

The Properties of Colloidal Systems. I.—The Osmotic Pressure of Congo-red and of some other Dyes.

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Experiments made in 1895 by Linder and Picton* upon solutions of arsenious sulphide indicated that colloidal solutions possess a real osmotic pressure, although the authors themselves claim no quantitative value for their results. In 1905† further experiments were made, but again great difficulties were met with, and, although it seemed evident that osmotic pressure was present, the numerical values obtained were irregular and small.

The first definite proof that certain colloidal solutions are able to exert a not inconsiderable osmotic pressure was given by Starling‡ in the case of the colloids of blood-serum. When separated by a gelatin membrane from a solution obtained by filtration of some of the same serum through Martin's gelatin filter, the pressure rose to about 30 mm. of mercury.

Waymouth Reid§ found that solutions of carefully purified hæmoglobin gave an undoubted osmotic pressure when separated from water by a membrane of parchment-paper, but regards this fact as evidence that hæmoglobin forms a true solution.

Moore and Parker|| determined the osmotic pressures of the colloids of white of egg, of serum, and of soap solutions, while Moore and Roaf¶ made measurements of those of serum-proteins, of gelatin, and of gum-acacia.

Hüfner and Gansser** and, somewhat later, Roaf,†† independently of one another, made careful determinations of the osmotic pressure of hæmoglobin solutions, to which reference will be made in a subsequent page.

It will be noticed that, in all these cases, with the exception of arsenious sulphide and soaps, the chemical constitution of the body investigated is uncertain, although the molecular weight of hæmoglobin has been calculated from its content in iron. The experience of those who had worked with arsenious sulphide and with soaps was not encouraging for further research,

* 'Chem. Soc. Trans.,' vol. 67, p. 72, 1895.

† 'Chem. Soc. Trans.,' vol. 87, p. 1909, 1905.

‡ 'Journ. Physiol.,' vol. 19, p. 322, 1896, and vol. 24, p. 318, 1899.

§ 'Journ. Physiol.,' vol. 33, p. 12, 1905.

|| 'Amer. Journ. Physiol.,' vol. 7, p. 261, 1902.

¶ 'Biochem. Journ.,' vol. 2, p. 34, 1907.

** 'Archiv f. Physiol.' (Engelmann), 1907, p. 209.

†† 'Physiol. Soc. Proc.,' 1908, p. i, in 'Journ. Physiol.,' vol. 39, 1909.

so that it seemed desirable to investigate the behaviour of colloids of known chemical constitution and molecular weight, the latter to be as small as possible, in order that the osmotic pressure should be sufficiently great. By this means it might be possible to estimate the number of molecules taking part in the formation of "solution-aggregates" or colloidal elements, and also to obtain more definite information as to the effect of electrolytes on the osmotic pressure.

Certain of the aniline dyes form colloidal solutions, if we may take Graham's criterion of non-diffusibility through parchment-paper as decisive. One of these dyes is congo-red, whose constitution and molecular weight are well known. My attention was first called to the fact that solutions of this body have a considerable osmotic pressure by phenomena met with in purifying it by dialysis. It was striking and, in fact, a matter of some inconvenience, to find that the contents of the parchment-paper tubes rapidly increased in volume by taking up water and, unless some of the fluid was removed, continuously overflowed. Congo-red, therefore, formed the starting-point of the observations to be recorded in the present paper.

Owing to their high colouring power, the aniline dyes present many advantages for the study of colloidal properties. In the investigation of osmotic pressure, for example, the slightest leak in the membrane of the osmometer is detected at once.

The particular form of osmometer used was that of Moore and Roaf,* modified in order to change at will the fluid on the side of the membrane opposite to the solution under investigation. Repeated changes of distilled water could be made until no further change in the osmotic pressure occurred, while the effect of the presence of various electrolytes or other bodies could be examined. A diagram of the apparatus is given in fig. 1.

Congo-red, as obtained from Kahlbaum, was found to contain an appreciable amount of sodium chloride. In order to remove this, hydrochloric acid was added until the red colour had vanished and the free acid, precipitated, was washed on a filter with distilled water. It was soon found that the free acid went into a beautiful deep blue colloidal solution, which passed through the filter. (This observation has been published by Pelet-Jolivet and Wild,† since my experiments were made.) I was obliged, therefore, to resort to prolonged dialysis against distilled water. This dialysed solution was placed in the osmometer. It gave a very small osmotic pressure, about 6 mm. Hg. Subsequent experiments, to be described below, were made to determine the osmotic pressure of this blue colloid

* *Loc. cit.*

† 'Kolloid-Zeitsch.,' vol. 3, p. 175, 1908.

more accurately. Dilute solution of sodium hydroxide was then run through the lower chamber in order to convert the free acid into the sodium salt. This solution was replaced at intervals of 24 hours until the outside solution remained permanently slightly alkaline. During this process the osmotic pressure rose gradually to about 40 mm. Hg. Repeated changes of

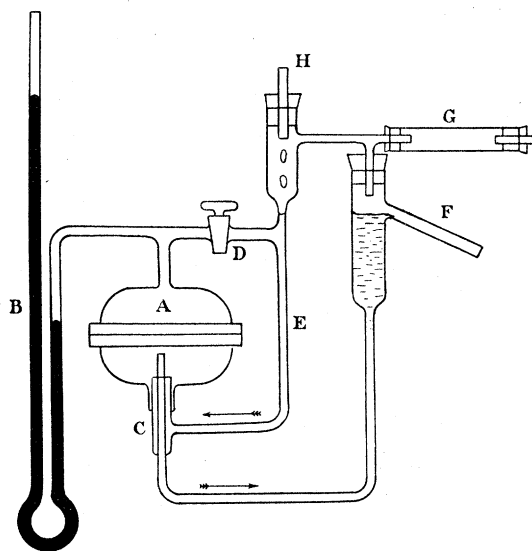


FIG. 1.

A, Osmometer of Moore and Roaf.

B, Mercury manometer, read by means of a reading microscope.

C, T-tube, with inner smaller tube, to allow a current of water, or other fluid, through the outer, lower, chamber of the osmometer.

D, Tube, connecting the two chambers when the stopcock is opened. This is done in order to control the zero of the manometer at any time.

E, Inlet tube to the lower chamber. F, Outflow.

G, Soda-lime tube to exclude atmospheric CO_2 .

H, Tube from flask of distilled water or other fluid.

The osmometer was immersed in a thermostat.

distilled water were then similarly run through as long as the pressure continued to rise. After ten days, equilibrium was attained with a pressure of 79.3 mm. Hg at a temperature of $30^{\circ}2$ C.

The molecular weight of congo-red (the di-sodium salt of benzidine-tetrazo-di-naphthylamine-di-sulphonic acid) is 696.47. On the basis of an osmotic pressure of 22.4 atmospheres for a molar solution (as true solution) at 0° , a 1-per-cent. (= 10 grammes per litre) solution at $30^{\circ}2$ should have a pressure of

$$22.4 \times 760 \times \frac{273 + 30.2}{273} \times \frac{10}{696.47} = 271.4 \text{ mm. Hg.}$$

At the end of the above experiment, the solution was pipetted out of the osmometer and its concentration determined by evaporating to dryness a known volume and drying the residue to constant weight in a toluene bath in the usual way. It was found to be 0.30 per cent., so that, if the dye had been present as separate molecules, the osmotic pressure should have been $0.30/1 \times 271.4 = 81.4$ mm. Hg. The actual value found was 79.3 mm. Hg, or 97 per cent.

In other experiments the agreement with theory was not so good, *e.g.* 207 mm., instead of a theoretical 228 mm., or 91 per cent., for a 0.84-per-cent. solution at $30^{\circ}2$; 77.4 instead of 84, or 92 per cent., for a 0.309-per-cent. solution at $30^{\circ}7$; and 128 instead of 146, or 88 per cent., for 0.58-per-cent. solution at 10° .

It is obvious that these values could only be obtained if the greater part of the elements responsible for the production of osmotic pressure were present as single molecules, since any value greater than one-half the theoretical implies that a part of the active elements consists of single molecules.

When the molecular weight of hæmoglobin is calculated from the content in iron, a value of about 12,000 to 14,000 is obtained.* Now this is the same number obtained from the osmotic pressure determinations of Hüfner and Gansser,† and of Roaf.‡ Hæmoglobin, therefore, exists in solution in single molecules, although, like congo-red, it does not pass through parchment-paper. The molecular weight of congo-red, however, is very much less than that of hæmoglobin, only about one-twentieth in fact, so that it is more surprising to find it to behave as a colloid.

On this account it is advisable to examine how far congo-red exhibits other properties associated with colloids. To what degree does a molecule of such dimensions show the characteristics of matter in mass, possessing surfaces?

In the first place, what appearance does a solution of congo-red show in the ultra-microscope? According to Michaelis§ the particles present are sub-microscopic, that is, resolvable into separate bright points. The same statement is made by Pelet-Jolivet and Wild.|| My observations are not entirely in agreement with those of the investigators mentioned. There are undoubtedly, a few scattered bright points to be seen, but these only

* See Schultz, 'Die Grösse des Eiweissmoleküls,' p. 31, Jena, 1903.

† *Loc. cit.*

‡ *Loc. cit.*

§ 'Deutsche Med. Wochensch.,' No. 42, 1904.

|| *Loc. cit.*

account for a very small part of the total quantity of the dye present in the solution, as can easily be shown as follows: The blue colloidal free acid of congo-red, even in extreme dilution, shows the track of the beam of light filled with shining points of a beautiful copper colour and of nearly equal size, so far as their diffraction images enable one to judge. If a drop of dilute alkali be added to this solution, the track of the beam suddenly vanishes, occasionally a bright point moves into the field and back again. These few particles seem to be slightly larger than those of the acid. When the illumination is carefully adjusted and made as brilliant as possible, close attention shows that the track of the beam is very faintly visible as a bluish grey haze, not resolvable into separate points, at all events not with the means at my disposal, viz., arc light, Zeiss D* objective. As I am inclined to interpret the phenomena, the faint haze is the optical expression of the part of the dye present in the molecular state, and the rare bright points are due to aggregates of a number of molecules, produced by the action of traces of electrolytes, to which congo-red is enormously sensitive, as will be shown below. The solutions described by previous observers as being resolvable into particles by the ultra-microscope were, in all probability, not sufficiently free from electrolytes.

The ultra-microscope, then, does not throw much light on the nature of solutions of congo-red, since, although it does not contain particles large enough to be visible by means of this instrument, other undoubted colloids, such as ferric hydroxide, are similarly non-resolvable, but show a faint haze.* Moreover, the phenomena described above in the case of congo-red are very like those seen by Michaelis† in certain protein solutions, namely, a part visible as granules and the rest not so resolvable.

The property of carrying an electric charge, not as an ion, but on undissociated molecules, is shared by congo-red with matter in mass. In Whetham's boundary apparatus the dye moves as a whole towards the anode and is, therefore, negatively charged. The origin of the charge is obscure, but is, perhaps, derived from electrolytic dissociation.‡ In accordance with its nature as an electro-negative colloid, congo-red is aggregated or precipitated by cations, especially powerfully by bi- and tri-valent ions. It is also precipitated by an electro-positive colloid, such as toluidine-blue or ferric hydroxide. The precipitate has the properties of an adsorption-

* Zsigmondy, 'Zur Erkenntniss der Kolloide,' Jena, 1905, p. 148.

† 'Virchow's Archiv,' vol. 179, pp. 205—208, 1905.

‡ The phenomena seen in the boundary apparatus are of some complexity, being accompanied by slight electrolysis. These will more properly form the subject of a separate paper.

compound, in that its composition varies with the relative concentrations of the two colloids present in the solution. As I have shown elsewhere,* the behaviour of congo-red in respect of adsorption by cotton and other materials is that of an electro-negative colloid.

The statement is sometimes made that colloids have no definite point of saturation. Congo-red, on the contrary, has, in a certain sense, a somewhat indefinite limit of solubility. It appears, however, that many colloids, especially the inorganic ones, tend to aggregate and deposit when their particles are brought into too close apposition; it may be that traces of electrolytes are responsible for this behaviour, which is thus not the same thing as the crystallisation of a super-saturated true solution.

Recent research tends to show that there is no real line of demarcation to be drawn between colloids and crystalloids. Congo-red is evidently one of those interesting cases which have some of the properties of both classes. In any case, it does not seem reasonable to expect fundamental differences as regards properties dependent on dimensions of the active elements between a large molecule such as congo-red, containing some 70 atoms, and a particle of colloidal gold containing a similar number of atoms. The properties referred to are those dependent on diffusion, such as osmotic pressure and those dependent on surface development.

The fact that, in the case before us, true solution and colloidal solution are one and the same thing suggests several interesting questions. At what molecular size do bodies begin to show properties due to surface development, although still in the condition of single molecules? Again, why should we not be able to reduce the number of molecules in the aggregates of colloidal gold until they consist of single molecules? In this case we should have a true solution of gold in the metallic state, not in the ionised condition. It is possible that differences of electric potential and surface-tension oppose obstacles to such a phenomenon.

The great sensitiveness of congo-red to traces of electrolytes has already been incidentally referred to and this fact makes it a matter of considerable difficulty to obtain the maximum readings of the osmotic pressure as given above. For example, a solution of congo-red, containing 0.84 per cent. of the dye, dialysed against repeated changes of ordinary distilled water until no further rise of pressure took place, gave an osmotic pressure of only 118 mm. Hg, whereas on using water which had been distilled after the addition of potassium permanganate and sulphuric acid and a *second time* after the addition of barium hydroxide, being kept from contact with air by soda-lime tubes, the pressure rose to 207 mm. Hg. The ordinary distilled water

* 'Biochem. Journ.,' vol. 1, pp. 175—232, 1906.

used above was a fairly good sample, having a conductivity of not more than 5 gemmhos at 18° while the purer water had a conductivity of 1.8 gemmhos. If still better water had been used, no doubt the full theoretical value of 228 mm. for the osmotic pressure would have been reached.

Since the ordinary distilled water presumably contained carbonic acid, I tried the effect of water through which carbon dioxide gas had been passed until its conductivity was 23 gemmhos. By titration, this solution was found to contain 0.19 gramme CO₂ per litre. The osmotic pressure fell from 207 to 120 mm. Hg.

The powerful action of so weak an acid as carbonic is rather surprising, and makes it unnecessary to subject stronger acids to detailed investigation.

When the solution of carbonic acid in the above experiment was replaced by a decinormal solution of sodium chloride, the osmotic pressure fell in the course of 24 hours to 15 mm. Hg. When equilibrium was established, the concentration of sodium chloride on both sides of the membrane would be about one-twentieth normal.

Linder and Picton* found that when aggregation of arsenious sulphide was brought about by an electrolyte, it was impossible to reverse the process by washing with distilled water. Similarly, although repeated changes of distilled water were passed through the osmometer after the sodium chloride, until the issuing water gave no reaction with silver nitrate, the osmotic pressure only rose to about three-quarters of its initial value. It is possible that very much more prolonged dialysis might have produced further effect, but it seemed more important to use the apparatus for other experiments, since all these experiments are of necessity of long duration. This washing with water was, in one case, continued for three weeks, and, although after this process the osmotic pressure had risen only to three-quarters of what it was before the action of sodium chloride, no further rise was to be detected on the mercurial manometer when a fresh change of water was added. If a more delicate manometer had been used, it is quite possible that the pressure would have been found to be still rising very slowly, since the extreme slowness of removal of the last traces of electrolyte is characteristic of adsorption phenomena.†

In order to test the action of alkali, a solution of the blue free acid was placed in the osmometer. Only a small pressure of a few millimetres was obtained, too small to read accurately on the mercurial manometer. When dilute sodium or ammonium hydroxide was run into the lower chamber, the pressure rose rapidly as long as the alkali combined with the dye-acid.

* 'Chem. Soc. Trans.,' vol. 87, p. 1911, 1905.

† Bayliss, 'Biochem. Journ.,' vol. 1, p. 182, 1906.

When excess was present, as shown by the permanent alkaline reaction of the solution, the pressure fell again. The explanation is, no doubt, that the aggregating action of the cation made itself felt. In view of the results of Moore and Roaf* on the augmentation of the osmotic pressure of protein solutions under the action of alkali, the fact noted by me is of some interest. It seems probable that, in the case of proteins, the rise of osmotic pressure is the result of the formation of new colloids by chemical action, these "salts" having smaller "solution-aggregates" than the original colloid.†

If we were unaware of the chemical nature of congo-red and observations were being made of the osmotic pressure of the solution of the free acid, we might imagine that alkali caused a large increase in the osmotic pressure of this body. The fact is that the blue colloidal solution of the free acid, as will be shown later, consists of large particles, easily resolvable by the ultra-microscope, and producing only a small osmotic pressure. When alkali is added, the salt, ordinary congo-red dye, is produced, and this, as shown above, exists in solution in single molecules with high osmotic pressure.

The fact that, as an electrolyte is added in stages, pausing sufficiently long between each addition to allow equilibrium to be established, there is a definite osmotic pressure for each step, so that a continuous smooth curve is obtained, shows that the action of a low concentration of electrolyte must be exerted on a part only of the molecules present. For example, it is not every molecule that unites with another one, since, if so, there would be no intermediate stages between full and half osmotic pressure. From the fact that these stages do exist it follows that a number of molecules are left single. The process is analogous to the association which takes place in ethyl alcohol when dissolved in benzene, where the apparent molecular weight of the alcohol rises from 50 to 208 in regular gradation as the concentration rises from 0.494 to 14.63 grammes to 100 of benzene.‡ This can only be explained by the assumption of a steadily increasing number of molecules becoming associated with others, while the rest remain free.

Ultra-microscopic observations of the actions of electrolytes on congo-red, although somewhat difficult to interpret, confirm the results given by measurements of osmotic pressure. A dilute solution, showing only a very few scattered bright points, on the addition of a solution of carbonic acid or a natural salt contains a greatly increased number of these bright particles, which vary considerably in size. In this respect they contrast with the particles seen in the colloidal solution of the free acid, which are strikingly

* *Loc. cit.*, p. 66.

† See also Lillie, 'Amer. Journ. of Physiol.', vol. 20, pp. 127—169, 1907.

‡ Walker, 'Introduction to Physical Chemistry,' 4th ed., p. 205, 1907.

uniform in size. It appears that the action of an electrolyte is, so to speak, selective, leaving some molecules free, while causing others to aggregate into particles, consisting themselves of very different numbers of molecules.

Faraday showed, more than 50 years ago,* that the ruby-red solutions of gold which he prepared by reduction of gold chloride were suspensions of minute particles of metallic gold. He also noticed that the colour of the solution became blue under the action of sodium chloride in dilute solution, and was precipitated by stronger solutions. Both these effects were absent if a small amount of "jelly" had previously been added to the gold solution. This latter "protecting" action of "stable" colloids is now well known, forming the basis of Zsigmondy's "gold number" as a characteristic of proteins. Congo-red behaves, as regards this protection from precipitation by electrolytes when a stable colloid is present, in the same way as the inorganic colloids. It may be noted, in passing, that this is a phenomenon usually ascribed to surface properties. The dye also is protected from adsorption by paper, under the influence of electrolytes, when a trace of gelatin is present.† It was, therefore, of interest to examine the influence of stable colloids on the reduction of osmotic pressure produced by electrolytes.

Since my experiments on adsorption above referred to indicated that this protective action was greater when the stable colloid had an electric charge of the same sign as that of the dye, or of opposite sign to that of the precipitating ion, I chose for the present experiments a dialysed solution of Grüber's serum-albumin, to which a trace of ammonium hydroxide was added in the first experiment. The solution in the osmometer contained about 0.18 per cent. of the dye and 0.25 per cent. of serum-albumin. When dialysed against distilled water, the osmotic pressure rose to 42 mm. of mercury. The water was then displaced by a decinormal solution of sodium chloride. To my surprise, the pressure fell to zero in about 14 hours. Moreover, as in the experiments without stable colloid, it was found that after 14 days' changes of distilled water the pressure could only be brought back to three-quarters of its original value. Measurements of the electrical conductivity of the water after interchange with the colloidal solution showed that the latter parted with its electrolytes very slowly. They were probably held in a state of adsorption by the protein as well as by the dye.

In another experiment I first tested the particular solution of serum-albumin used and found that in the proportion of 5 c.c. to 50 c.c. of one-thousandth normal congo-red solution, precipitation by one-hundredth

* 'Phil. Trans.,' vol. 147, 1858: As to the Nature of the Solutions, see pp. 160 and 172; Precipitation by Salt, p. 165; Protection from Action of Salt by "Jelly," p. 175.

† 'Biochem. Journ.,' vol. 1, p. 201, 1906.

normal calcium sulphate was prevented, although in the absence of the protein complete precipitation occurred. In order to be quite certain of adequate protection, I added 10 c.c. of the albumin solution to 50 c.c. of the dye and placed the mixture in a Schleicher and Schüll diffusion thimble of parchment-paper tied on to a glass tube fitted with a cork and a long narrow tube to act as manometer. This was placed in water contained in a large test-tube and then immersed in a thermostat at 30°·7 C. The osmotic pressure rose to 200 mm. of water in the course of 30 hours, being 94 per cent. of the theoretical value. Calcium sulphate in one-hundredth normal solution was then put into the outer tube instead of the water. In about 24 hours the pressure had fallen to 50 mm. and no further fall took place. In this case a certain protection was shown, since, without the albumin, the pressure would have gone down nearly to zero.

The apparent disagreement between the results of osmotic pressure measurements and the naked eye appearances are, I think, to be explained in the following way. When the contents of the osmometer in the last experiment were poured into a glass vessel and observed carefully, it was obvious that, although no precipitation had taken place, the solution was distinctly more turbid than a similar one to which no calcium sulphate had been added. On examination under the ultra-microscope, the former was resolvable into a multitude of distinct, but not brilliant, particles; whereas solutions of congo-red itself, as already shown, are not resolvable. It is clear, therefore, that a certain degree of aggregation had in reality taken place, although the particles formed are much smaller than those formed when calcium sulphate acts upon congo-red in the absence of a protecting colloid. In this latter case, they are large enough to fall as a precipitate. The actual values of the osmotic pressure observed, 200 mm. and 50 mm., show that, under the conditions of this experiment, aggregates of four molecules are formed. It appears that, so far as congo-red is concerned, the mode of action of a stable colloid is to form, under the influence of an electrolyte, a colloidal complex with the dye, which complex, although consisting of several molecules and therefore, when formed, causing a large fall in the osmotic pressure of the solution, is yet in particles sufficiently minute not to fall as a precipitate. The reason why the aggregates formed are small is, no doubt, connected with the lowering of surface-tension caused by the protein. It is to be noted that, unless the solution of dye+albumin +electrolyte had been compared with a similar solution without the electrolyte, it would have been supposed that complete protection from the action of the electrolyte had been brought about, since no true precipitation occurs.

Results similar to the above have been obtained in the case of arsenious sulphide, aggregation without precipitation also occurs here. In order that the albumin shall be efficient as a protecting colloid it is necessary that it be of the same sign as regards its electric charge as the arsenious sulphide, that is, electro-negative; if electro-positive, it tends to aid the action of the electrolyte.

I am unable, at present, to state definitely whether the same considerations apply to the action of electrolytes and stable colloids on gold hydrosols, the "gold number" in fact. I cannot make out any difference in the appearances under the ultra-microscope of mixtures of gold hydrosols and serum-albumin, with and without calcium sulphate. To the naked eye, there is perhaps a slight tendency to a more purple colour in the case of the former, but nothing approaching the blue of the mixture of gold and electrolyte in the absence of the albumin. The ultra-microscope shows also that the particles in the blue solution are larger and less numerous than in the ruby-coloured ones. It is possible that complexes of gold particles and protective colloid, of the kind described by Zsigmondy,* may be formed without change of colour of the gold. The whole series of phenomena in these cases of protection are of much complexity and in need of further investigation.

It is somewhat remarkable that congo-red is the only dye which I have been able to make use of which shows this combination of non-diffusibility through parchment-paper with existence of single molecules in solution. The greater number pass through parchment-paper with more or less rapidity, although showing many colloidal properties, so that no permanent osmotic pressure can be obtained. Aniline-blue, with a molecular weight of 734, does not pass through. A solution containing 0.266 per cent. gave an osmotic pressure of 30.4 mm. Hg instead of the theoretical one of 68.8 mm. Hg, indicating a mean "solution-aggregate" of two molecules. It must be stated, however, that this solution was apparently not free from electrolyte, since, although when the experiment ceased no further permanent rise in osmotic pressure was produced by change of water, there was still a certain amount of "initial osmosis." The displaced water, also, showed a fairly high conductivity, 40 gemmhos, which did not diminish during the last five days. This outer solution was always stained faintly blue, due, as I shall show in another paper, to dissociation of the dye and permeability of the membrane to the products of this dissociation. In a similar way, the dialysate of congo-red is very faintly red. In the ultra-microscope the appearance of the solution removed from the osmometer was very like that

* 'Zur Erkenntnis der Kolloide,' p. 116, Jena, 1905.

of congo-red, as described above; a faint haze, with bright particles here and there, these bright points being much more numerous than in congo-red. I am inclined, therefore, to attribute the low osmotic pressure found to incomplete removal of electrolytes, the solution really being one of single molecules, but, owing to the electrolytes, a large number of these are aggregated to particles, the rest being single.

The free acid of congo-red, as already stated, forms a deep blue colloidal solution, with comparatively large particles, easily resolved by the ultra-microscope. These particles are of a very nearly uniform size. In view of the debated question as to the source of osmotic pressure, it is of interest to see whether an unquestionable suspension, such as this is, has an appreciable osmotic pressure and, at the same time, to determine the number of particles present in unit volume. Since, as shown above, the salts of this acid have so considerable an osmotic pressure, while that of the particles of the acid would probably only be small, it is obviously of the greatest importance to ensure the absence of traces of alkali, whether in the water used or from glass vessels. The apparatus shown diagrammatically in fig. 2 was devised for this purpose. It will probably be found of use in the investigation of other colloids sensitive to traces of electrolytes, such as arsenious sulphide. All the tubes in contact with the fluids in the osmometer are of quartz and the containing vessel of glass is thickly coated with paraffin inside. The membrane is a Schleicher and Schüll parchment-paper thimble, as used by Waymouth Reid and by Hüfner. It is necessary to test these thimbles very carefully for holes and, for my purpose, to extract thoroughly before use with hydrochloric acid and distilled water to remove soluble salts. In future experiments it would perhaps be better to use membranes of collodion.

The distilled water must be carefully prepared. I found it best to distil tap water (New River) after adding potassium permanganate and sulphuric acid, and again after the addition of barium hydroxide. When essential to exclude carbon dioxide entirely, the water from the quartz condenser drops directly into the osmometer, as shown in the figure.

In an actual experiment with congo-red acid, the outer water was at first made acid with hydrochloric acid to ensure the absence of any salt of congo-red in the contents of the osmometer. The solution in the inside had been dialysed for a long time against distilled water. The concentration at the end of the experiment was 0.465 per cent. of the free acid. At the beginning of the experiment no measurable osmotic pressure was obtained; the acid was in the form of a precipitate, due to the presence of hydrochloric acid. Distilled water, from potassium permanganate and sulphuric acid, was then allowed to flow from the condenser, gradually displacing the hydrochloric

acid. It is interesting to note that the osmotic pressure rose distinctly, while the outflowing water still gave reactions with silver nitrate and with

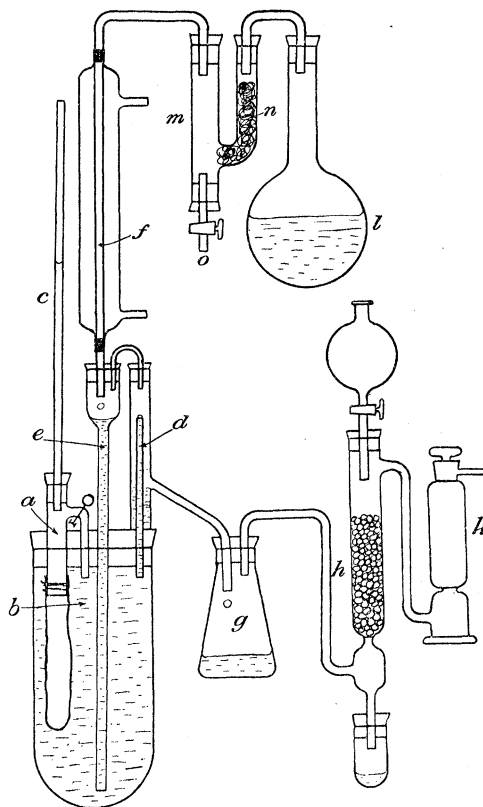


FIG. 2.

a, Tube on to which the parchment-paper thimble is tied. This tube has a side branch, connected by a short piece of rubber tubing to *b*, and thus, when the clip on the tubing is released, the inner and outer chambers are put in connection. This is of importance to control the zero point.

c, Narrow bore tube, serving as manometer. *d*, Outlet tube from outer chamber.

e, Tube conducting water from the quartz condenser, *f*.

g, Flask to collect the outflowing water, apart from contact with the atmosphere.

h, Tower containing dilute sulphuric acid. *k*, Soda-lime tower.

l, Flask for boiling water. *m*, Trap to collect spray. *n*, Glass wool.

o, Outlet for water condensed in *m*.

The containing vessel is thickly coated with paraffin and kept in a thermostat. The tubes *a*, *c*, *d*, *e*, and *f* are of quartz.

Günzberg's test, and, in fact, the pressure had risen to 60 mm. of water when 60 c.c. of the water neutralised 1.4 c.c. of decinormal ammonia. The distilled water, containing carbonic acid but no basic substance, was run in at intervals

of 24 hours until no further rise in the osmotic pressure took place. The value attained was 91 mm. of water, or 6.8 mm. Hg at 30°-9 C. There seems no possibility whatever of the presence of a salt of the dye in the above conditions. In any case, there was a fairly large pressure when free hydrochloric acid was detected in the outer fluid. A further reason for denying the presence of alkaline bodies in the water used is that, if this had been so, the pressure would have continued to rise as long as fresh water was run in, until finally the great pressure of the salts of congo-red would have been reached and the contents of the osmometer converted entirely into the salt.

The conclusion must be that a definite osmotic pressure can be exerted by a solution consisting of an undoubted suspension of particles, resolvable under the ultra-microscope. When water distilled from over barium hydroxide was run in, there was not much further rise in the osmotic pressure, the maximum being only a few millimetres higher. It appears, therefore, that this colloid is not particularly sensitive to traces of free acid.

If now we proceed to calculate, from the concentration of such a colloidal solution as that of the above experiment, what the osmotic pressure should be if the dye were present in single molecules, we find that it is 20 times that actually found. Assuming for the present that the kinetic theory of the osmotic pressure of colloids is correct, this means that the average number of molecules forming a colloidal particle of the free acid of congo-red is 20.

This being so, and the separate particles being easily seen in the ultra-microscope, it seemed to be a point of interest to attempt to estimate the dimensions of the particles in the manner described by Siedentopf and Zsigmondy.* For this purpose a part of the contents of the osmometer at the end of the experiment was diluted 1300 times, so that the particles might be sufficiently far apart to be counted. This counting was somewhat difficult, owing to the rapid movement of the particles. The mean of a number of determinations was between 8 and 9 in a volume of 56×10^{-6} cubic millimetre. The undiluted solution, therefore, contained 2×10^{11} in 1 c.c. There is, however, a possibility not to be lost sight of. As I shall show in a later paper, this acid of congo-red appears to be very slightly soluble in water at 100°, in true solution, ionised. Although the amount dissolved at room temperature is infinitesimal, it may be sufficient to vitiate conclusions drawn from solutions necessarily very dilute. Accordingly, I have made determinations, similar to the above, with solutions of one and a-half and of twice the dilution of that one. The three values are placed together in the table for comparison. It will be seen that the values are, within the limits of error, proportional to the dilution.

* 'Drude's Ann.,' vol. 10, pp. 17, 21, and 22, 1903.

Dilution (= number of litres containing 1 gramme).	Number of particles in 1 c.c.
280	14—16 × 10 ⁷
420	10·7—12·5 × 10 ⁷
560	8·9 × 10 ⁷

Now, the total weight present in 1 c.c. of the original solution is 4·65 milligrammes, so that the weight of each particle is

$$\frac{4\cdot65}{2 \times 10^{11}} = 2\cdot3 \times 10^{-11} \text{ milligramme.}$$

Further, the specific gravity of the solid acid is 1·46, determined by weighing under toluene in a pycnometer.* Hence, the diameter of each particle is 310 μμ.

We may, perhaps, go even further still. According to the osmotic pressure measurements and assuming the kinetic origin of this pressure, each particle contains on the average some 20 molecules; so that, if this theory be correct, we ought to be able to obtain an approximate value for the molecular dimensions of this body. When calculated from the data given, the weight of a molecule of congo-red acid comes out to be

$$1\cdot16 \times 10^{-12} \text{ milligramme,}$$

or nearly 10⁹ times that of hydrogen.† And the diameter

$$111 \mu\mu.$$

The molecular weight being 652·372, the number of molecules contained in 1 gramme-molecule comes out as

$$\frac{652\cdot372}{1\cdot16 \times 10^{-15}} = 5\cdot6 \times 10^{18}.$$

The number of molecules in a gramme-molecule of a perfect gas is usually estimated at about

$$6 \times 10^{23}.\ddagger$$

Considering the many sources of error, the result obtained for the molecular dimensions of our colloid does not seem very far out. This being so, the hypothesis of the kinetic origin of osmotic pressure is, so far, supported.

The chief difficulty in the estimation of the number of particles under the ultra-microscope is, in the case before us, the lively movements which they

* Ostwald-Luther, 'Phys.-Chem. Mess.,' 2te Aufl., p. 147, 1902.

† Walker, 'Introd. to Phys. Chem.,' 4th ed., p. 217.

‡ See Perrin, 'Comptes Rendus,' vol. 147, p. 531, 1908.

manifest. In order to stop this movement, I mixed a part of the diluted solution, as used for the previous measurements, with an equal volume of a 2-per-cent. solution of gelatin, warmed just sufficiently to liquefy it. This method was used by J. Duclaux,* in his investigations of ferric hydroxide. Although the gelatin used by me had been soaked in repeated changes of toluene-water, it retained a certain amount of its adsorbed electrolytes; so that on adding the blue colloid to it, a distinct change of colour towards purple resulted. Although the particles were seen to be immobilised, it did not seem worth while to proceed further with the laborious determinations, since the change of colour indicated a change in the colloid.

The determinations of molecular dimensions given above are intended to show the possibilities of the method. The exact numerical data are, no doubt, capable of correction when a more satisfactory means of immobilising the particles has been found. The values obtained appear high, even for a molecule containing 70 atoms, such as the one in question. From Zsigmondy's observations with colloidal gold it would seem that particles of these dimensions should be resolved by the ultra-microscope. It is true that the impression given to the observer is that the solutions of congo-red are just on the limit. Moreover, the fact that molecules of congo-red are unable to pass through parchment-paper shows that they far exceed in dimensions those of crystalloid bodies.†

Further experiments are in progress, as also others in the manner of those of Perrin‡ with suspensions of gamboge. It would be premature to draw conclusions from the results of the preceding pages as to whether the particles as wholes are responsible for the osmotic pressure, or whether only a part of each one, such as adsorbed ions, alone is active in this respect. So much may be said, that my observations speak decidedly in favour of the kinetic theory of the osmotic pressure of colloids. According to this theory the "Brownian movement" of the particles corresponds to the molecular movement assumed in the kinetic theory of gases.

Important recent confirmation of this view is to be found in the experiments of Perrin already alluded to, which show that the kinetic energy of a colloidal particle is identical with that of a molecule. This observer shows that, if we take the number of molecules contained in one gramme-molecule of a perfect gas to be

$$6 \text{ or } 7 \times 10^{23},$$

* 'Comptes Rendus,' vol. 147, pp. 131—138, 1908.

† As regards size of pores in parchment-paper, see Bechhold, 'Zeit. f. Phys. Chem.,' vol. 64, pp. 328—342, 1908.

‡ 'Comptes Rendus,' vol. 146, pp. 967—970, and vol. 147, pp. 530—532, 1908.

as given by the kinetic theory, the osmotic pressure of a solution containing n molecules per unit volume is

$$n \times 40 \times 10^{-15} \text{ atmos.}$$

When deduced from the rate of fall of the particles in a gamboge suspension, assuming Stokes' formula to apply, and taking n to refer to particles, the osmotic pressure works out to be

$$n \times 36 \times 10^{-15} \text{ atmos.}$$

When deduced from the distribution of particles in a vertical column, after attainment of equilibrium, the formula becomes

$$n \times 42 \times 10^{-15} \text{ atmos.}$$

From my observations, determining the concentration of particles by direct enumeration under the ultra-microscope, the formula becomes

$$n \times 44 \times 10^{-15} \text{ atmos.}$$

Such close approximations to the theoretical value must be more than mere coincidence.

Ramsay and Senter* also concluded, from experiments on the density of arsenious sulphide solution taken by different methods, that the particles behave as if in true solution.

On the other hand, it is evident that my experiments lend no support to the theory according to which the osmotic pressure of a colloidal solution is due, in some way not very clear, to ions associated with the colloidal particles. It is difficult to understand how these ions can still exert their osmotic pressure when forming part of a complex system, which must move and act as a whole. This much may be said, congo-red gives an osmotic pressure which is at its highest when foreign electrolytes are most effectively excluded. This must be understood as in no way excluding, as the ultimate source of the negative charge, electrolytic dissociation of the colloid itself.

It is very doubtful whether electrolytes in the state of adsorption are ionised at all. Ruer† finds that the chlorine present in colloidal zirconium hydroxide gives no reaction with silver nitrate. Similarly in the case of ferric hydroxide, the chlorine can only be detected after destruction of the colloid by nitric acid.

The general conclusion to be drawn is, I think, that whether a body present in solution be in the form of particles, molecules or ions, each of

* B. A. Reports, 1901. In 1905 ('Journ. Phys. Chem.,' vol. 9, p. 319) Senter also made the suggestion that Brownian movement in colloids is equivalent to molecular movement in true solutions.

† 'Zeits. f. Anor. Chem.,' vol. 43, pp. 83—93, 1905.

these acts as an individual and equivalent element in the production of osmotic pressure.

Summary.

Congo-red, although a colloid in the sense of not being diffusible through parchment-paper and exhibiting certain other colloidal properties, has an osmotic pressure equal to that which would be given if it were present in true solution in single molecules.

The solutions are not resolvable into particles by the ultra-microscope.

The theoretical osmotic pressure is only to be obtained in the complete absence of extraneous electrolytes. Even the carbonic acid present in ordinary distilled water is sufficient to cause a marked fall in the osmotic pressure.

The manner in which electrolytes produce this fall is by causing aggregation of molecules to particles. This is the same whether acid, alkali, or neutral salt be in question.

The action of a stable colloid in protecting against the effect of electrolytes is shown to consist, in the cases of congo-red and arsenious sulphide, in the production of minute aggregates, which, although causing fall in osmotic pressure by diminution of effective concentration, are not of sufficient size to precipitate. Hence the protective power can only be regarded as a limited one, due probably to the formation of complex colloids.

The free acid of congo-red forms a blue colloidal solution when dialysed. This is easily resolvable under the ultra-microscope, but gives a definite and measurable, though small, osmotic pressure, about 14 mm. Hg for a 1-per-cent. solution. Assuming the kinetic theory to be correct, this means that the aggregates contain, on an average, 20 molecules.

Estimation of molecular dimensions are given on the basis of enumeration of the number of particles in unit volume by means of the ultra-microscope. The values found are considerably larger than the accepted ones for water, etc.

The whole of the results are capable of explanation on the assumption that colloidal particles possess the kinetic energy of molecules, but do not lend support to any view which postulates the necessary presence of foreign electrolytes.

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