUCHOWSKI'S relation

$$\zeta = \frac{4\,\pi\,\eta}{\varepsilon}\,.\,v \qquad (1)$$

or with the refinements given by HENRY[1] and others.

This relation holds for compact particles whose dimensions are large compared with the thickness of the double layer. For smaller particles various corrections must be applied of which especially the one for the relaxation of the ion cloud still contains difficulties[2].

Next one must calculate the charge from the ζ potential and this brings two new difficulties with it. As is well known the ζ potential is the potential on the "slipping-plane" between particles and liquid. In general a layer of liquid of one or more molecules thick will stick hydrodynamically to the particle and quite a portion of the ions from the outer coating of the double-layer may be present in this layer. As a result of this the charge calculated from the ζ potential is always lower than the charge of the particle itself and this lowering can be important especially at higher electrolyte concentrations. A second difficulty is formed by the fact that calculation of the charge from ζ calls for accurate knowledge of the shape and size of the particle and this is not always easy to obtain. Determinations of the molecular weight and the velocity of diffusion leave two interpretations open if the diffusion is slower than corresponds to a compact sphere. Either one assumes that the particle is spherical but enlarged by hydration or that there is no hydration but the particle has an elongated form. And the two hypotheses lead to different values of the charge for the same value of ζ[3].

As a general conclusion one may state that, provided the ζ potential, and thus the electrophoretic velocity, is small ($\zeta < 25\ mV$, $v < 1\ \mu\ cm/Vsec$) the charge of the particles is directly proportional to ζ and therefore also to v. The exact indication of the proportionality factor (which also depends on the electrolyte concentrations of the system) still presents difficulties.

The above statements hold for the electrophoresis of compact particles. These considerations cannot be applied to a charged skein molecule without further discussion. Apart from the motion of the particle with respect to the surrounding liquid, the motion of the liquid through the meshes of the skein molecule has certainly to be taken into account. No theory has yet been given for the electrophoresis of clewed molecules. The only thing which one may expect with high probability here is that, again for the region of small velocities charge and electrophoretic velocity are proportional to one another and that electrophoretic stand-still thus signifies that the macromolecule carries no charge. But in this case we are even worse informed regarding the proportionality factors between charge and electrophoretic velocity than in the case of compact particles.

A much used expedient in the electrophoretic investigation of macromolecules is the determination of the *E.V.* not of free molecules but of rather coarse particles

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[1] D. C. HENRY, *Proc. Roy. Soc. London*, 133 (1931) 106.

[2] See Chapter V, part I and J. TH. G. OVERBEEK. *On the interpretation of the electrophoretic velocity, in Advances in Colloid Science III*, Interscience New York, in the press.

[3] See H. A. ABRAMSON, L. S. MOYER, and M. H. GORIN, *Electrophoresis of proteins*, New York 1942, p. 151 *et seq.*

(quartz, carbon, oil drops etc.) which are covered to saturation with macromolecules on their surface. The *E.V.* of these coarse particles can then be determined very readily by microelectrophoretic methods.

Since now we are dealing with very large particles, SMOLUCHOWSKI's electrophoresis equation (1) can be applied with confidence and the ζ potential is therefore calculable. From ζ the charge per cm^2 can then be calculated if the electrolyte concentration is known but to find the charge per macromolecule from this, the density of covering of the surface must be known and that is in general not known. Thus with this method also we do not get any further than to a relative measure for the charge of the macromolecules in which an influence of the absorbing particle on the electrochemical properties of the absorbed substance can possibly also occur as a complication.

b. Some examples

In the first place we give some comparisons between the electrophoretic velocity of corpuscular proteins in solution and of the same proteins absorbed on various particles under the same conditions as regards pH and salt concentration. It appears that in various cases these two *E.V.* are identical (serum albumin, Fig. 5, pseudoglobulin, Fig. 6) and that in a case such as ovalbumin (Fig. 7) where a definite difference exists, this difference is nevertheless small and can practically be described as a shift in the direction of denaturation which results in the *E.V.* of the absorbed ovalbumin resembling more that of the denatured protein.

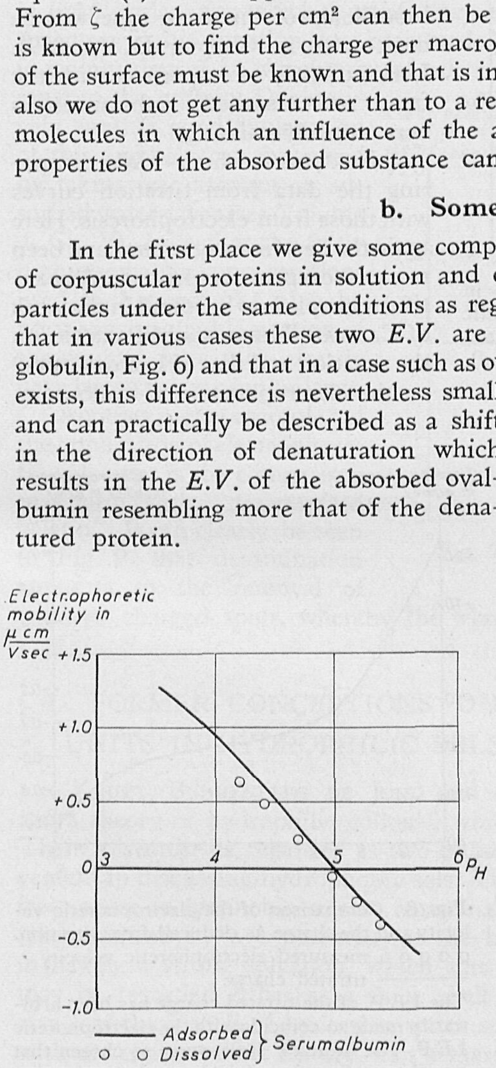

Fig. 5. Electrophoretic velocity of dissolved and adsorbed serum albumin.

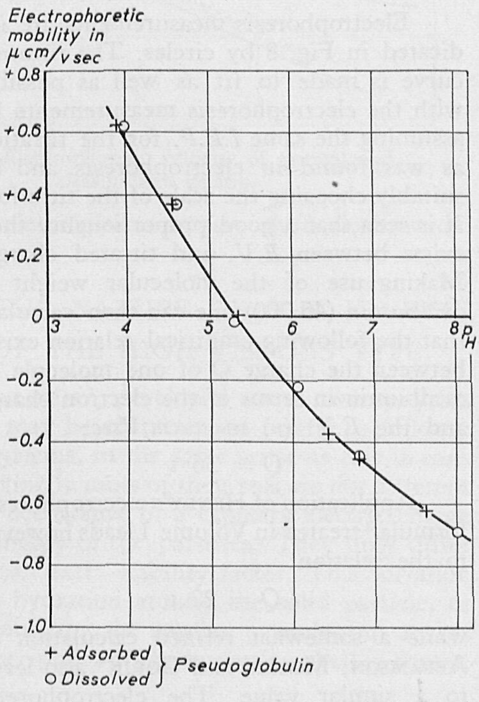

Fig. 6. Electrophoretic velocity of dissolved and adsorbed pseudoglobulin.

Although fundamentally this identity or great similarity is unexpected and not at all explained, one can nevertheless conclude from it that one obtains valuable information by electrophoretic investigations on adsorbed macromolecules even on the behaviour of these substances in solution, which is of great importance since the measurements with the adsorption method are so much simpler.

Electrophoretic measurements by this adsorption method on many biocolloids of the skein type will be dealt with in Chapter IX (p. 259). Unfortunately a comparison with the free, non-adsorbed, substances is here entirely missing.

Next, it is worth while comparing the data from titration curves with those from electrophoresis. Here again the best investigations have been made on corpuscular proteins. A beautiful example is to be found in the work of CANNAN, KIBRICK and PALMER[1] on the titration and that of LONGSWORTH[2] on the electrophoresis of ovalbumin.

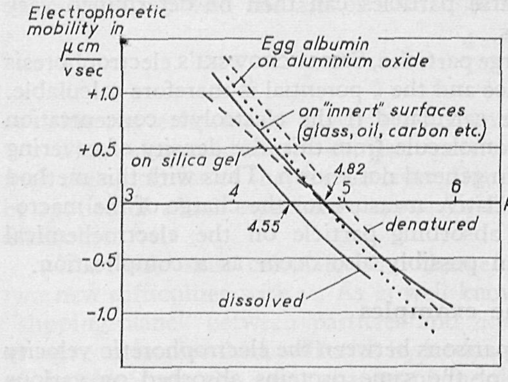

Fig. 7. Electrophoretic velocity of ovalbumin, in the dissolved state, adsorbed on particles of acidic (silica gel), basic (Al_2O_3) and inert (oil, carbon etc.) nature and in the denatured state (surface denatured).

Electrophoresis measurements are indicated in Fig. 8 by circles. The titration curve is made to fit as well as possible with the electrophoresis measurements by assuming the same *I.E.P.* for the titration as was found in electrophoresis and by suitably choosing the scale of the titration. It is seen that a good proportionality then exists between *E.V.* and titrated charge. Making use of the molecular weight of ovalbumin (45 000) one can then calculate that the following empirical relation exists between the charge Q of one molecule of ovalbumin in terms of the electron charge and the *E.V.* (v) in μ *cm/Vsec*:

$$Q = 36\, v$$

Application of HENRY's electrophoresis formula[3] treated in Volume I leads however to the relation:

$$Q = 22\, v$$

while a somewhat refined calculation of ABRAMSON, MOYER, and GORIN[4] also leads to a similar value. The electrophoretic

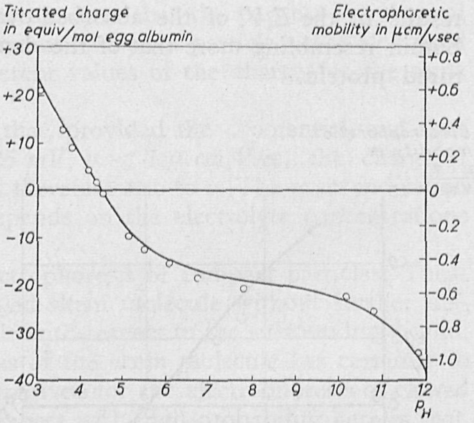

Fig. 8. Comparison of the electrophoretic velocity and the charge as deduced from titration.
o o o o o measured electrophoretic velocity
——— titrated charge
The I.E.P. of the titrated charge has been arbitrarily made to coincide with the electrophoretic I.E.P. The ordinate scales were so chosen that the electrophoretic velocities and the titration approximately coincide.

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[1] R. K. CANNAN, A. KIBRICK, and A. H. PALMER, *Ann. New York Acad. Sci.*, 41 (1941) 243.

[2] L. G. LONGSWORTH, *ibid.*, p. 267.

[3] For details see the article by LONGSWORTH quoted above.

[4] H. A. ABRAMSON, L. S. MOYER, and M. H. GORIN, *Electrophoresis of proteins*, New York 1942, p. 152 *et seq.*

velocities, which play a part here, are still so small that one can indeed neglect the relaxation correction.

Thus in the best investigated case in this line there is an important difference between the electrophoretic charge and the titrated charge and of such a kind that the titrated charge is appreciably larger.

An explanation which is satisfactory in all respects has not yet been given for this. Attempts in this direction have been made by various workers[1], in which the suggestion is mainly that of an attachment of other ions than H^+ and OH^- (for example Cl^- ions) to the protein. Qualitatively another good explanation of this anomaly can be given by taking into account an adsorbed water-layer (see Volume I, Ch. V). Indeed at the fairly high electrolyte concentrations used in this case (0.1 n) a by no means negligible portion of the counter ions may already have been taken up in this layer.

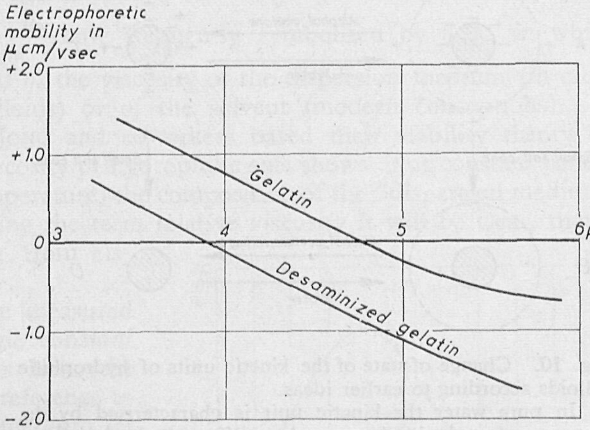

Fig. 9. Electrophoretic velocity of normal and desaminised gelatin.

Another pretty example[2] of the application of electrophoretic velocities is the comparison of gelatin with "desaminised gelatin". It can clearly be seen in Fig. 9 that desamination amounts to the removal of positive charged spots, whereby the whole electrophoresis curve shifts downwards.

§ 4. FORMER CONCEPTIONS ON THE NATURE OF THE KINETIC UNITS IN HYDROPHILIC SOLS OF THE HIGH VISCOUS TYPE

Kruyt, Bungenberg de Jong and coworkers[3], developed some twenty years ago a theory of hydrophilic colloids, which may be characterised briefly as follows: Their sols must be regarded as two-phase systems, in the same sense as this is convenient in discussing hydrophobic sols. The kinetic units of their sols are not different from those in lyophobic sols as regards the occurrence of a capillary electric charge (characterised by a ζ potential) at the boundary of the particles. They only differ in having a strong solvation, which acts as an extra stability factor. This solvation may be regarded either as a thick shell of hydration around the solid particle, or as a less thick shell of hydration water surrounding the swollen particle, but always with the characteristic feature, that a sharp periphery of the hydration shell is absent.

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[1] H. A. Abramson, L. S. Moyer, and M. H. Gorin, *l.c.*, p. 158. L. J. Longsworth, *l.c.* J. Steinhardt, *Ann. New York Acad. Sci.*, 41 (1941) 287.

[2] H. A. Abramson, *J. Gen. Physiol.*, 15 (1932) 575.

[3] H. R. Kruyt and H. G. Bungenberg de Jong, *Kolloidchem. Beih.*, 28 (1928) 1 and other publications with coworkers in the *Kolloidchem. Beihefte* under the title *Zur Kenntnis der lyophilen Kolloide.*

Only such a "diffuse solvation shell" could account for the solvation being a stability factor.

"Hydrophobic sols" have only one stability factor: the capillary electric charge, "hydrophilic sols" however have two stability factors: capillary electric charge and hydration. Flocculation of the latter will only occur if both factors are sufficiently suppressed. This condition can be reached in steps along two ways: see Fig. 10.

A. by first removing the charge and afterwards the hydration, as by adding $MgSO_4$ in increasing quantities to an agar sol. In small concentrations the capillary electric charge is suppressed, in much higher concentrations dehydration sets in, the colloid is "salted out".

B. by first dehydrating and then removing the charge, as can be realised with sufficiently diluted agar sols, with alcohol and afterwards adding a little of an indifferent salt.

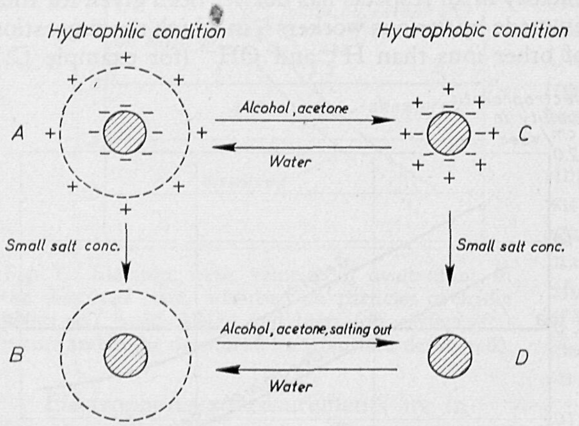

Fig. 10. Change of state of the kinetic units of hydrophilic colloids according to earlier ideas.
A. In pure water the kinetic unit is characterised by the presence of a double layer and a diffuse hydration (here represented as an external shell, but can also be taken up for a great part inside the particle). B. After addition of a small amount of salt: the double layer (not shown) has receded inside the hydration shell. C. and D. are similar to A and B as regards charge but do not possess the diffuse hydration shell. A possesses two, B and C one and D no stability factors.

This stability theory, which was also extended to amphoteric colloids — at first proved a useful guide in experimental work (e.g., also in the first stages of the investigations on coacervation, see p. 243, Chapter VIII, § 3). But gradually facts were met with which are not compatible with it. Its usefulness became less and less and nowadays we may say it has only historical value.

Indeed at the present time there seems no longer to be any real need for a "stability" theory of hydrophilic sols, as we now regard the colloid substance as truly dissolved, and its electrical properties as caused by ionisation of ionogenic groups in the dissolved macromolecules. Flocculations or coacervations are now regarded as transgressions of solubility. Many of the facts, the above stability theory seemed to explain have received other explanations. See for instance for the opalescent sols with lyophobic character obtained by adding alcohol to the sol Chapter VIII § 1c (p. 234), and for the flocculation or coacervation with alcohol + indifferent salt Chapter X § 3f (p. 396).

The viscosimetric data, upon which for a greater part the now discarded stability theory was based, remain, and they need a reinterpretation from the macromolecular point of view.

Before doing so, we give in the next subparagraph first a survey of these data (and their bearing to the former conceptions).

§ 5. CHANGES IN $(\eta_s - \eta_o)/\eta_o$ AT CONSTANT COLLOID CONCENTRATION, CAUSED BY ALTERING THE COMPOSITION OF THE SOLVENT

In all theories which try to connect viscosity of sols with the state or structure of its kinetic units, this connection is always made through relative viscosity η_s/η_o or relative viscosity increase $\frac{\eta_s - \eta_o}{\eta_o}$ now frequently symbolized by η_{spec}, in which η_s is the viscosity of the sol and η_o the viscosity of the dispersion medium (in older conceptions of hydrophilic colloids) or of the solvent (modern conceptions).

Kruyt, Bungenberg de Jong and coworkers based their stability theory on *the changes that the relative viscosity of hydrophilic sols shows if at constant colloid* concentration (and constant temperature) the composition of the "dispersion medium" is systematically altered. In using the term relative viscosity it will be clear, that it has here a different meaning from elswhere in physical chemistry.

Instead of comparing the measured viscosities with that of a single constant calibration liquid, η_s/η_o means here the values to be calculated with reference to the viscosity of the dispersion medium (at the same temperature), which medium each time has a different composition. Thus below in Fig. 12 the influence of increasing concentrations of alcohol on $\frac{\eta_s - \eta_o}{\eta_o}$ of a dilute agar sol is given. This figure is obtained by calculation from the two sets of viscosity determinations represented in Fig. 11, *viz.*, from the viscosities of a dilute agar sol containing increasing amounts of alcohol (upper curve), and from the viscosities of the corresponding alcohol water mixtures (lower curve). In these two sets of determinations water is taken as calibration liquid. By division of the corresponding viscosity values a series of relative viscosities in the sense as above indicated is obtained, and by subtracting unity from them, the values of $\frac{\eta_s - \eta_o}{\eta_o}$ used as ordinates in Fig. 12 are obtained.

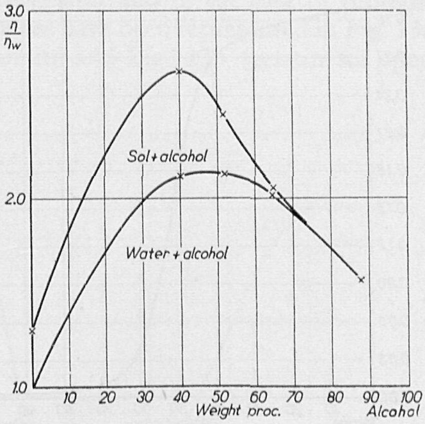

Fig. 11. Viscosity measurements. (45° C) required for the calculation of the curve of Fig. 12.
Lower curve: alcohol-water mixtures.
Upper curve: alcohol-water mixtures which contain 0.134 g agar per 100 ml.
Ordinates: Relative viscosities with water at 45° as calibration liquid.
Abscissae: Alcohol content in weight percents.

Al further viscosity changes to be discussed below refer to relative viscosities in the above sense; we keep further to a constant low colloid concentration and a constant temperature and only vary the composition of the dispersion medium.

The now following survey falls into two sections, which relate to obviously different causes for the changes in relative viscosity. We have labelled them provisionally "*Solvation*" and "*Electric Charge*", thus following more or less the interpretations suggested in the stability-theory of § 4 (p. 197). A reinterpretation from the macromolecular point of view will be postponed till later (p. 209 § 6).

A: "*Solvation*".

For an interpretation of viscosity data of sols obeying POISEUILLE's law KRUYT and BUNGENBERG DE JONG took EINSTEIN's equation

$$\eta_s = \eta_o\,(1 + 2.5\,\varphi) \quad \text{or} \quad \frac{\eta_s - \eta_o}{\eta_o} = 2.5\,\varphi$$

as a starting point. In this formula φ is the ratio of the volume of the dispersed phase and the volume of the whole sol.

Determinations performed with dilute sols (the formula indeed has been deduced for values of φ not exceeding a few per cent (by volume) of many colloids (e. g., agar, gum arabic, amylum solubile, gelatin and others) have shown that frequently the value of φ as calculated from the above formula with the factor 2.5, appears to be many times (e.g., in some cases more than 100 ×) larger than could be expected from the volume of the dispersed substance in the dry state. Now the formula is valid for rigid particles of spherical shape only. With other shapes of particles we must write $(\eta_s - \eta_o)/\eta_o = k\,\varphi$ (SMOLUCHOWSKI[1]), where $k > 2.5$.

In looking for an interpretation of the much too great values of φ it is consequently uncertain whether this should be attributed to the non-spherical shape, or to a swelling (solvation) of the particles.

So long as only one dispersion medium is used, there is no possibility of distinguishing between these two cases for a given temperature and given concentration of the dispersed substance.

If the too high values for φ originated from the first cause (rigid non-spherical particles), according to the EINSTEIN formula, determinations performed with various dispersion media (variation of η_o), provided both temperature and colloid concentration were kept constant, would always lead to the same value of $(\eta_s - \eta_o)/\eta_o$.

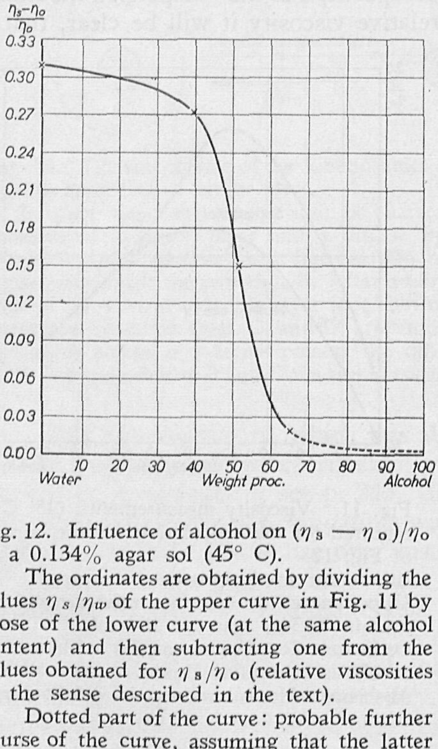

Fig. 12. Influence of alcohol on $(\eta_s - \eta_o)/\eta_o$ of a 0.134% agar sol (45° C).

The ordinates are obtained by dividing the values η_s/η_w of the upper curve in Fig. 11 by those of the lower curve (at the same alcohol content) and then subtracting one from the values obtained for η_s/η_o (relative viscosities in the sense described in the text).

Dotted part of the curve: probable further course of the curve, assuming that the latter at 100% alcohol ends at the value 0.002 which follows from EINSTEIN's formula (with factor 2.5) for dry agar (density about 1.5).

Experimentally this appears not be the case. Here then we find a starting point

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[1] M. VON SMOLUCHOWSKI, *Kolloid-Z.*, 18 (1916) 190.

for experimental work. By systematically changing the dispersion medium, results may be obtained which will show us changes in solvation (or in solvation + amount of occlusion liquid).

For a number of negatively charged hydrophilic sols curves of the type represented in Fig. 12 are obtained if alcohol or acetone is gradually substituted for water (the diagram reproduced refers to an agar sol in water-alcohol mixtures). It is seen that in the concentration range 40—60% alcohol there is a strong decrease of $(\eta_s - \eta_o)/\eta_o$. At the same time the stability changes in a conspicuous way. Whereas the sol in aqueous surroundings appears stable against the addition of salts such as KCl or $BaCl_2$, this is no longer the case after the descent of the curve. It is now exceedingly sensitive to the addition of electrolytes, minute quantities of which will bring about flocculation or coacervation. It has thus obtained the character of a lyophobic sol (see stability theory p. 198, § 4).

The decrease of $(\eta_s - \eta_o)/\eta_o$ from the point of view of EINSTEIN's equation must be interpreted as a process of *desolvation* (the much too high values of φ ascribed to solvation, being reduced to values nearer the calculated ones).

Besides the type mentioned there is a second type of curve[1] which is obtained upon addition of tannin, (sols of agar, amylum, gelatin and in the case of proteins also upon addition of lower phenols). Some instances have been represented in Fig. 13a (agar sol at 40° C and 50° C upon addition of tannin) and Fig. 13b (gelatin sol upon addition of resorcinol).

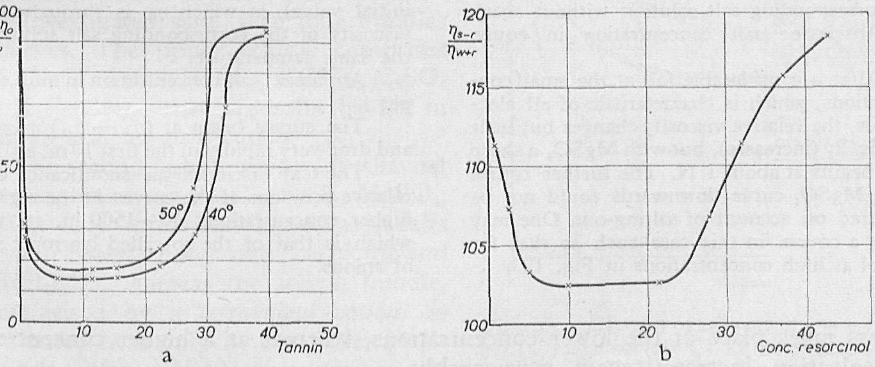

Fig. 13. Influence of tannin on a dilute agar sol (a, 40° and 50° C) and of resorcinol on a dilute isoelectric gelatin sol (b, 42° C).

Ordinates: $(\eta_s - \eta_o)/\eta_o$ (in % of the initial value), or η_s/η_o (η_o is in this case always the viscosity of the corresponding tannin - water or resorcinol-water mixture).

Abscissae: gr. tannin or resorcinol per 100 ml.

The curves drop fairly steeply at relatively small concentrations, rise afterwards in the region of large concentrations. With gelatin the curve rises above the initial value. The clearly noticeable lowering of the pH as a consequence of the very high resorcinol concentration may contribute to this effect.

The effect produced by tannin and polyphenols is fairly strongly dependent on the temperature; it decreases as the temperature rises. This is also evident from the relative positions of the two curves in a.

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[1] H. G. BUNGENBERG DE JONG, *Rec. trav. chim.*, 42 (1923) 437; 43 (1924) 36; 46 (1927) 727; 84 (1929) 494.

In this case we first have a strong decrease of $(\eta_s - \eta_o)/\eta_o$ for relatively small concentrations of the added substance and afterwards an increase at much higher concentration.

As appears also from the changes in stability we must conclude that a desolvation process takes place at the lower concentrations, whereas at a higher concentration the solvation increases again considerably.

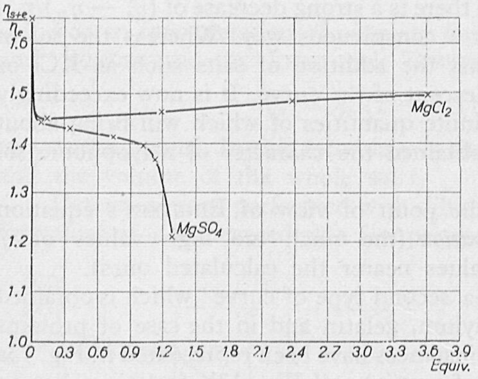

Fig. 14. Influence of $MgCl_2$ and $MgSO_4$ on the relative viscosity of a 0.14% agar sol (50° C).

Ordinates: relative viscosities (relative to the corresponding salt solution without agar).

Abscissae: salt concentration in equiv. per l.

After a considerable fall at the small concentrations, which is characteristic of all electrolytes, the relative viscosity changes but little for $MgCl_2$ (increases), but with $MgSO_4$ a sharp drop begins at about 1 N. The further course of the $MgSO_4$ curve downwards could not be measured on account of salting-out. One may expect a course in this case such as that for alcohol at high concentrations in Fig. 12.

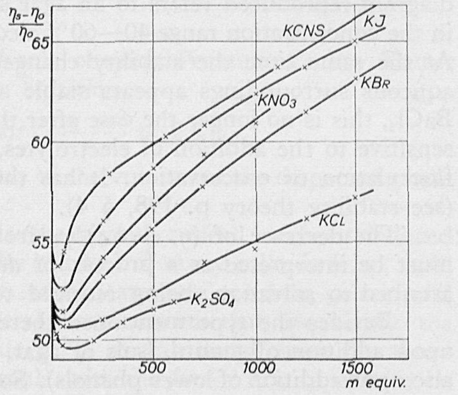

Fig. 15. Influence of a number of K Salts on $(\eta_s - \eta_o)/\eta_o$ in a 0.5% sol of amylum solubile (MERCK) at 25° C.

Ordinates: $(\eta_s - \eta_o)/\eta_o$ (in % of the initial value), in which η_o is throughout the viscosity of the corresponding salt solution at the same temperature.

Abscissae: salt concentration in milli equiv. per l.

The curves begin at $(\eta_s - \eta_o)/\eta_o = 100$ and drop very rapidly in the first 10 m. eq. per l.

The text discusses the significance of the relative positions of the curves in the region of higher concentrations (10—1500 m. eq. per l) which is that of the so-called lyotropic series of anions.

Not only non-electrolytes (alcohol, acetone, tannin, resorcinol etc.) but also electrolytes may alter the "degree of solvation". An example of curves obtained in this way has been given in Fig. 14, representing the effect of $MgSO_4$ and $MgCl_2$ on the relative viscosity of a diluted agar sol.

After a decrease at very small electrolyte concentrations, the meaning of which will be discussed in B (p. 203), further addition of $MgCl_2$ changes the specific viscosity very little, $MgSO_4$ on the other hand causes a second decrease at higher concentration. Now $MgCl_2$, even in the highest concentrations does not flocculate the agar sol; salting out with $MgSO_4$, however, occurs exactly at the point where the curve could not be measured further downwards. Hence from the point of view of the EINSTEIN equation: this second decrease in specific viscosity is due to a desolvation process. $MgCl_2$, instead, seems to increase the solvation slightly.

With other sols electrolytes may give similar effects. After a strong decrease at very low concentrations, to be discussed below, at higher concentrations $(\eta_s - \eta_o)/\eta_o$ may increase, which must be interpreted as increase of solvation. Fig. 15 gives an example, referring to amylum solubile[1] and a number of potassium salts. The interpretation, that these changes in spec. viscosity are really related to changes in solvation seems fortified by the order of the curves, which is that of the so-called lyotropic series of the anions: CNS > J > NO_3 > Br > Cl > SO_4

B: "*Electric Charge*" *(Electroviscous Effect)*

In Figs. 14 and 15 we found a peculiar descent of $(\eta_s - \eta_o)/\eta_o$ at very low electrolyte concentrations, which descent can hardly be interpreted as a variation of the degree of solvation. Indeed, if the effect of various types of electrolytes is measured, there is no indication of the appearance of a lyotropic order. SCHULZE-HARDY's rule on the other hand, comes conspicuously to the front: the curves are arranged in narrow bundles, for each of which the valency of one of the ions is characteristic. See Fig. 16, which, for the agar sol [2], shows the results obtained within the concentration range 0—4 m.eq. p. l. It appears, that the fourteen investigated salts give four bundles of curves. The upper bundle comprises KCl, NaCl, LiCl, NH_4Cl, KCNS, K_2SO_4 and $K_4Fe(CN)_6$, that are salts having in common a *monovalent cation*.

The second bundle unites salts having a *divalent cation*, viz., $BaCl_2$, $SrCl_2$, $MgSO_4$, $CdSO_4$. The third bundle contains salts with a *trivalent cation* viz., $La(NO_3)_3$ and $Co(NH_3)_6Cl_3$, whereas the fourth bundle, characterised by a *tetravalent cation*, is represented here only by a complex platinum salt $[Pt(en)_3](NO_3)_4$ where (en) stands for ethylenediamine.

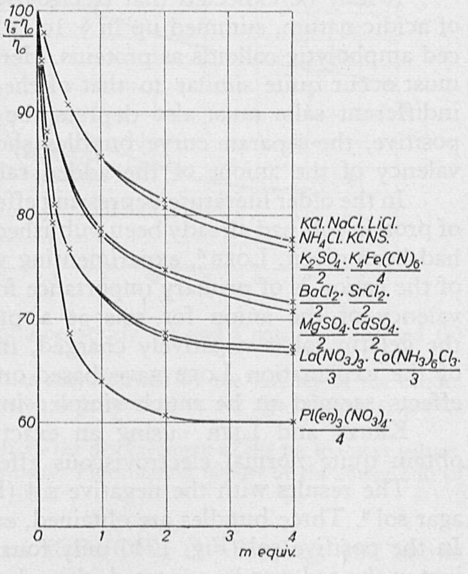

Fig. 16. Influence of salts on $(\eta_s - \eta_o)/\eta_o$ of a 0.14% agar sol (50° C) in the region of small concentrations (Neutralisation of the electroviscous effect).

Ordinates: $(\eta_s - \eta_o)/\eta_o$ in % of this value without added salt.

Abscissae: salt concentration in m. eq. p. l.

The salts arrange themselves in four bundles each characterised by the valency of the cation.

With the complex Pt salt opalescence appears along the lower portion of the curve (see § 10).

The results obtained strongly recall the influence of indifferent salts on a negatively charged "lyophobic" sol, where also valency of the cations is of primary importance in lowering the capillary electric potential (see Volume I). As in the electric field agar moves to the anode, the kinetic units in

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[1] H. R. KRUYT and H. J. EDELMAN, *Kolloidchem. Beihefte*, 36 (1932) 350.

[2] H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloidchem. Beihefte*, 37 (1928) 1. Preliminary publication *Z. phys. Chem.*, 100 (1921) 250.

the agar sol bear indeed a negative charge. Further it was shown that added salts really decrease the electrophoretic velocity of agar.

Thus it seemed quite natural to correlate the sharp fall in viscosity by added salt with the decrease of the charge on the kinetic units, the higher relative viscosity of the original agar sol being caused by the electric charge of the kinetic units.

For this increasing effect of the charge on the viscosity the term "*Electroviscous effect*" was proposed.

If the conclusion drawn is correct, a similar behaviour of other negative "hydrophilic" sols as to the influence of indifferent salts on the relative viscosity was to be expected. Indeed the electroviscous effect has been found to exist in all cases investigated, viz., gum arabic[1], soluble starch[2], mucilage of semen lini[3] and of carrageen[3], sodium thymus nucleate[4] and sodium yeast nucleate[5]. Pecularities shown by some of these examples will be discussed later p. 223 § 10 and p. 227 § 11.

It may be expected that electroviscous effects are not restricted to the colloids of acidic nature, summed up in § 1c (p. 186), but must also occur in such pronounced ampholytic colloids as proteins. Here at a pH higher than the *I.E.P.* a behaviour must occur quite similar to that of the agar sol. At pH values lower than the *I.E.P.* indifferent salts must also depress the relative viscosity but as the charge is here positive, the separate curve bundles should each be characterised by the different valency of the anions of the added salts.

In the older literature depressing effects of indifferent salts on the relative viscosity of protein sols had already been published and various explanations of this phenomenon had been given. LOEB[6], experimenting with gelatin sols, could show that the valency of the cation is of primary importance for sols at a pH higher than the *I.E.P.*, and the valency of the anion for sols at a pH lower than the *I.E.P.* In the first case the gelatin sol is negatively charged, in the latter positively charged. Thus instead of the explanation LOEB gave based on the DONNAN equilibrium, these depression effects seemed to be much simpler interpretated as electroviscous effects.

KRUYT and LIER[7] using an exact technique of viscosity measurement could obtain quite normal electroviscous effects with casein sols.

The results with the negative sol (Fig. 17 a) did not differ from those with the agar sol[8]. Three bundles are obtained, each characterised by the valency of the cation. In the positive sol (Fig. 17b) only four salts were investigated, but their curves lay just in the order to be expected, the valency of the anion being of primary importance.

The only complication occurring here is that the spreading of the curves for

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[1] H. R. KRUYT and H. J. C. TENDELOO, *Kolloidchem. Beihefte*, 29 (1929) 396.

[2] H. G. BUNGENBERG DE JONG, *Rec. trav. chim.* 43 (1924) 189.

[3] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 29 (1929) 436.

[4] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte*, 31 (1930) 89.

[5] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 49 (1930) 658.

[6] J. LOEB, *Proteins and the theory of colloidal behaviour*, Mc Graw-Hill Publishing Co. Ltd., London, 1922.

[7] H. R. KRUYT and H. LIER, *Kolloidchem. Beihefte*, 28 (1929) 407.

[8] The curve for NH_4Cl lies quite abnormally to the left of that for $Co(NH_3)_6Cl_3$, but the pH change (10.5 → 9.7) which this salt of a weak base (NH_4OH) brings about in the original sol accounts for this abnormal behaviour. The other salts used did not alter pH in the concentrations used. That NH_4Cl, behaves as other salts of the type 1 — 1 in the agar sol (Fig. 16) is due to the fact that small pH changes in the neighbourhood of pH 6 — 5, have not yet any detectable influence on the dissociation state of the acidic ionogenic groups of agar, which are ester sulphate groups.

the tri- and tetravalent anion is very small, to which peculiarity we shall return later in § 10.

The primary importance of the anion valency can be seen to apply also for positively charged ichthyocoll sols [1] from the relative positions in Fig. 18 of the curves

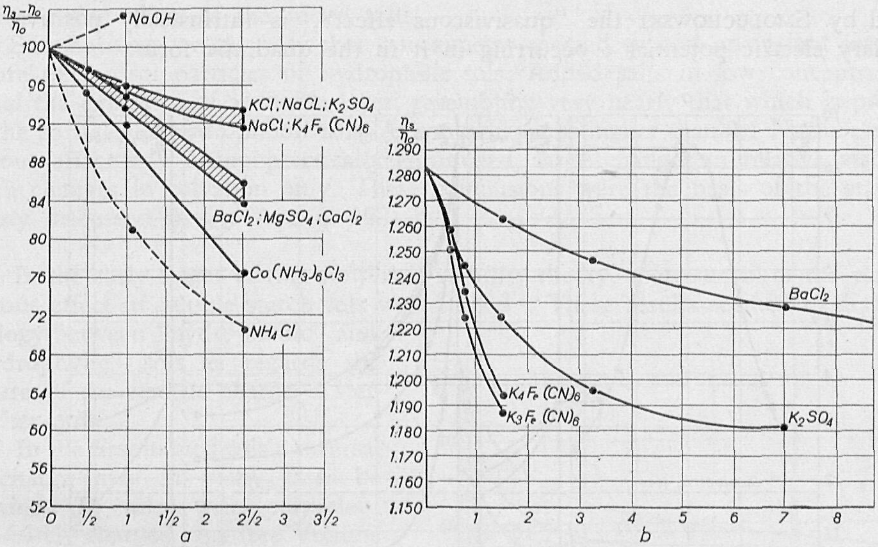

Fig. 17. Influence of salts on the relative viscosity of a negatively (a) and a positively charged (b) 1% casein sol (50° C.).
a: at pH 10.5 the curves arrange themselves in bundles characterised by the valency of the cation.
b: at pH 2.75 on the other hand the valency of the anion is determinative for the relative sequence of the curves.
In a the curve for NH_4Cl is discrepant; which results from the displacement of the pH to lower values. NaOH addition does in fact increase the relative viscosity. Flocculation occurs at the lower end of the curves for $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (see § 10).

obtained with KCl (1—1), K_2SO_4 (1—2) and $K_3Fe(CN)_6$ (1—3). Fig. 18c shows moreover, that in these positive sols the cation influence is of no importance, the curves for $CaCl_2$ (2—1) and $Co(NH_3)_6Cl$ (3—1), nearly coinciding with the curve for KCl (1—1).

For the influence of salts on η_s/η_o of positively charged clupein sols [2] see later in § 12.

In 1916 SMOLUCHOWSKI [3] gave an extension of EINSTEIN's formula for the case when the (undeformable) particles bear a capillary electric charge:

$$\frac{\eta_s-\eta_o}{\eta_o}=k\varphi\left[1+\frac{1}{\eta_o\varkappa r^2}\left(\frac{D\zeta}{2\pi}\right)^2\right]$$

---

[1] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 50 (1931) 238.

[2] H. G. BUNGENBERG DE JONG, W. A. L. DEKKER and P. VAN DER LINDE, *Rec. trav.. chim.* 54 (1935) 1.

[3] M. VON SMOLUCHOWSKI, *Kolloid-Z.*, 18 (1916) 190, c.f. also W. KRASNY-ERGEN, *Kolloid-Z.* 74 (1936) 172. F. BOOTH, *Nature*, 161 (1948) 83.

From the formula it can be seen that a positive as well as a negative charge on the particles increases the relative viscosity. For the additional term

$$\frac{1}{\eta_o \varkappa r^2}\left(\frac{D\zeta}{2\pi}\right)^2$$

called by SMOLUCHOWSKI the "quasiviscous effect", is intrinsically positive, the capillary electric potential ζ occurring in it in the quadratic form.

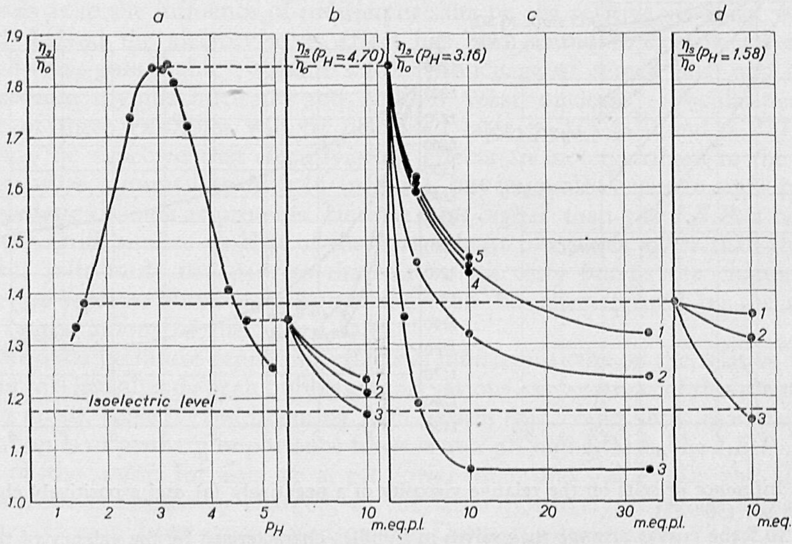

Fig. 18. Influence of salts on the relative viscosity of positive 0.42% ichthyocoll sols (37° C).
a: Part of the pH-viscosity curve at pH values lower than the I.E.P. (see Fig. 20 curve A).
b, c & d: influence of salts in which a point is chosen respectively on the rising branch, at the maximum and on the falling branch of the curve in a as working point.
1 = KCl, 2 = K_2SO_4, 3 = $K_3Fe(CN)_6$, 4 = $CaCl_2$, 5 = $Co(NH_3)_6Cl_3$.
The $K_3Fe(CN)_6$ curves fall to below the value of η_s/η_o corresponding to the I.E.P. (lowest dotted horizontal line). This is associated in c with the occurrence of highly cloudy systems, which on microscopic examination is found to be based on coacervation. (On this point see also § 10).

This theoretical deduction of SMOLUCHOWSKI seems to give at least a qualitative interpretation of the experimental results discussed above.

If we assume, that the kinetic units are undeformable, which in the small concentrations used in Fig. 16, 17 and 18 do not alter their volume, then in the above formula r the radius of the particles and φ, the total volume of the particles, expressed as a fraction of the volume of the sol, will remain constant. The only variables which may alter the relative viscosity, are then $\varkappa$, the conductivity of the dispersion medium and ζ the electrocapillary potential.

The increase of $\varkappa$ by added salts to a sol relatively free from electrolyte impurities will in itself already cause the "quasiviscous" term or — as KRUYT and BUNGENBERG DE JONG preferred to call it — the *electroviscous* term to become very small. Thus the decrease in relative viscosity by added salts within the first 100 m. eq. p. l. strictly speaking does not yet mean a discharging effect, but only that the charge can no longer manifest itself in the relative viscosity at salt concentrations higher than 0.1 N.

The fact however, that distinct bundles of curves appear, each characterised by the valency of only one of the ions of the added salts (cations in the case of negative sols and anions in the case of positive sols), and the relative position of these bundles (the higher the valency of the ion, the lower the position of the bundle), does indicate a discharging effect of the added salts.

Summarising we may say that viscosimetry seemed to give an insight into the nature of the sol particles of hydrophilic sols: Added salts in low concentrations reveal the existence of a double layer, resembling very nearly that which is present on the particles of hydrophobic sols. At concentrations higher than 0.1 N, the electroviscous effect now being practically eliminated, large changes in relative viscosity mean changes in solvation only. These conclusions were the basis of the stability theory discussed in § 4 (see p. 198).

In the early stages of this simplistic stability theory, the removal of the electroviscous effect in soluble starch sols was studied [1]. These results seemed to draw the analogy between "hydrophobic" and "hydrophylic" sols as regards the nature of the electric charge a step further still.

In the first named sols a reversal of charge may in many cases be obtained by salts with polyvalent oppositely charged ions (see Volume I). We then meet with a zône of flocculation around the point of charge reversal („Unregelmäszige Reihen" i.e., "irregular Series"). If this reversal of charge should occur also in hydrophilic sols, no flocculation zône is to be expected according to the above named stability theory. For the hydration shell in itself would assure stability though a capillary electric charge were absent.

Assuming that such a reversal of charge would occur in such low salt concentrations, that by the smallness of $\varkappa$ electroviscous effects may still manifest themselves, then the presence of such a reversal of charge will show itself in a peculiar form of the corresponding viscosity curve.

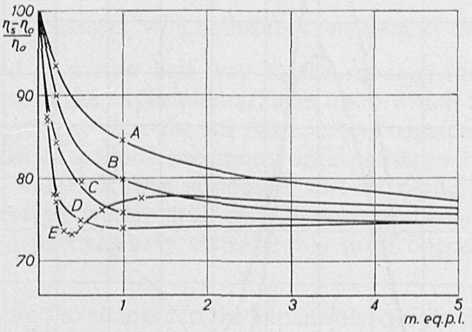

Fig. 19. Influence of salts on $(\eta_s - \eta_o)/\eta_o$ of a 1.97% sol of amylum solubile (20° C) in the region of small concentrations.
Ordinates and abscissae: as in Fig. 16. A = KCl, B = $BaCl_2$, C = $Co(NH_3)_6Cl_3$ and D = $[Pt(en)_3](NO_3)_4$ fit completely with the behaviour in Fig. 16. E = hexol nitrate (composition, see note 1 on page 208) gives a curve with a minimum. At this minimum reversal of charge of the sol takes place from negative to positive. Flocculation does not take place with any of the salts, even not around the minimum of the hexol nitrate curve.

For as the "electroviscous term" (formula on page 205) contains ζ in the quadratic form, this term can be either zero (if $\zeta = 0$) or have a positive value (ζ being either negative or positive).

The curve for the relative viscosity must therefore show a minimum, which

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[1] H. G. Bungenberg de Jong, *Rec. trav. chim.*, 43 (1924) 189.

corresponds to the concentration at which reversal of charge occurs. At higher concentrations the relative viscosity must thus increase, but because of the simultaneously increasing $\varkappa$ it must soon reach a maximum and then decrease further.

This state of affairs has been shown to exist in the sol of soluble starch using hexol nitrate (a complex cobalt salt with hexavalent cation[1]).

Fig. 19 shows the results obtained. The curves for the salts with mono- di- tri and tetravalent cations, have the same relative positions and shapes as with the agar sol (Fig. 16). Hexol nitrate — in accordance with the still higher valency of the cation — initially lowers the relative viscosity still more than the complex Platinum salt, and shows further the above predicted curve form.

By electrophoresis it could indeed be shown that reversal of charge takes place. Further flocculation nowhere occurs (as also neither with the other salts). But as was to be expected, with an appropriate amount of added alcohol maximum flocculation around the minimum of the hexol nitrate curve could be obtained.

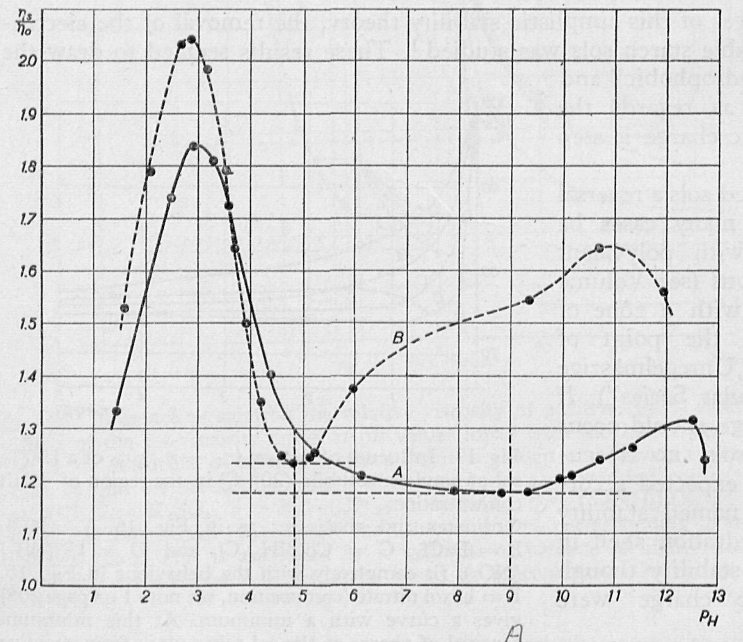

Fig. 20. pH-viscosity curves of a 0.42% ichthyocollsol (37° C) and of a B⇒ 0.67% gelatin sol (42° C). The relative viscosity is a minimum at the I.E.P. (gelatin pH 4.8; ichthyocoll pH 9) and rises on either side (fairly rapidly with gelatin, much less steeply with ichthyocoll). The viscosity falls again at sufficiently low and sufficiently high pH so that a maximum is encountered on either side of the I.E.P.

Though the underlying mechanism of the reversal of charge with proteins by changing the pH is quite different, the minimum of the well known viscosity-pH curve is comparable to the minimum discussed for soluble starch with hexol nitrate. This minimum, occurring at the *I.E.P.*, is here quite easily detectable, because of the still low values of $\varkappa$.

The shape of the viscosity pH curve (see Fig. 20: gelatin and ichthyocoll[2]) may than be interpreted as follows: Starting from the *I.E.P.* i.e., $\zeta = 0$, a pH change originates a positive or negative charge. By the smallness of $\varkappa$ in this range electroviscous effects can manifest themselves. Thus viscosity is increased. By

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[1] Hexol nitrate = $[\mathrm{Co}\{(\mathrm{OH})_2\mathrm{Co}(\mathrm{H_2N{-}CH_2{-}CH_2{-}NH_2})_2\}_3]\,(\mathrm{NO_3})_6$.

[2] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 50 (1931) 238.

still adding more HCl or more NaOH, $\varkappa$ becomes so great, that electroviscous effects must be gradually suppressed. The viscosity-pH curve must therefore show two maxima. See also Fig. 37 on p. 229, referring to clupein.

The different forms of the curves for gelatin and ichthyocoll in the region of the *I.E.P.*'s, which is shown in a completely analogous way in the titration curve may be explained by the supposition that the histidine content of gelatin is much larger than that of ichthyocoll. As mentioned in § 2b, the dissociation of the imidazol groups varies strongly just in this region of pH.

§ 6. REINTERPRETATION FROM THE MACROMOLECULAR POINT OF VIEW OF THE CHANGES OF $(\eta_s - \eta_o)/\eta_o$ CAUSED BY ALTERING THE COMPOSITION OF THE SOLVENT (AT CONSTANT COLLOID CONCENTRATION)

As a result of further investigations, especially those studying various properties of colloids as functions of the sol concentration Bungenberg de Jong, Kruyt and Lens[1] came to an interpretation of $\frac{\eta_s - \eta_o}{\eta_o}$ changes, which differs considerably from the original ideas and can now be valued as a step half way to the modern interpretations. We shall not enter into detail as to the experimental facts upon which this change in ideas was based. Let it suffice to say that the sol particle was considered as a very loose structured material system of colloid substance, interpenetrated by for the greater part very loosely bound water. It was supposed that in taking up this large amount of intramicellar hydration water, still enough energy becomes available to expand the loosely structured particle very considerably until opposing elastic forces set an end to it.

All changes in $\frac{\eta_s - \eta_o}{\eta_o}$ were now attributed to changes in the expansion equilibrium. In "desolvation processes" the loosely built structure contracts, and the circumscribed volume of the particle decreasing, $\frac{\eta_s - \eta_o}{\eta_o}$ diminishes.

But also the electroviscous effect can be seen from the same point of view. For if ionogenic groups situated at the periphery of the particle dissociate, establishing a diffuse double layer, the ionised groups attached to the structure will by mutual repulsion help to expand the particle. Thus more water can be taken in till equilibrium with the opposing elastic forces is reached at a now greater volume of the expanded particle.

By adding an indifferent salt, the diffuse double layer is compressed, with the result that the repulsion between the ionised groups is diminished so that the additional expansion factor of electrical nature is greatly lessened. The swollen particle must thus expell part of the water it contains until a new equilibrium is reached at a lower total volume. The electroviscous effect and its removal by added salts are thus seen in the first place as volume changes of the expanded particles.

The picture of the sol particles as expanded very loosely built structures, has

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[1] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloidchem. Beihefte*, 36 (1932) 429; 37 (1933) 395.

many advantages. Still it possesses a grave difficulty, which disappears however completely in introducing the randomly kinked macromolecule as its intrinsic structure.

This difficulty may be formulated in the form of the following controversy.

A. Because of the rapid decrease of hydration forces with distance a hydration layer can hardly be thicker than a few molecules of water. It is therefore very improbable that the large amount of water taken up by the dry particle is firmly bound by hydration forces. The remaining quantity must therefore rather be considered as "free" water, which is only present between the expanded structure as occlusion water.

B. The dry particle brought into water takes up a definite amount of water, shown by the definite $(\eta_s - \eta_0)/\eta_0$ values. This amount can be shifted reversibly to other definite values by changing the composition of the medium. To explain these definite amounts of water taken in there seemed no other escape than the assumption that an equilibrium is set up between expanding forces and opposed contracting forces — hydration forces versus elastic forces of the expanded particle structure. In this picture therefore we were forced to assume that all the water taken in is hydration water somehow (small amount of firmly bound water + very large amount of "diffuse hydration" water). As the residual hydration forces which bind the diffuse hydration water can only be very small, it followed that the internal structure of the kinetic unit must be very loose, enabling a great expansion before opposing elastic forces set an end to it.

In introducing the concept of the randomly kinked macromolecule as the intrinsic structure of the sol particle the above mentioned controversy no longer exists. If the dry macromolecule is brought into a medium that acts as a solvent, it will expand to the randomly kinked form described in Chapter IV, occluding a relatively large amount of solvent. The "expansion" takes place by itself, the "expanded state" being the most probable form (state of maximum entropy). Thus no work has to be done on elastic forces proper and no special forces to do this work are needed. The solvent taken in is thus not bound by solvation forces but can be described as free occluded solvent, a very small part being excluded which serves for true solvation of for instance polar groups of the macromolecule.

We have now to consider the case that the macromolecule carries ionised groups along its length. Then instead of assuming its most probable form, the macromolecule will assume as a result of the repulsive forces between the ionised groups a less probable and more voluminous form. If now pH is changed so that electrolytic dissociation of the ionogenic groups is suppressed, the volume of the macromolecule will decrease to its normal value.

A decrease of this volume will however also occur at constant pH on adding a small concentration of indifferent salts, which will diminish the mutual repulsion of the ionised groups. Here the valency of the oppositely charged ions of the added salt will be of primary importance in screening off the ionised groups.

Thus in principle the *electroviscous effect* finds a simple explanation. All other facts discussed in the preceeding section may also be explained in an analogous way.

Thus in introducing the randomly kinked macromolecule or macromolecular ion as the kinetic unit in the hydrophilic sols, (indeed all the preceding examples of colloids do belong to this class and not to the corpuscular type, which is restricted to native proteins), volume changes of the kinetic units as postulated by BUNGENBERG DE JONG, KRUYT and LENS do certainly occur, but they need not in the ideal case to give exclusively the basis for an interpretation of the $(\eta_s - \eta_o)/\eta_o$ values experimentally found.

Indeed this volume effect enters here only as a correction factor, in the case in which the macromolecular skein is relatively dense, so that the occluded solvent acts for a part as hydrodynamically immobilized. Taking the ideal case, that the occluded solvent is not immobilized, theoretically the specific viscosity increase is given by the formula (see Chapter IV p. 108) $\frac{\eta_s - \eta_o}{\eta_o} \backsim N^2 . A^3 . G$ in which A is the length of a chain element, N the number of chain elements constituting the macro- molecule and G the number of macromolecules per unit of volume.

In this equation the factor NA^2 forms a measure for the density of the skein, the factor NA representing the resistance caused by the different chain elements. (See Ch. IV). If by introduction of ionised groups the density of the skein is diminished, the factor NA^2 should be replaced by a larger value. Consequently the specific viscosity is increased above the theoretical value[1].

Now in reviewing in short the changes in $(\eta_s - \eta_o)/\eta_o$ represented in the preceding figures, we must conclude that minor changes in the true hydration of polar groups situated along the macromolecule, may give an explanation of the relatively small increases in $(\eta_s - \eta_o)/\eta_o$, caused by various indifferent salts (Fig. 15 and $MgCl_2$ in Fig. 14, p. 202) in the range of higher concentration (an enforced hydration probably diminishing the flexibility of the macromolecule, cf. p. 109, 110).

The sharp fall in $(\eta_s - \eta_o)/\eta_o$ occurring with $MgSO_4$ in Fig. 14 means then, that the solvent is changed by adding this salt in a direction in which the ideal solvability, first existing, is lost. In this "salting out" medium, the macromolecule can no longer expand to the ideal statistical form, but the higher the salt concentration, the more loops of the macromolecule in the interior of the skein mutually adhere, followed soon by adhering of loops between adjacent macromolecules: the colloid substance is "salted out".

In the same way the large decrease of $(\eta_s - \eta_o)/\eta_o$ by addition of alcohol is explained (Fig. 12, p. 200). In higher alcohol concentrations the macromolecular substance is not soluble, the opalescent systems obtained with small colloid concentrations really being systems of quite other nature than originally supposed (see Chapter VIII, § 1c p. 234: "Apparent single systems of composite nature").

Further, still another factor enters here, namely the influence of alcohol in diminishing the dissociation of the ionogenic groups, this influence aiding also in the direction of interlinking loops in the interior of the macromolecule and between adjacent macromolecules (see Chapter X § 3g, p. 400).

The results obtained with tannin and simple phenols (Fig. 13, p. 201) must also be interpreted as a departure from ideal solvability. This occurs in relatively low concentrations. The renewed increase of $\frac{\eta_s - \eta_o}{\eta_o}$ in higher concentrations is simply

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[1] See footnote 1 on page 185.

explained by the fact that the colloids considered are soluble in these higher concentrations of tannin or resorcinol.

The macromolecular skein can in these solvents re-expand and thus $\frac{\eta_s - \eta_o}{\eta_o}$ increases to the order of magnitude, comparable with that occurring originally in only watery medium.

The changes of $(\eta_s - \eta_o)/\eta_o$ occurring with indifferent salts (Fig. 16, 17, 18 and 19, p. 203-207) in small concentrations (the removal of the electroviscous effect) have already been discussed above (the decreased flexibility of the chain molecule caused by the presence of the ionised groups, is increased by the added salts).

It will not be difficult to understand also the occurrence of viscosity minima at reversal of charge points (Fig. 20 and hexol nitrate in Fig. 19) from analogous points of view. We shall however consider this point in more detail in § 9.

§ 7. INFLUENCE OF TEMPERATURE ON $(\eta_s - \eta_o)/\eta_o$

As discussed in Chapter IV, p. 108 theoretically $(\eta_s - \eta_o)/\eta_o$ should be independent of temperature. A small influence of the temperature should be ascribed to changes of the flexibility of the macromolecule. As both the direct influence of the temperature (more intense Brownian motion, less pronounced preference for configurations that are energetically favourable) and the indirect influence via the hydration (higher temperature, less hydration, greater flexibility) tend to diminish the number of monomeric groups in the chain element a small decrease of $(\eta_s - \eta_o)/\eta_o$ with increasing temperature must be expected. That in this respect a typical hydrophilic colloid such as agar (which according to its composition as the salt of a polyose-sulphuric acid[1], can hardly be otherwise seen as a long chain macromolecular ion) behaves according to the expectation, is seen from the following figures [2] relating to three agar sols. Each sol was successively measured in the order 50° → 45° → 40° → 45° → 50° C.

TABLE 2

temp. ° C.	$\frac{\eta_s}{\eta_o}$ ($^2/_7$ % sol)	$\frac{\eta_s}{\eta_o}$ ($^1/_7$ % sol)	$\frac{\eta_s}{\eta_o}$ ($^1/_{14}$ % sol)
50	2.364	1.652	1.322
45	2.393	1.662	1.325
40	2.419	1.674	1.330
45	2.393	1.663	1.326
50	2.362	1.652	1.321

From the figures may be concluded that in the short time of measurement no irreversible changes have occurred, so that the figures may be used to calculate the change of $(\eta_s - \eta_o)/\eta_o$ by increasing the temperature from 40° to 50° C.

We then calculate a decrease of 3.95% (2/7% sol); 3.26% (1/7% sol) and 2.49% (1/14% sol). Obviously this percentage decrease is still a concentration function and linear extrapolation to zero agar concentration then gives a decrease of approximately 2%.

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[1] NEUBERG and OHLE, *Biochem. Z.*, 125 (1921) 311.

[2] H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloidchem. Beihefte*, 28 (1928) 1.

§ 8. THE ELECTROVISCOUS EFFECT AS A DISTURBING FACTOR IN THE DETERMINATION OF THE LIMITING VALUE OF $(\eta_s - \eta_o)/\eta_o C$ AT COLLOID CONCENTRATION ZERO

It often has been claimed that it would be characteristic for hydrophilic colloids, that the viscosity concentration curve will deviate even at small concentrations in the positive direction from linearity. So for instance from the EINSTEIN formula the viscosity concentration curve should be linear up to values of about $\eta_s/\eta_o = 1.05$ and at higher concentrations the curve should bend upwards (see Fig. 21).

In investigating the agar sol, KRUYT and BUNGENBERG DE JONG[1], using an accurate method of viscosity measurement, found that even up to values of $\eta_s/\eta_o = 2.4$ only slight deviations from a linear

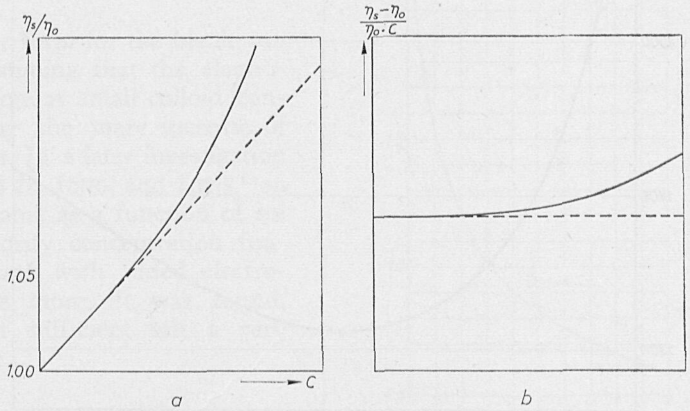

Fig. 21. Schemes to illustrate the relation to be expected between viscosity and sol concentration in the simplest case (see text).

a: the relative viscosity starts with a linear portion but at higher concentrations deviations in the positive sense occur.

b: The curve for $(\eta_s - \eta_o)/\eta_o \cdot C$ will, proceeding toward smaller concentrations, approaches a horizontal level asymptotically.

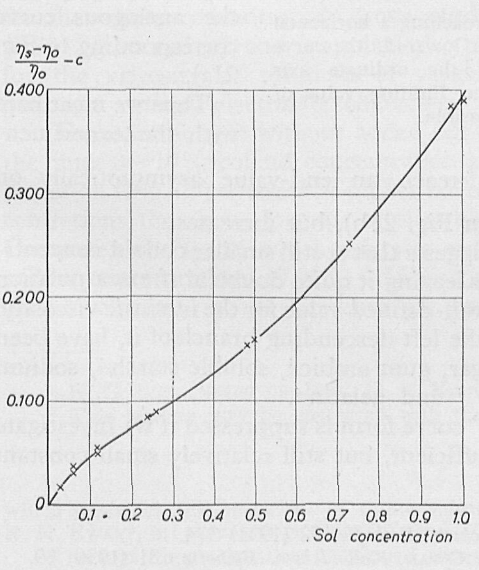

Fig. 22. Viscosity and sol concentration in agar (50° C).

Abscissae: Sol concentration expressed in terms of that of the highest concentrated sol (= 0.279% agar) as unit.

Ordinates: If the experimentally found values of the relative viscosity η_s/η_o are plotted against C, there results a relation between them which is visually near enough linear.

The curve is however S-shaped. To bring out this S-shaped character more clearly in a small scale figure the corresponding values of C (in the above mentioned sense) are each time subtracted from the values $(\eta_s - \eta_o)/\eta_o$.

Compare this figure with Fig. 21a.

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[1] H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloidchem. Beihefte*, 28 (1928) 1.

function occur. These deviations had however a quite different character, the curve obtained being of the third degree, having an inflexion point at about $\eta_s/\eta_o = 1.5$ The deviations from linearity are so slight, that they will hardly be visible if the original figures of the measurements are used in a small scale figure. To enlarge them we have used in Fig 22 the values $[(\eta_s - \eta_o)/\eta_o]$ - C as ordinates. Already without introducing artifical ordinate units the deviation from expectations is plainly visible if we take $\frac{\eta_s - \eta_o}{\eta_o C}$ as ordinates, i.e., the increase in relative viscosity divided by the colloid concentration. This has been done in Fig. 23 (Fig. 21b is the analogous curve corresponding to Fig. 21a).

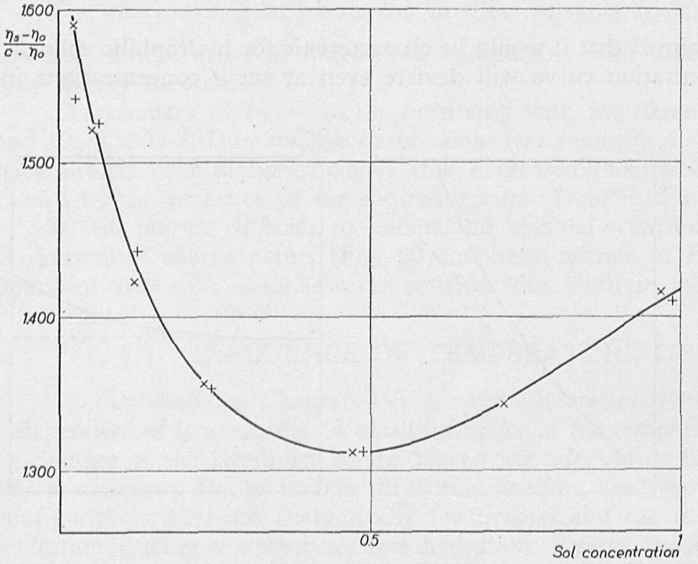

Fig. 23. The expression $(\eta_s - \eta_o)/\eta_o C$ as a function of the agar concentration.
Abscissae: agar concentration expressed in the same units as in Fig. 22.
Ordinates: The values of $(\eta_s - \eta_o)/\eta_o$ obtained from the original experimental figures, divided by the sol concentration C (in the same units as along the abscissa axis).
Compare this figure with Fig. 21b. Instead of approaching a horizontal level asymptotically as the sol concentration is lowered, the curve exhibits a minimum and seems to approach the ordinate axis asymptotically. A graphical determination of the limiting value of $(\eta_s - \eta_o)/\eta_o C$ for $C = 0$ is consequently impossible.

Thus we meet here with the experimental fact that $\frac{\eta_s - \eta_o}{\eta_o C}$ does not decrease to reach an end-value asymptotically on decreasing the colloid concentration (as in Fig. 21b), but increases.

The shape of the curve Fig. 23 even suggests that at still smaller colloid concentrations $(\eta_s - \eta_o)/\eta_o C$ increases further, thus leaving it quite doubtful if extra-polation to zero colloid concentration would give a well-defined value for the intrinsic viscosity.

Curve forms as in Fig. 23, or at least the left descending branch of it, have been found in all sols that were investigated: agar, gum arabic[1], soluble starch[1], sodium thymus nucleate[2], sodium yeast nucleate[3] and gelatin[4].

It has been shown, that the "abnormal" curve form is suppressed if we investigate the viscosity concentration function at a sufficient, but still relatively small constant concentration of an added salt.

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[1] H. R. Kruyt and K. C. Winkler, *Kolloidchem. Beihefte*, 32 (1931) 374.

[2] H. G. Bungenberg de Jong and Ong Sian Gwan, *Kolloidchem. Beihefte*, 31 (1930) 89.

[3] H. G. Bungenberg de Jong and N. F. de Vries, *Rec. trav. chim.* 49 (1930) 658.

[4] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloidchem. Beihefte*, 36 (1932) 429.

As an example Fig. 24 gives the results for the thymus nucleate sol, the upper curve relating to sols without added salt, the lower to sols containing 5 m. eq. p. l. KCl.

This latter curve corresponding to the type of Fig. 21b is thus quite normal. The change of "abnormal" to "normal" curve forms is thus obtained in the region of salt concentrations in which the electroviscous effect is suppressed for the greater part.

The abnormal curve form for the blank can thus be explained by *assuming that the electroviscous effect, being large at small colloid concentration, decreases by the mere increase of the colloid concentration*. In a later investigation by Kruyt, Bungenberg de Jong, and Lens[1] on the viscosity of gum arabic as a function of sol concentration, the viscosity concentration function for sols without and with added electrolytes was studied once more. It was found, that in the presence of sufficient salt a very simple relation[2] exists:

$$\log \frac{\eta_s - \eta_o}{\eta_o\, C} = k_1 C + k_2$$

the logarithm of the specific viscosity increase being a linear function of the colloid concentration (see Fig. 25). This simple relation holds for the whole concentration range studied (up to 30% gum arabic), and it applied also perfectly for the viscosity of solutions of various high polymers without electrolyte character.

Gum arabic sols without added salt gave in the range 0—10% colloid concentration a strong deviation (Fig. 25) whereas at higher colloid concentrations the above formula applies here also. Compare also Fig. 26 which refers to only small colloid concentrations (0.028—2.558%) without added salt and in the presence of small KCl concentrations.

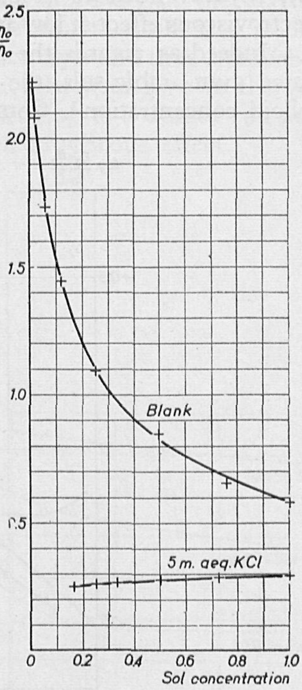

Fig. 24. $(\eta_s - \eta_o)/\eta_o$ C as a function of the concentration of the Na thymus nucleate sol (42° C). Abscissae: nucleate concentration expressed in that of the highest investigated concentration (= 0.164%) as unit.
Upper curve: experimental results of the sols diluted with distilled water.
Lower curve: similar experimental results for which care is taken that 5 m. eq. p. l. KCl is always present in the sols (see text.)

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[1] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloid Beihefte*, 36 (1932) 429.

[2] This relation may be compared with the very analogous relation

$$\frac{\eta_s - \eta_o}{\eta_o\, C} = k_2 + k_1 C$$

which describes the behaviour of dilute solutions of uncharged macromolecules very well. Cf. R. H. Ewart, in: *Advances in Colloid Science II*, 197, Interscience, New York 1946.

The logarithmic equation mentioned above however is valid up to very high concentrations (at least 30%) whereas the linear equation which has a better theoretical foundation is valid only up to concentrations of a few per cent.

In this first concentration range (up to 10% gum arabic) we have therefore once more the same abnormality as already discussed above occurring in agar (Fig. 23), thymus nucleate (Fig. 24) and other sols. It is caused by the presence of the electroviscous effect at low and the self-suppressing of it at high colloid concentrations.

Indeed as regards the influence of indifferent salt on less and more concentrated gum arabic sols, the fall in relative viscosity becomes smaller the higher the colloid concentration[1]. Compare the curve bundles in Fig. 27.

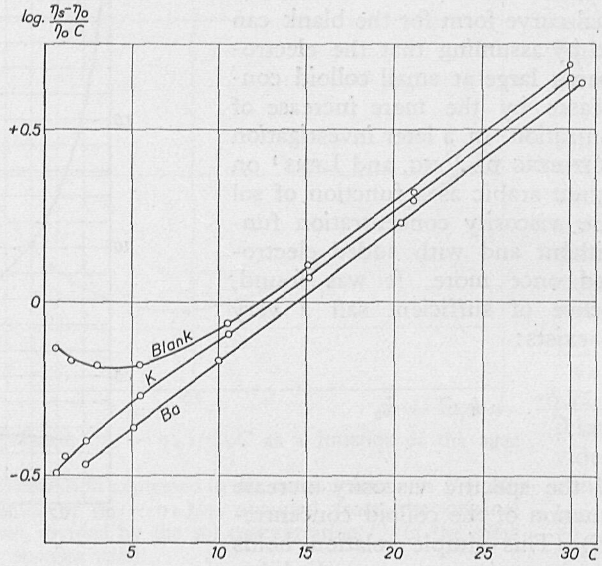

Fig. 25. log $(\eta_s - \eta_o)/\eta_o C$ as a function of the sol concentration in gum arabic (42° C).
Abscissae: gum arabic concentration expressed in gram per 100 ml.
Upper curve: experimental results of the sols diluted with distilled water.
The curves below; ditto at constant presence of 10 m. eq. p. l KCl or $BaCl_2$.
The abnormal shape of the blank curve has given place to a simple linear course, which offers a chance of extrapolation to the sol concentration = O. The slight deviations from the linear course of the KCl curve at the smaller concentrations are possibly derived from experimental errors. The downward bend is not present in Fig. 26.

Thus it seems, that already crowding together of the sol particles into a smaller volume (i. e., increase of colloid concentrations) removes part of the large electroviscous effect existing at low colloid concentrations. This crowding together has thus in principle the same result as the addition of an indifferent salt at constant colloid concentration.

Though for these high sol concentrations the simple formulation $(\eta_s - \eta_o)/\eta_o \backsim N^2 . A^3 . G$ will no longer hold, it may be supposed that among the factors determining the actual (high) viscosities, the flexibility of the macromolecule will still play a similar rôle.

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[1] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloidchem. Beihefte*, 37 (1933) 395.

For an explanation of the "self suppression" of the electroviscous effect we may then suppose that the macromolecular electrolyte itself acts as indifferent electrolyte and thus diminishes the interaction between its own charged spots thus leading to a denser coil and a smaller electroviscous effect.

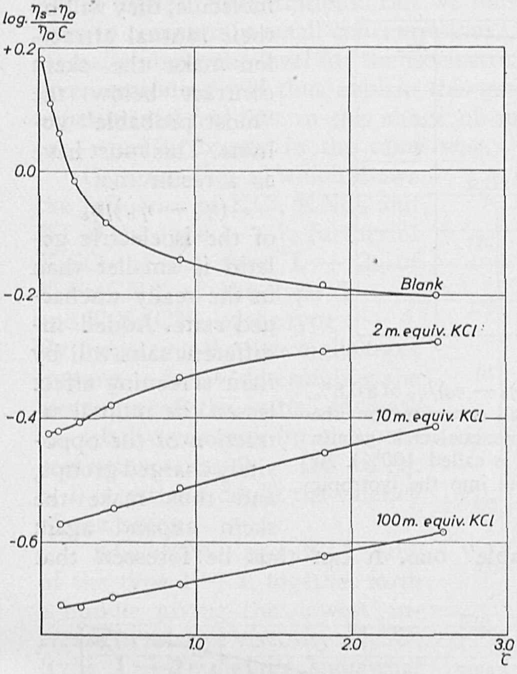

Fig. 26. log $(\eta_s - \eta_o)/\eta_o C$ as a function of the sol concentration with gum arabic (25° C). Abscissae: gum arabic concentration expressed in gram per 100 ml.
Top curve: without added salt. On account of the shape of the curve an extrapolation to the sol concentration = O is very uncertain.
Below: The abnormal course of the blank curve disappears on addition of sufficient KCl, as a result of which the above mentioned extrapolation does appear to be permissible.
(at 2 m. eq. p. l KCl the course of the curve is not yet quite linear).

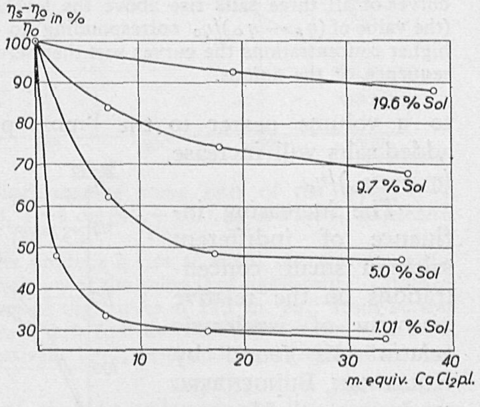

Fig. 27. Neutralisation of the electroviscous effect by $CaCl_2$ for a number of gum arabic sols of increasing concentrations.
The sol concentrations are expressed in grams per 100 ml.

§ 9. REVERSAL OF CHARGE AND ELECTROVISCOUS EFFECT

The occurrence of a viscosity minimum in the viscosity-pH curve of gelatin (see p. 208 Fig. 20) can be simply explained from the macromolecular point of view from changes in the flexibility of the macromolecule, for both a positive and a negative charge will diminish this flexibility and thus $(\eta_s - \eta_o)/\eta_o$ will then be greater than in the uncharged condition.

However we must thereby take into consideration that according to modern points of view an amphoteric protein is not really uncharged at the I. E. P., but contains an equal amount of positively and negatively ionised groups.

If ionised groups of opposite sign are thus present side by side on the macro-

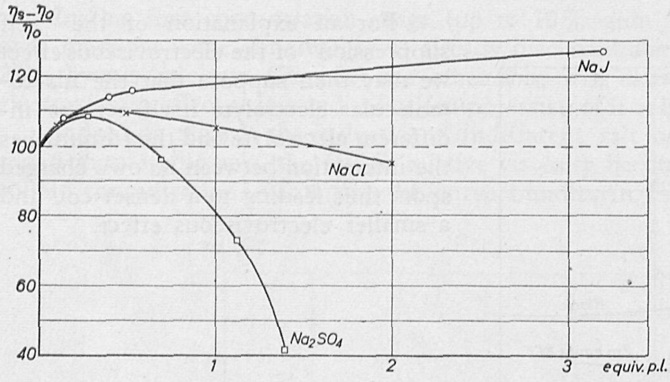

Fig. 28. Influence of NaI, NaCl and Na_2SO_4 on $(\eta_s - \eta_o)/\eta_o$ of a 0.8% isoelectric gelatin sol (50° C). In the region of small concentrations the curves of all three salts rise above the level of the isoelectric gelatin (the value of $(\eta_s - \eta_o)/\eta_o$ corresponding to this is called 100%). At higher concentrations the curves sort themselves out into the lyotropic sequence of the anions.

molecule, they will by their mutual attraction make the skein contract below the "most probable" volume. This must have as a result that $(\eta_s - \eta_o)/\eta_o$ of the isoelectric gelatin is smaller than in the really uncharged state. Added indifferent salts will by their screening effect lessen the mutual attraction of the oppositely charged groups, and thus make the skein expand again to a volume nearer to the "most probable" one. It can thus be foreseen that added salts will increase $(\eta_s - \eta_o)/\eta_o$.

The increasing influence of indifferent salts in small concentrations on the relative viscosity of isoelectric gelatin sols found by HOLLEMAN, BUNGENBERG DE JONG, and MODDERMAN[1] can be explained in this way. Fig. 28 shows the influence of Na_2SO_4, NaCl and NaI up to relatively high concentrations.

· Fig. 29 shows the same for KI, KBr, KCl and KF. In both figures so-called lyotropic influences of the anions are clearly seen, these causing the curves to bundle

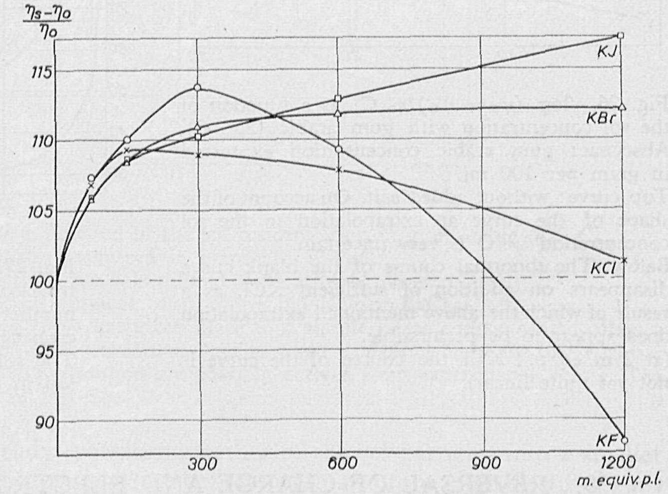

Fig. 29. Influence of some K salts on $(\eta_s - \eta_o)/\eta_o$ of a 2.5% isoelectric gelatin sol (50° C). As in Fig. 28: at smaller concentrations rise of all the curves above the level 100%, at higher concentrations arrangement in the sequence of the lyotropic series of the monovalent anions. For the course of the KF curve, see note. 1 on page 219.

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[1] L. W. J. HOLLEMAN, H. G. BUNGENBERG DE JONG, and R. S. TJADEN MODDERMAN, *Kolloidchem. Beihefte*, 38 (1933) 439.

The explanation given in this publication was not yet based on the macromolecular point of view, but already used the idea of expansion and contraction of the loosely built colloid particles.

out at higher concentrations. But we must direct our attention to the effect all salts have in common in small concentrations[1], this effect being an increase of the relative viscosity above the level for the isoelectric gelatin sol without added salts. The above given reasoning will thus explain this general effect of salts, but it foresees also the experimental fact that in this range of small concentrations not all salts will increase $(\eta_s - \eta_0)/\eta_0$ exactly in the same way.

Compare Fig. 30 which shows the influence of KCl, KNO_3 and KI of the type 1 — 1, further of K_2SO_4 of the type 1 — 2, of $K_3Fe(CN)_6$ of the type 1 — 3 and $K_4Fe(CN)_6$ of the type 1 — 4. By inspecting the figure, the most important factor determining the steepness of the initial increase in relative viscosity (in the concentration range 0 — 150 m. eq. p. l) is without doubt the valency of the anion.

KCl, KNO_3 and KI[2], salts of the type 1 — 1, together form a bundle giving the lowest increase in relative viscosity. K_2SO_4 (type 1 — 2) mounts somewhat higher (compare at 150 m. eq. p. l), whereas $K_3Fe(CN)_6$ (type 1 — 3) gives a still more rapidly mounting curve which is far surpassed by $K_4Fe(CN)_6$ (type 1—4).

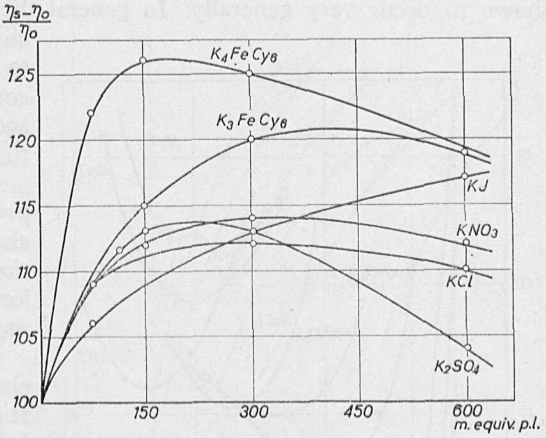

Fig. 30. Influence of some salts of the types 1—1, 1—2, 1—3, 1—4 on $(\eta_s - \eta_0)/\eta_0$ of a 0.8% isoelectric gelatin sol (50° C).

All the salts produce a rise at small concentrations for which the valency of the anion is of significance. Compare the sequence of the curves at 150 m. eq.: from bottom to top there follow first the three salts of the type 1—1, then successively 1—2, 1—3 and 1—4 (see further text).

This experimentally found importance of the ion valency can be quite easily foreseen from the general explanation given above.

We must then first state, that the above reasoning will apply in its purest form only for such a salt, of which the cation will screen off the negative ionised groups of the macromolecule to exactly the same extent as its anion screens off the positively ionised groups of the macromolecule.

Such an ideal salt will not alter the isoelectric condition, and we shall assume that salts of the type 1 — 1 come near to this ideal salt[3]. With salts of the type 1 — 2, 1 — 3 and 1 — 4, the polyvalent anion will in the given order screen off the positively charged groups of the macromolecule to an increasingly greater extent than the monovalent cation screens off the negative ionised groups.

Thus also an unbalancing of the original electrical compensation characteristic of the isoelectric state, will be the result, which will increase in the order 1—2, 1—3, 1—4. Of course this unbalancing will in itself diminish the efficiency of the mutual

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[1] The irregular course of the KF curve in Fig. 29 in the range of smaller concentrations is probably caused by an increase of the pH, KF being the salt of the weak acid HF.

[2] The very low value for KI at 75 m. eq. p. l seems suspect and may be due to an experimental error.

[3] The differences between KCl, KNO_3 KI in Fig. 30 show already, that besides the valency other factors of specific nature play a rôle. They will not be considered here. See Chapter IX, p. 259.

attraction of the oppositely charged ionised groups of the macromolecule and the sequence of the curves 1—4 > 1—3 > 1—2 > 1—1 in Fig. 30 may be explained by this.

Reversal of charge with hexol nitrate of negatively charged macromolecular sols — first met with in the case of amylum solubile (see p. 207 Fig. 19) — has been shown to occur very generally. In general they do not lend themselves to a viscosimetric analysis because, in contrast to amylum solubile, flocculation or coacervation phenomena in most cases accompany the reversal of charge (see p. 223 § 10).

Also lower valent cations share this property of bringing about reversal of charge, but the required salt concentrations are then increasingly higher the lower the valency of the cation (see p. 276 Chapter IX § 2).

If such a colloid and such salts are chosen, that form favourable combinations for viscosimetric investigation, — favourable here meaning that no flocculation or coacervation accompanies the reversal of charge — then it may be expected that, similar to the combination amylum solubile + hexol nitrate minima in the$(\eta_s - \eta_o)/\eta_o$ curves must also occur.

The viscosimetric detection of these minima for salts, which bring about reversal of charge at not very low concentrations, will however not be very easy. For at these higher salt concentrations (where activity coefficients are already low) it may be expected that the branch ascending from the minimum at the side of the higher salt concentrations will be still much less steep than for hexol nitrate in Fig. 19.

The ordinary technique of viscosimetry, which gives figures accurate to a few 0.1%, will at most be able to detect the existence of the predicted minima in the viscosity curves, but will not suffice to study them in more detail. Holleman and Bungenberg de Jong[1]

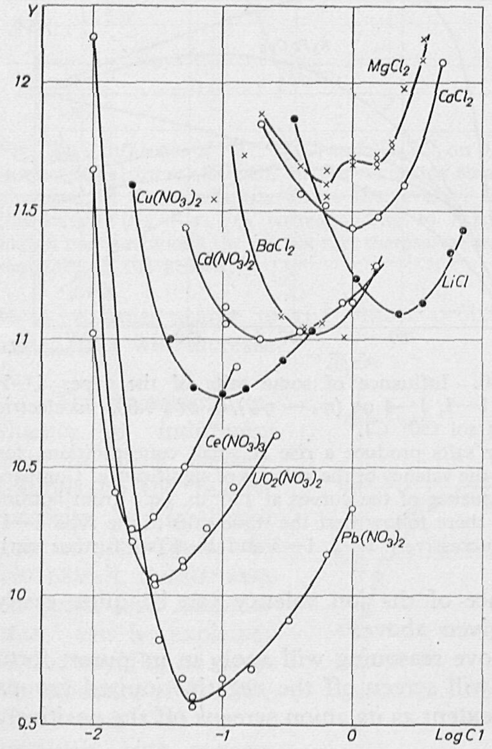

Fig. 31. Influence of a number of salts on $(\eta_s - \eta_o)/\eta_o$ of a 1% Na arabinate sol (25° C). Ordinates: Y = quantity which apart from a small correcting factor (which is however constant) corresponds to $(\eta_s - \eta_o)/\eta_o$ expressed in % of the corresponding value for the sol without added salt. Abscissae: logarithm of the salt concentration, the latter expressed in eq. per l (thus — 2 = 0.01 N, — 1 = 0.1 N, 0 = 1N).
With all the salts a minimum curve is obtained which lies very close to those concentrations at which reversal of charge occurs (compare with Fig. 32 and 33. See further text).

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[1] L. W. J. Holleman and H. G. Bungenberg de Jong, *Kolloidchem. Beihefte*, 46 (1937) 113.

used for that purpose a technique permitting the measurement of the time of flow with a reproducibility of a few 0.01%. With the sodium arabinate sol they could detect the existence of these minima in the relative viscosity, using one trivalent, seven divalent and one monovalent cations.

The results are given in Fig. 31, in which y represents $\frac{\eta_s - \eta_0}{\eta_0}$ in per cent of the blank. This quantity is given as a function of the logarithm of the salt concentration, (the use of log *C* is here of advantage to make the minimum character of the viscosity curves for all salts plainly visible in one and the same figure).

The reversal of charge concentrations were also determined electrophoretically on the movements of suspended SiO_2 particles in the arabinate sol, see Fig. 32. Comparing the curve minima with the reversal of charge, a direct correlation is plainly visible, see Fig. 33. The order in which the minima follow from left to right is the same as that of the reversal of charge concentrations, but in general the latter lie somewhat (mean 0.2 units) to the right.

This investigation has thus shown that indeed minima in the relative viscosity occur as predicted for salts bringing about reversal of charge, though the systematic non-coincidence of minimum and reversal of charge experimentally found, still needs an explanation[1].

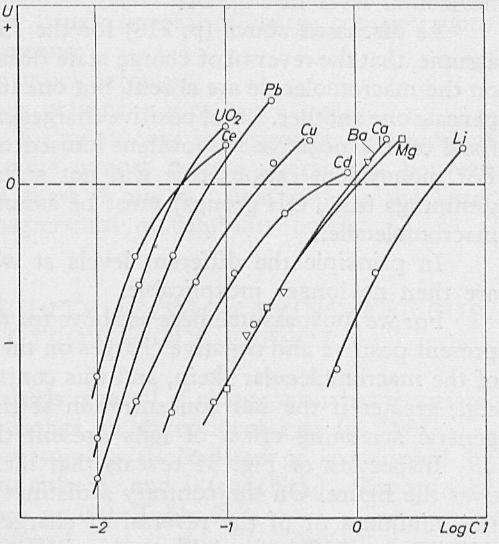

Fig. 32. Reversal of charge of 1% Na arabinate with the salts of Fig. 31.
Ordinates: Electrophoretic velocities measured on suspended SiO_2 particles, expressed in arbitrarily chosen units.
Abscissae: log of the salt concentration (expressed in equiv. per l).

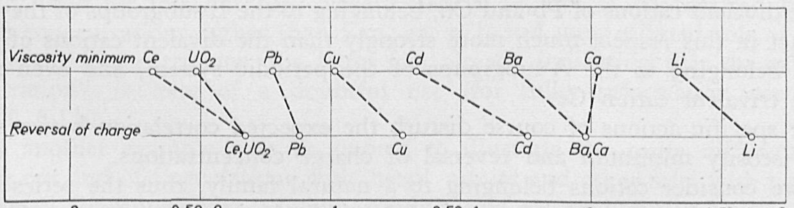

Fig. 33. Relative positions of the viscosity minima of Fig. 31 and of the reversal of charge concentrations of Fig. 32.
Abscissae: logarithms of the salt concentrations (expressed in equiv. per l).
The reversal of charge concentrations lie in general somewhat higher (average 0.2 logarithmic units).

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[1] For instance it may be asked if the reversal of charge of the adsorbed arabinate film on SiO_2 does coincide with that of the arabinate particles free in solution, or lies systematically too high. Other possible causes are discussed in the original publication.

Of more importance are here the different levels, at which for every salt used the relative viscosity reaches its minimum value.

If at the reversal of charge point the macromolecule was really uncharged, we would expect that the minima with all salts investigated would lie on the same horizontal level in Fig. 31.

As discussed above (p. 218) for the *I.E.P.* of gelatin, we can however here also assume, that the reversal of charge state does not represent a state in which all charges on the macromolecule are absent, but one in which positive and negative charges compensate one another. Local positive charges can for instance arise from polyvalent cations fixed on the negative monovalent ionised carboxyl groups of the arabinate molecule. For monovalent cations this will not suffice, but an extra fixation on other polar groupings (e.g., OH groups) must be assumed to create local positive charges on the macromolecule.

In principle the different levels at which the minima are situated in Fig. 31 are then no longer inexplicable.

For we must assume here as above for the *I.E.P.* of gelatin that the simultaneously present positive and negative charges on the macromolecule will cause a "contraction" of the macromolecular skein, and this contraction can be of different grades, it being e.g., greater if the salt concentration at the viscosity minimum is lower (a smaller general screening effect of ions present then opposes this "contraction").

Inspection of Fig. 31 reveals that these minima are not distributed at random over the figure. On the contrary a distinct correlation between y *min* and log *C* (of the minimum or of the reversal of charge) exists. Low values of y *min* are found with $Ce^{\cdot\cdot\cdot}$, $UO_2^{\cdot\cdot}$ and $Pb^{\cdot\cdot}$ showing low values for log *C*, whereas high values of y *min* are found with $Mg^{\cdot\cdot}$, $Ca^{\cdot\cdot}$ and $Li^{\cdot}$, having high values of log *C*.

We shall see later that reversal of charge is directly connected with fixation of cations on the ionised groups of the colloid and that as regards this fixation specific properties of the cations play a great rôle. Here no longer valency of the cation is the only important quality, but others such as volume, polarisability and polarising power of the cation come into play. See Ch. IX, § 2, p. 276.

Now in Fig. 31 such specific features also occur, the valency of the cation not being the only factor which determines the $\frac{\eta_s - \eta_0}{\eta_0}$ value at the minimum.

The divalent cations of Pb and Cu, belonging to the B subgroups of the periodic system act in this respect much more strongly than the divalent cations of Ba, Mg and Ca, belonging to the A subgroups of the periodic system, and even stronger than the trivalent cation Ce.

The specific actions of course disturb the expected correlation between height of the viscosity minimum and reversal of charge concentrations.

If we consider cations belonging to a natural family, thus the series Ba, Ca, Mg or the series Pb, Cu, Cd, then we find that indeed with a higher reversal of charge concentration there corresponds a higher $\frac{\eta_s - \eta_0}{\eta_0}$ value in the minimum.

We may conclude that the different $(\eta_s - \eta_0)/\eta_0$ values, which occur in Fig. 31 at the viscosity minima must be connected with different grades of contraction of the macromolecular skeins, caused by electrical attraction forces, set up by localised attachment of cations on the macromolecule, especially on its negatively charged ionised groups.

Such attractions may also exist between adjacent macromolecular skeins. Thus it is probable that at and around the viscosity minima associations of macromolecules exist.

With the cations studied here the arabinate sol still remains perfectly "stable", but with 4 and 6 valent cations flocculation or coacervation occurs. These higher valent cations have lower or even very low (true) reversal of charge concentrations (see p. 259 Chapter IX § 1 and 2), that means:

a. that the attachment of cations is here much stronger,
b. that the general screening effect of ions present is now much less.

Both factors will thus be favourable for producing still stronger contractions in the macromolecular skeins and much more intense mutual interactions between adjacent macromolecules. Obviously with these 4 and 6 valent cations the latter reach such a magnitude that actual transgressions of solubility occur.

§ 10. TRANSGRESSIONS OF SOLUBILITY ACCOMPANYING THE SUPPRESSION OF THE ELECTROVISCOUS EFFECT

The behaviour of soluble starch sol towards reversal of charge with hexol nitrate, without flocculation accompanying it, is rather an exceptional case (p. 208).

As a rule negative colloids of acidic nature are flocculated or coacervated by small amounts of hexol nitrate, rhodochrome chloride and $Pt(en)_3(NO_3)_4$, that are salts with 6, 5 or 4 valent complex cations (see p. 270, Table 2)

In the simplistic stability theory discussed in § 4, this "instability" occurring as a result of "discharging" at very low concentrations, does not fit in. One would have to assume, that simultaneously with the reduction of ζ a dehydration occurs, the reason why being obscure. For at these low concentrations (e.g., of a few m. eq. p. l or less) there is no reason to explain this "dehydration" as a "salting out effect", comparable for instance with the action of $(NH_4)_2SO_4$ on protein sols or of $MgSO_4$ on agar sol (see Fig. 14), which occurs as a rule at relatively high concentrations.

That these flocculations (or coacervations) with high valent cations are of a quite different nature is clearly shown in the case of gum arabic. Its sols, as Pohl[1] has already shown, cannot be salted out even by the highest concentrations of salts commonly used for this purpose. Nevertheless a 1% sol is coacervated by hexol nitrate in concentrations higher than 5 m. eq. p. l, and more diluted sols at proportionally lower concentrations, the latter fact already indicating that the expression "concentration" is here of a doubtful use (for fuller information see p. 262, Chap. IX § 1b).

Still another example may be quoted to illustrate the quite different nature of salting out and of flocculations with hexol nitrate and other salts with polyvalent ions in small concentrations: amylum solubile sols are readily salted out e.g., with certain sulphates, whereas its sols do not flocculate with the named 6, 5, or 4 valent complex cations.

Salting out must be considered as being caused by changing the solvent in such a direction, that the macromolecule becomes difficultly soluble along larger parts of

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[1] Pohl, *Z. physiol. Chem.*, 14 (1890) 155.

its length, irrespective of the presence or absence of ionised groups. The flocculations (coacervations) with the named polyvalent ions on the other hand are primarily connected only with fixations of these ions on the ionised groups of the macromolecule and one very important factor is thereby the frequency of occurrence of the ionised groups along the macromolecule.

If this frequency is very small, as in soluble starch, even the highest valent cation will not suffice to cause flocculation or coacervation.

In gum arabic the frequency is much greater and it allows of coacervation with salts of the types 6—1, 5—1, 4—1, yet not with lower valent cations.

Other colloids have still greater frequency of occurrence of the ionised groups and here also transgression of solubility is possible with 3—1 or even with 2—1. (For fuller information on the above statements see Chapter IX in particular § 1d, p. 269 and Chapter X in particular § 3d, p. 392).

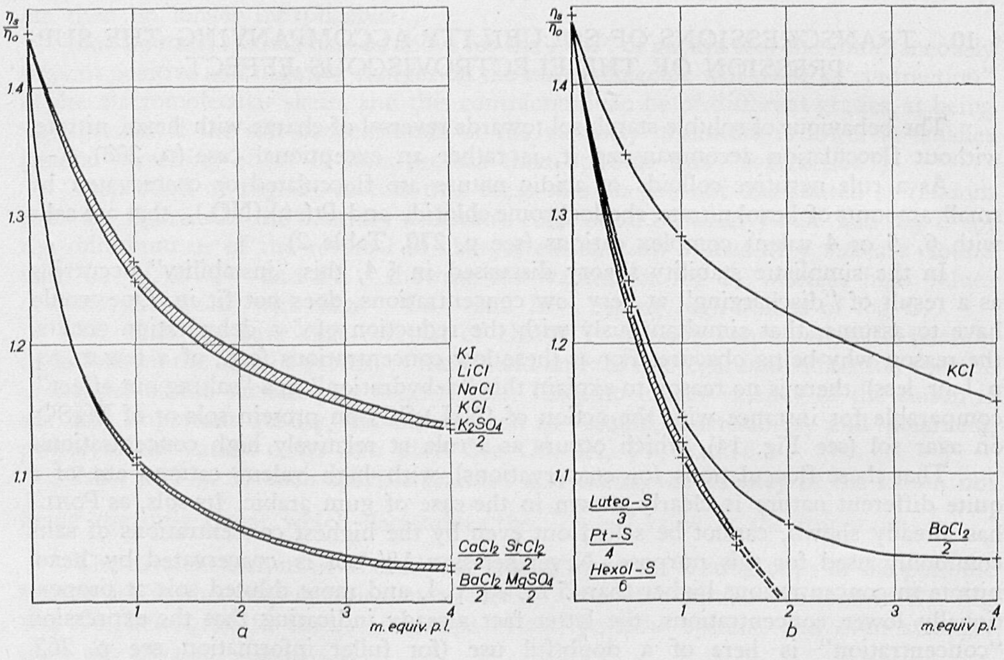

Fig. 34. Influence of salts in small concentrations on the relative viscosity of 0.1% Na thymus nucleate. (42° C).
Ordinates: relative viscosity.
Abscissae: salt concentration in m. eq. p. l.
a: The given salts divide into two groups, characterized by the valency of the cation. K_2SO_4, although of the type 1—2, acts as strongly as the four given salts of the type 1—1. In the same way $MgSO_4$ (2—2) fits with the three salts of the type 2—1.
b: The normal grouping of the curves according to the valency of the cation is restricted to 1—1, 2—1 and 3—1 but 3—1, 4—1 and 6—1 are practically equivalent and with these flocculation occurs at the bottom of the curves.

Luteo-S	abbreviation for	$Co(NH_3)_6Cl_3$ (type 3—1).
Pt-S	,, ,,	$[Pt(en)_3](NO_3)_4$ (type 4—1).
Hexol-S	,, ,,	$[Co\{(OH)_2\ Co(en)_2\}_3](NO_3)_6$ (type 6—1).

At very small concentrations $BaCl_2$ also fits closely with 3—1, 4—1 and 6—1.

An interesting feature in the course of the viscosity curves for such cases in which flocculation (or coacervation) accompanies the depression of the electroviscous effect, *is the crowding together of these curves.* As examples in Fig. 34 we give the influence of some indifferent salts on the relative viscosity of the sodium thymus nucleate sol[1] and in Fig. 35 the same for the carrageen sol[2].

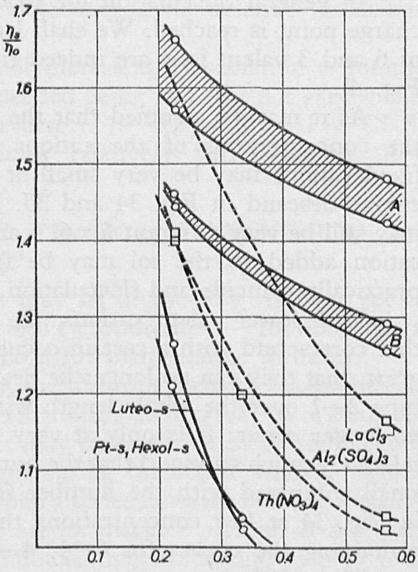

Fig. 35. Influence of salts in very small concentrations of the relative viscosity of 1/90% carrageen sol (42° C). Ordinates, abscissae, luteo-s, Pt-s and hexol-s as in Fig. 34.
The curves start at $\eta_s / \eta_o = 2.3$ for the salt concentration zero; no measurements have however been made in the concentration range 0—0.22 m. eq. per l.
In the shaded strip A lie the curves for: LiCl, NaCl, KCNS, KCl, K_2SO_4, $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ thus salts with monovalent cations (types 1—1, 1—2, 1—3, 1—4).
In the shaded strip B lie the curves for: $Ba(CNS)_2$, $Ba(NO_3)_2$, $BaCl_2$, $Mg(NO_3)_2$, $MgCl_2$, $MgSO_4$, $CaCl_2$ and $SrCl_2$, thus salts with divalent cations (types 2—1 and 2—2).
Luteo—s (3—1), Pt—s (4—1) and Hexol—s (6—1) act practically equally strongly and give flocculation at the lower end of the curves.
The three dotted curves run quite discrepantly which is attributable to disturbances, connected with the strong tendency to hydrolysis of the salts in question.

In the first named sol, we see in Fig. 34b the normal sequence 1 — 1 . . . 2 — 1 . . . 3 — 1 . . ., but 3 — 1, 4 — 1 and 6 — 1 give approximately the same curve and all three flocculate the nucleate sol. The curves here fall to extremely low values of

$$\frac{\eta_s - \eta_o}{\eta_o}.$$

In principle the same is shown by the carrageen sol, though here the bundles corresponding to the salts with monovalent or divalent cations are very broad. Here also 3 — 1 . . . 4 — 1 . . . and 6 — 1 form one bundle, ending in flocculation and very low values of relative viscosity.

This carrageen sol is interesting in further respects. It belongs to the extremely high viscous type, the 1/90% sol showing a relative viscosity of 2.3. The very great electroviscous effects may partly be caused by the small sol concentration (see § 8), partly be connected with the abnormal viscosity character, this sol not following POISEUILLE's law, the viscosity varying in the domain of the low shearing stresses commonly used in the OSTWALD viscometer.

For an explanation of the crowding together or even practical coincidence of the curves for polyvalent ions, we must first state that this recalls very much the

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[1] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte,* 31 (1930) 89.
[2] H. G. BUNGENBERG DE JONG and ONG SIAN GWAN, *Kolloidchem. Beihefte,* 29 (1929) 436.

so-called *equivalent flocculation* known in lyophobic sols (see Volume I). Electro-equivalent amounts of added 6, 4 or 3 valent cations in Fig. 34 and 35 lower the relative viscosity to the same extent, and lead to transgression of the solubility of the macromolecular colloid. These facts are much in favour of the above postulated fixation of the cations on the ionised groups of the macromolecular colloid, as the cause of flocculations and of reversal of charge phenomena.

In general flocculation (or coacervation) sets in already before the reversal of charge point is reached. We shall later see an example in which equivalent amounts of 6 and 3 valent ions are indeed fixed at the reversal of charge point (see p. 265 Fig. 4).

As it may be assumed that the fixation of these polyvalent ions is very strong, the concentrations of the cations free in the solvent in equilibrium with the fixed cations may be very small at that gross concentration in which the viscosity curves descend in Fig. 34 and 35. In this case, though the amounts of free cations may still be very different for 6, 4 and 3 valent cations, nevertheless practically every cation added to the sol may be fixed on the colloid. So that the curves may practically coincide and flocculation may occur at the same apparent concentration.

With lower valent cations, the concentrations of the ions free in the solvent, that correspond with a certain occupation of the ionised groups with cations, are so great, that they can no longer be neglected. Coincidence of the curves for salts of the type 2—1 over the whole length with the curves for 6—1, 4—1 and 3—1 will then no longer occur. It is only at very low concentrations (where fixation of cations is relatively much stronger) that the number of the cations free in the solvent may become small compared with the number fixed on the colloid. This explains the fact that in Fig. 34 at low concentrations the $BaCl_2$ curve comes very near to the bundle containing the curves for 6—1, 4—1 and 3—1.

With KCl, the conditions are still more unfavourable, so that the KCl and $BaCl_2$ curve do directly take different courses.

Reviewing the action of salts on negatively charged colloids, we may discern three cases:

A. *Simple screening effect* by the cation of the added salt, this effect giving a typical spreading of curves in the suppression of the electroviscous effect according to the valency of the cation. With increasing salt concentration sooner or later, depending on the nature of the cation and the nature of the colloid, this effect emerges into B.

B. *Fixation of cations on the ionised groups.* This effect gives crowding together of curves in the suppression of the electroviscous effect, is often accompanied by transgression of solubility, and can further lead to reversal of charge phenomena.

C. *Salting out effet*, for which the presence of ionised groups on the macromolecule is not a *conditio sine qua non* as in A and B. Relatively large concentrations are needed (see the bend in the $MgSO_4$ curve in Fig. 14 on p. 202) and the position of both cation and anion in particular of the latter, in the lyotropic series is here of primary importance.

For the action of salts on positively charged colloids the same division into three cases applies, for A and B now the valency and specific[1] properties of the anion being of primary importance. As for C (salting out) as well the action of the anion preponderates over the action of the cation, it may sometimes be difficult to discern if a flocculation or coacervation of a positive sol, e.g., with monovalent anions, is the result of the actions B or C.

Discrimination is then often possible by determining the relative intensities of flocculation with other monovalent anions.

Indications for C are then, that the order of increasing flocculation is found: CNS — I — Br — Cl — (F), the concentration needed being as a rule not very small. Indications for B, that the order is just the reverse (F) — Cl — Br — I — CNS, the concentrations needed being here sometimes relatively small (See p. 299, Chapter IX § 3b and p. 407, Chapter X § 3j).

§ 11. VISCOUS BEHAVIOUR OF SHORT CHAIN-MACROMOLECULAR "COLLOIDS"

Sols of long chain macromolecular colloids may be of the low viscous type if the kinetic units in their sols consist of densely built corpuscules — the long chain molecule being folded up — as is assumed to hold for many native proteins.

If the kinetic unit is not built up in that way, the macromolecular colloids belong to the high viscous type, their long chain molecules forming statistical skeins in solution.

From the formula $(\eta_s - \eta_o)/\eta_o \propto N^2A^3G$ it may be seen that in a "homologous polymeric series" viscosity will increase with increasing molecular weight, (which is proportional to NA). The earliest members of such a series however must necessarily give low viscous sols. Such short chain molecules are for instance present in *Na-yeast nucleate* (which is generally formulated as a tetranucleotide, consisting of only four mononucleotides linked together by ester phosphate linkages. *Na-thymus nucleate* is constituted on similar lines (abstraction made from certain details, which are not important here) but here not only four nucleotides but a very great number are linked together by ester phosphate linkages (see formula on p. 188 § 1c).

Thus it is interesting to compare the viscous behaviour of yeast nucleate and thymus nucleate.

We have already seen in § 10, that the latter is indeed a colloid of the very high viscous type (compare the relatively high values of η_s/η_o at very small sol concentration), this being in agreement with the high polymeric nature of its molecules.

The large decrease in η_s/η_o by suppressing the electroviscous effect, is also to be expected, considering that in the backbone of the molecule — probably $(C\text{-}C\text{-}C\text{-}O\text{-}P\text{-}O)_n$ — the phosphorus atoms recurring at short distances are the centres of monovalent ionised phosphate groups. The macromolecule may then be symbolized by $-\ominus-\ominus-\ominus-$ etc., that is a long chain molecule in which negative charges are situated at short intervals. This of course will contribute to a markedly diminished flexibility of the macromolecule.

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[1] The normal crowding together but now of polyvalent anions, is to be seen in Fig. 17b (p. 205), with the positive casein sol for the curves obtained with $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$. They also end in flocculation.

It may even occur, that specific properties are of so great importance that an inversion of the normal sequence of the curves in the suppression of the electroviscous effect is the result. See Fig. 38 (p. 230) in which $K_3CH(SO_3)_3$ (type 1—3) decreases η_s/η_o more than $K_4Fe(CN)_6$ (type 1—4).

Thus a large electroviscous effect may be expected and consequently a large decrease in relative viscosity on adding indifferent salts (see p. 224 Fig. 34).

Now turning to Na-yeast nucleate, we meet with a "colloid" which only slightly increases the viscosity of the solvent. Compare Fig. 36, where we see that the 0.83% sol has only a relative viscosity of 1.052 (The 0.1% thymus nucleate however gives $\eta_s/\eta_o = 1.45$).

For an accurate measurement of the influence of salts on the viscosity of yeast-nucleate it was necessary to use a technique which gave a reproducibility of a few 0.01% in the time measurements.

The results[1], see Fig. 36, gave a normal sequence of the curves for KCl, $BaCl_2$ and $Co(NH_3)_6Cl_3$ and we may interpret this decrease of the relative viscosity as a suppression of the electroviscous effect.

The yeast nucleate molecule is however relatively so small that its four mononucleotide residues possibly do not suffice to form a chain element. Thus a skein proper will not be present in solution. Nevertheless in principle the decrease in viscosity by added salt may be explained on similar lines from the increased flexibility of the (short) chain molecule. Without added salt the latter will by the mutual repulsion of the ionised phosphate groups assume a more or less stretched form. If this "electrical stiffening" is removed by added salts, which screen off the ionised phosphate groups, the chain molecule may assume a more bent form.

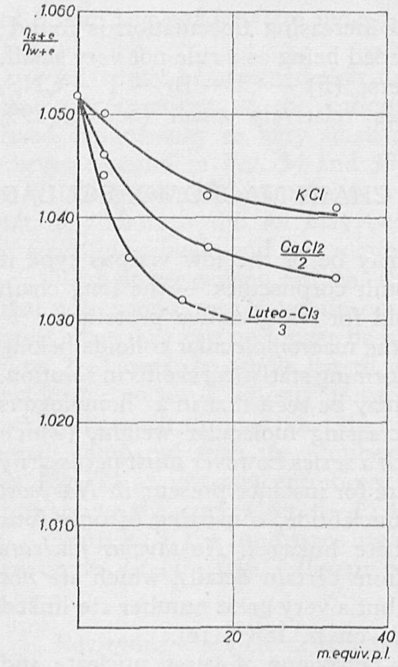

Fig. 36. Neutralisation of the electroviscous effect for a 0.83% sol of Na yeast nucleate (42° C). Beyond 13.3 m. eq. per l. Luteo—s (= $Co(NH_3)_6Cl_3$) flocculation occurs. This figure should be compared with Fig. 34 from which it appears that, notwithstanding the fact that yeast nucleate belongs to the low viscous, thymus nucleate to the very high viscous type of macromolecular colloids, the character of the bundle of curves is the same and also $Co(NH_3)_6Cl_3$ causes flocculation with both (and this occurs with both with $CaCl_2$ also at higher concentrations, see p. 270).

It is interesting that yeast nucleate also gives flocculation with $Co(NH_3)_6Cl_3$. Later (see p. 395) we shall see that as regards transgression of solubility by adding different cations the low polymeric yeast nucleate and the high polymeric thymus nucleate behave very similarly.

§ 12. LOW VISCOUS BEHAVIOUR AS A RESULT OF EXISTING "COMPLEX RELATIONS"

Investigations of the viscous behaviour of clupein sulphate sols have shown that these also give relatively low viscous sols, so that here also the measurement had to be

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[1] H. G. BUNGENBERG DE JONG and N. F. DE VRIES, *Rec. trav. chim.*, 49 (1930) 658.

performed using a technique allowing reproducibility of time measurements to a few 0.01%.[1]

The viscosity-pH curve (Fig. 37) shows in principle the expected form, if one takes into consideration that the *I.E.P. is situated at a high pH value, the only point which* calls for explanation being the very low order of the η_s/η_o values which are obtained.

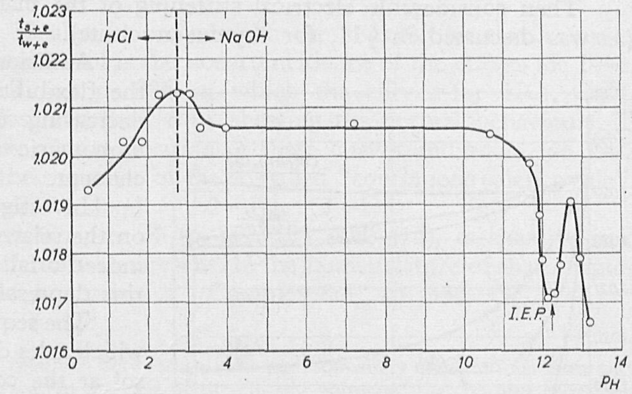

Fig. 37. pH-viscosity curve of a 0.292% clupein sulphate sol (25° C). (See text).
The ordinate gives the quotient of the times of flow in an OSTWALD viscometer for the sol (t_{s+e}) and a liquid of the same electrolyte composition but containing no clupein (t_{w+e}).
As the addition of the clupein has only a slight influence on the density, the ordinate gives practically the relative viscosity η_s/η_o.
The shape of the curve for this colloid of the low viscous type is in principle the same as those of proteins of the high viscous type (comp. Fig. 20). Minimum at the I.E.P. and fall of the curves at very high or very low pH values. As a consequence of the extreme position of the I.E.P. at already fairly high pH values, the maximum in the curve to the right of the I.E.P. is here very narrow, the maximum left of the I.E.P. on the other hand very broad.
The very flat course of the curve between pH = 10 and pH = 4 and the extra bump at pH = 3 can be directly explained from the amino acid composition of the clupein. Between pH = 10 and pH = 4 all guanidino groups are positively and the one carboxyl group negatively charged. The charge and thus the viscosity is constant in this range. In the neighbourhood of pH = 3 the dissociation of the carboxyl group is suppressed so that there the charge increases by one unit and the viscosity rises somewhat before it decreases again at lower values of the pH, due to high concentration of the ions present.
Turbidity (which is due to coacervation) occurs at the I.E.P.

There are sufficient indications that this low viscous character is not connected with its kinetic units being densely built corpuscules as in native proteins. There are for instance the absence of *denaturation phenomena* (by heat or otherwise) and the great ease with which coacervates of a distinctly liquid character can be obtained. Compare Ch. VIII § 3, p. 247 and Ch. X § 3j, p. 406.

The low viscous character could further be connected (as above in the case of Na-yeast nucleate) with the macromolecules being very short, consisting only of a few monomeric residues. This once more does not strictly apply for clupein, the latter though a protein of relative low molecular weight (a few thousands) still consists of more than 30 amino acid residues linked together by peptide groups.

We may expect that this comparatively small number of monomeric residues will not give very viscous solutions, but we hardly think it possible that the very low η_s/η_o value found experimentally can be explained wholly in this way.

Considering that $^2/_3$ *of the amino acids consists of arginine, we might* expect reasonable depressions of the relative viscosity on adding salts.

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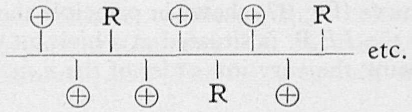
[1] H. G. BUNGENBERG DE JONG, W. A. L. DEKKER, and P. VAN DER LINDE, *Rec. trav. chim.* 54 (1935) 1.

For assuming a more or less regular distribution of the arginine side-chains along the macromolecule the latter may be symbolised by



$$\begin{array}{cccccccccc} \oplus & & R & & \oplus & & \oplus & & R & \\ | & & | & & | & & | & & | & \\ \hline & | & & | & & | & & | & & \text{etc.} \\ & \oplus & & \oplus & & R & & \oplus & & \end{array}$$

Then considerable electrical stiffening of the macromolecule may be expected (as was discussed in § 11, for thymus nucleate).

Addition of salts will then increase the flexibility of the macromolecule, now decreasing considerably the number of monomeric residues constituting a chain element.

Investigation of the influence of salts on the relative viscosity (see Fig. 38) shows indeed a fall of η_s/η_o with some salts, but this depression is certainly not very great.

The sequence KCl — K_2SO_4 is normal, which salts did not coacervate the clupein sol at the concentrations used.

The decrease in η_s/η_o is greater with $K_3CH(SO_3)_3$ (1—3) and $K_4Fe(CN)_6$ (1—4) but the curves end here in coacervation. As discussed in § 10 such transgressions of solubility indicate "fixation" of ions on the ionised groups of the macromolecule. In such fixations the valency of the ions is no longer the only important factor, but specific factors of the ions concerned and of the nature of the ionised groups also enter. This gives a first explanation of the reversed order of the curves 1—3 and 1—4 in Fig. 38.

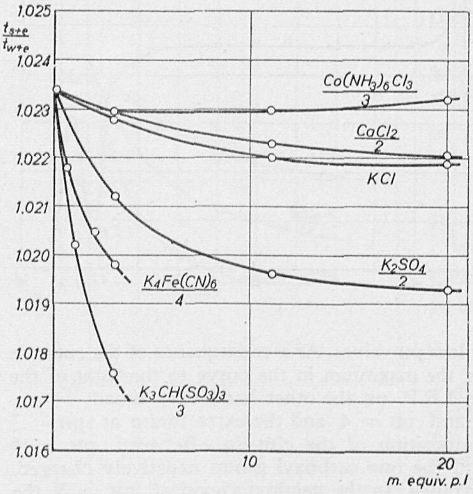


Fig. 38. Influence of small salt concentrations on the relative viscosity of a 0.32% clupein sulphate sol (pH 2.76, 25° C).
Some deviations, which are discussed in the text, here occur from a normal course of the curves in the suppression of the electroviscous effect of a positively charged sol.

It is further important that K_2SO_4 at higher, but still relatively low, concentrations than used in Fig. 38 also coacervates the clupein sulphate sol, showing that such intimate relations between anions and positively charged ionised groups of clupein are already possible in the case of sulphate ions.

This gives us a clue for explaining the low viscous character of the clupein-sulphate sol. What we said above about the electrical stiffening of the macromolecule does not apply; this could only be the case if the sulphate ions were to be found at relatively great distances from the positively charged arginine side-chains. On the contrary we have to assume that already in the original sol an appreciable fraction of the sulphate anions take up positions very close to the positively charged ionised groups of the clupein (what in the previous paragraphs was called "fixation"), which necessarily will contribute to rolling up of the macromolecule into a relatively dense skein.

In this direction the divalence of the sulphate ions helps, as one SO_4 ion can then connect two monovalent positive ionised groups of the clupein molecule.

We might thus explain the low viscous character by the existence of so-called "complex relations" (see Chapter X: Complex Colloid Systems) between the positive ionised groups of the macromolecule and the dissociated sulphate ions[1].

In accordance with it is the fact, that clupein sulphate is only slightly soluble in water, addition of more clupein sulphate to its saturated solution giving two-phase systems, a liquid rich in clupein sulphate (coacervate) and a liquid poor in it (see p. 406 Chapter X § 3j).

A further detail in Fig. 38 also in accord with the supposition that complex relations are present in the original sol is the non-coincidence of the curves for 1—1, 2—1, 3—1, (compare Fig. 18c on p. 206, in which the curves for KCl, $CaCl_2$ and $Co(NH_3)_6Cl_3$ are very close together, thus showing the normal behaviour).

Considering in Fig. 38 the relative position of these curves and those for 1—2 and 1—3, we easily recognize the sequence of the so-called "continuous valency rule".

1—3 ... 1—2 ... 1—1 ... 2—1 ... 3—1

which, as will be discussed in Chapter X (p. 352, 388, 408, 415), is characteristic of systems in which complex relations are present. In the nomenclature of that chapter the clupein sulphate sol should be named a "Complex sol" (p. 336, 337).

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[1] In most other cases complex relations between colloids and oppositely charged ions arise only upon addition of electrolyte to the sol. In the case of the clupein sulphate, the complex relation is strong enough to be manifest in the pure sol.