

down and rendered permeable by oxidation; they may be repaired subsequently, when assimilatory processes become ascendant.

We are extending our observations to animal tissues.

We have to thank Mr. Mummery for the assistance he has rendered to us in carrying out a number of the experiments.

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*The Properties of Colloidal Systems. III.—The Osmotic Pressure of Electrolytically Dissociated Colloids.*

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In a previous paper\* I showed that the osmotic pressure of solutions of Congo red, as measured directly in an osmometer with a membrane of parchment-paper, is about 90—95 per cent. of that which they should have if the dye were present as undissociated single molecules, such as those of glucose or urea. Attention was chiefly directed, in the paper referred to, to the fact that a body behaving as a colloid gives as high an osmotic pressure as if it existed in solution as single molecules and not as aggregates. It is to be remembered, however, that Congo red is the sodium salt of a fairly strong acid and as such must be dissociated to a considerable degree in solutions of the concentration employed. On this account, the interpretation of the experimental results required further work. Subsequent investigations have shown that there are many difficulties in the way of a satisfactory explanation.

\* 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 269.

As will be seen later, the close correspondence between the osmotic pressure found and that of the dye if undissociated must be due to the chemical nature of this particular dye as a disodium salt of a dibasic acid. Other dyes of a similar constitution, but of different sodium content, such as Chicago blue, do not show this property. I regard it as a somewhat unfortunate accident that Congo red was chosen as the object of the first investigation. Attention was thereby diverted from the more essential facts.

As it will frequently be necessary to refer to the osmotic pressure as it would be shown by a body present in solution in undissociated single molecules, I propose, for convenience, to speak of it as the "molecular" osmotic pressure, although of course the expression is not strictly correct.

#### *Hydrolytic Dissociation.*

It is fortunate, as an initial simplification of the problem, that no trace of hydrolytic dissociation can be detected in solutions of Congo red. When such solutions are separated from water by parchment-paper, no free alkali diffuses out, such as happens, for example, from solutions of sodium oleate. In this result I find myself in agreement with other observers.\* The acid of Congo red, in fact, behaves as a strong one, no doubt owing to the two sulphonic acid groups contained in its molecule. Its solutions attack metallic zinc. In its use as an indicator it is well known that the second sodium atom can only be displaced by strong mineral acids. It is evident that the  $\text{NH}_2$  groups of the naphthylamine residues are practically neutralised by the sulphonic acids. The basic properties of this amino-acid are so weak as to be negligible. I have been unable, indeed, to find any evidence that it forms salts even with hydrochloric acid.

It is interesting to note incidentally that even sodium caseinogenate appears to be hydrolytically dissociated only to a minute degree. Roaf† makes the same statement with respect to the sodium salts of the serum proteins. Hardy‡ also found the hydrolysis of the sodium salt of globulin to be very slight.

#### *Electrolytic Dissociation.*

Although, since my former experiments were made, measurements of the conductivity of Congo-red solutions have been published,§ I thought it best to determine that of the particular sample of dye used for the experiments

\* Pelet and Wild, 'Kolloid. Zeits.,' 1908, vol. 3, p. 174.

† 'Quart. Journ. Exp. Phys.,' 1910, vol. 3, p. 175.

‡ 'Journ. Physiol.,' 1905, vol. 33, p. 276.

§ 'Die Theorie d. Färbeprozesses,' von L. Pelet-Jolivet. Dresden, 1910, p. 27.

to be described below. The dye was Kahlbaum's best preparation, but was found to contain appreciable quantities of sodium chloride and a small amount of sodium sulphate. It was therefore purified by "recrystallising" from dilute alcohol. The hot saturated solution on cooling deposited a considerable part of its contents. Although this deposit did not seem to be actually crystalline, it was possible to purify the dye in this way, naturally with considerable loss. After repetition of the process for five times, the conductivity of solutions of equal concentration from two successive treatments became identical, so that no foreign electrolyte was present. Several determinations of conductivity in successive dilutions were made and a curve made for future use in order to obtain the concentration corresponding to a known conductivity.

The following numbers (Table I) will serve as an example of an experiment:—

Table I.

Dilution in litres.	Concentration in millimols. per litre.	Specific conductivity in recip. ohms $\times 10^6$ .	Molar conductivity in recip. ohms $\times 10^4$ .	Dissociation, per cent.
14	71.4	6772	950	45.6
28	35.7	3782	1060	51
56	17.86	2086	1166	56
112	8.93	1154	1293	62
224	4.46	662	1482	71.2
448	2.23	367	1645	79
896	1.12	201	1800	86.5
4480	0.223	47	2083	100

The determinations were made at 25° C., the same temperature at which the osmotic pressure measurements were made. The dilutions were made in quantities of 40 c.c. in a series of flasks, as it was found that the usual method of removing solution from the conductivity vessel and replacing with water was apt to lead to inaccuracy with the more concentrated solutions, owing to their viscosity. Further dilution beyond 4480 litres gave only slightly increased values of the molar conductivity, and from curves it appeared that the limiting value at infinite dilution would be  $2100 \times 10^{-4}$  recip. ohms, which was accordingly taken as the basis of calculation for the degree of dissociation of the various dilutions.

It will be noticed that, although the acid of Congo red is stronger than acetic acid, the degree of ionisation in the more concentrated solutions is less than that of sodium acetate. For example, in a dilution of 32 litres sodium acetate is dissociated to the extent of 86 per cent., whereas Congo red at the

same dilution is only dissociated to 52 per cent. This is probably due to colloidal association of molecules in the latter case. If so, it presents an interesting case for investigation, since ionisation would be some function of the surface of the aggregates.

Owing to the fact that this dye is the disodium salt of a conjugated disulphonic acid, it is of interest to compare its conductivity with that of disodium sulphate. The molar conductivity of this latter at infinite dilution, under the same conditions as Congo red above, was found to be  $2600 \times 10^{-4}$  recip. ohms. Sodium chloride, with a corresponding value of  $1200 \times 10^{-4}$ , and sodium naphthylamine-sulphonate with that of  $1150 \times 10^{-4}$ , may be compared.

From the values given it will be seen that in moderately dilute solutions, such as those whose osmotic pressure was given in my previous paper, Congo red may be considered to be a strong electrolyte. The migration velocity of the anion, although it has a molecular weight of 650, must be not much less than that of  $\text{SO}_4'$ . Biltz and v. Vegesack\* calculate this migration rate as 57 in the same units in which  $\text{SO}_4'$  is 67.

Congo red does not obey the Ostwald dilution law, the values of the "constant" showing steady and considerable diminution with increasing dilution.

It was found impossible to obtain at 25° C. solutions more concentrated than 1 molecule in 14 litres, or 4.975 per cent. At this dilution the salt was dissociated to the extent of 45 per cent., whereas sodium sulphate is dissociated to 74 per cent.

#### *The Factors of the Osmotic Pressure.*

The experimental facts which have to be accounted for may be realised by taking the case of Congo red at a dilution of 120 litres. I find that the osmotic pressure of such a solution is slightly lower than what it would be if the salt were undissociated. But measurements of the electrical conductivity show that it is dissociated to the extent of at least 60 per cent. at this dilution, so that, if the dissociation is into two  $\text{Na}'$  and one bivalent anion, the concentration of osmotically active elements within the membrane is  $m/66$  in ions and  $m/300$  in non-dissociated salt, or  $m/54$  in all.

A simple explanation of the discrepancy has been advocated by Biltz and v. Vegesack,† viz., that the  $\text{Na}'$  ions to which the membrane is permeable do not take any part in the production of osmotic pressure; so that, since only

\* 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 481.

† 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 490.

one anion is formed and the membrane is impermeable to it, the undissociated and dissociated fractions of the salt are equivalent in osmotic activity. On this view it is therefore immaterial whether the salt is or is not dissociated.

Unfortunately there are many reasons, both theoretical and experimental, which show that such an explanation is inadmissible.

It was pointed out by Laqueur and Sackur\* that the Na<sup>+</sup> ion in the case of the sodium salt of caseinogen is kept within the membrane of a dialyser by electrostatic forces alone. A similar state of affairs exists in the case of Congo red. The Na<sup>+</sup> ions tend to pass through the membrane in obedience to osmotic force. But they cannot travel beyond the distance at which the electrostatic attraction of the opposite ions, which are unable to pass the membrane, is equal and opposite to the osmotic pressure of the Na<sup>+</sup> ions. The osmotic pressure produced by the non-diffusible elements, the anions and the non-dissociated salt, shows itself in virtue of the mechanical constraint exerted by the membrane, which allows water to pass freely while holding back the bodies in question. In a similar way, the Na<sup>+</sup> ions are prevented from escaping owing to the constraint of the membrane on the opposite ions, so that the membrane must have to bear the pressure of both ions.† To put it in another way, the pull of the anions on the cations could not be effective unless the constraint of the membrane gave the former some *point d'appui*, so to speak.

We see, then, that what, at first sight, seems to be a reasonable view, viz. that the fact that the electrostatic forces balance the osmotic pressure of the diffusible ions prevents the manifestation of this pressure in an osmometer, will not stand examination.

Experimental evidence of various kinds, moreover, shows that all ions within the membrane are osmotically active. If direct measurements of osmotic pressure with membranes of parchment-paper did not give the full osmotic pressure of the whole of the bodies in solution inside the membrane, there would be a large discrepancy between values obtained in this way and those obtained in other ways—by vapour-pressure determinations, for example. The simple method of Barger‡ is sufficiently accurate for the present purpose. I found indeed, no difficulty in distinguishing between dilutions of cane-sugar of 45 and 50 litres. Two experiments were made with Congo red in dilutions of 30 and 50 litres. In the first case, it was found that the dye solution had a vapour pressure very slightly higher than

\* 'Beitr. z. chem. Phys. und Path. Hofmeister,' 1903, vol. 3, p. 203.

† See the forthcoming monograph on 'Colloids,' by W. B. Hardy, for a more detailed discussion of this question.

‡ 'Trans. Chem. Soc.,' 1904, vol. 86, p. 286.

that of saccharose in a dilution of 30 litres. The drops of dye solution at first took up a trace of water from the sugar solution and were then in equilibrium with it. Estimating from the change in dimensions of the drops, the dye solution had the same osmotic pressure as that of saccharose in a dilution of 29.4 litres. In the second experiment the drops of dye solution increased slightly in size when alternating with drops of saccharose at 50 litres dilution, but decreased when sugar at 45 litres dilution was substituted. The decrease in the latter case was greater than the increase in the former, so that we may take the actual osmotic pressure, or rather vapour pressure, of the dye solution to have been the same as that of sugar at a dilution of 48 litres. The results are sufficient to show that the direct measurements give correctly the total osmotic concentration. A very minute impurity, if of small molecular weight, would be capable of accounting for the very slightly higher vapour pressure of the dye solution than would be expected from the osmometer values; this, being diffusible, would not be shown by the direct method of estimation.

If the  $\text{Na}^+$  ions were inactive osmotically, it is impossible that pressures higher than what I have called "molecular" could ever be obtained, even in solutions of great dilution. Now Biltz and v. Vegesack\* themselves have obtained such. I have myself in dilutions of about 1000 litres seen on one or two occasions pressures of 101—102 per cent. of the "molecular"; but, since the actual values did not exceed 21 mm. Hg, I do not feel justified in drawing conclusions from them. On the other hand, with Chicago blue and with sodium caseinogenate pressures are always obtained higher than could be accounted for on the theory of Biltz.

Chicago blue is the tetrasodium salt of a substituted dinaphthylamine-tetrasulphonic acid.† If normally dissociated, it should give four sodium ions and one quadrivalent anion. If the former were inactive, the osmotic pressure of this dye should be the same as that of Congo red. In point of fact, a dilution of 353 litres gave a value of 93 mm. Hg or practically *double* the "molecular" osmotic pressure. It was the same, however, as that of a Congo-red solution of the same electrical conductivity. Another experiment, with a dilution of 1003 litres, gave an osmotic pressure of 35 mm. Hg, again double the "molecular" pressure.

The sodium salt of caseinogen, containing sufficient base to be faintly alkaline to phenolphthalein, gave an osmotic pressure of 313 mm. Hg for a solution of 3.23 per cent. If the anion alone were active, the maximum:

\* 'Zeit. f. physik. Chem.,' 1910, vol. 73, p. 490.

† I am indebted to the kindness of the Berlin Aniline Company for a supply of this dye.

pressure could only be 155 mm. Hg, if it were quadrivalent only, and it may be higher in valency.\*

On the other hand, benzo-purpurin, which is of similar constitution to Congo red, but with tolidine in place of benzidine, gave me values of osmotic pressure only about 0.87 of the "molecular." The preparation used was that supplied by Kahlbaum, "recrystallised" by me five times. An analysis of its sodium content showed that it only contained 0.81 of what it should have contained if it had been the neutral (disodium) salt. It was probably a mixture of the mono- and di-sodium salts or of free acid and neutral salt.† Its osmotic pressure corresponded to that of a Congo-red solution of the same conductivity, as would be expected if the acid salt were in large colloidal aggregates and osmotically inactive, as was no doubt the case.

These sodium salts of non-diffusible organic acids appear to give osmotic pressures related to their sodium-content, although less than would be expected if they were dissociated normally.‡

\* See Laqueur and Sackur, 'Hofmeister's Beiträge z. Chem. Phys. und Path.,' 1903, vol. 3, p. 199. The fact that its osmotic pressure is double the "molecular," similar to that of Chicago blue, suggests that it is tetrabasic, like the acid of the dye.

† An interesting fact worth mention is that, if to a solution of Congo red there be added half that amount of a strong acid which is necessary for complete decomposition of the salt, instead of the acid salt being formed, as in the case of sodium sulphate, half the salt is decomposed with production of the free acid, while the other half remains in its original state of disodium salt. The solution becomes at first turbid and of a purple-brown colour. On standing, the blue free acid is deposited, leaving a clear solution of the bright red colour of the neutral salt. This behaviour appears to be due to the fact that the free acid is insoluble in water. If the whole be evaporated to dryness, the deposit extracted with methyl alcohol, in which the free acid is slightly soluble, the extract filtered, the filtrate evaporated again to dryness and taken up in water, a permanent colloidal solution of the acid salt is formed, turbid and of a purple-brown colour. It is difficult to say, however, whether this is a true salt or a colloidal complex, since it has to be remembered that it is in very dilute solution and contains no foreign electrolyte, facts which favour its stability.

As regards the free acid itself, it has been stated that the sodium salt may possibly not be decomposed even by strong acids, owing to the presence of the two sulphonic acid residues. I have recently tested this by precipitating the salt, by addition of sulphuric acid, and determining the sodium in the two phases. It was found to be entirely in the fluid phase as sulphate. The precipitate was merely the free acid, containing no salt.

‡ I have made one or two experiments recently with a dye known as "Primulin," which may be shortly described as the monosodium salt of a poly-thiazol-sulphanilic acid. It has a molecular weight of 608.5, and passes through parchment paper with extreme slowness. Its osmotic pressure appears to be between the "molecular" and half this value. In a dilution of 168 litres the pressure observed was 84 mm. Hg, the "molecular" being 118 mm. The sodium content of the preparation was, however, greater than corresponded to that of the salt of a monobasic acid. Its conductivity was that of a Congo-red solution giving an osmotic pressure of 140 mm. Hg. Further experiments with this dye are in progress.

Notwithstanding these facts, it is undoubtedly remarkable that the osmotic pressure of Congo-red solutions should correspond so closely to the "molecular" through a very wide range of concentration. Table II gives a number of experimental values corresponding to particular concentrations. In order to obtain those of the higher concentrations, it was found necessary to determine the difference between one of lower concentration outside the membrane and one of higher concentration within it. The osmotic pressure of the former was known from previous measurements.

Table II.

Conductivity in recip. ohms $\times 10^6$ .			Osmotic pressure in mm. Hg.
Inside.	Outside.	Difference.	
6220	5070	1150	229
5545	5060	485	134.7
5033	4335	698	176
4360	3130	1230	223
3440	2470	970	172
2498	1762	736	122
1762	135	1627	245
1670	32	1638	263.5
1185	20	1165	178
705	18	687	108
409	22	387	33 (?)
406.5	18.75	387.75	19.5
319	146.4	172.6	27.5
277.5	21.5	256	27
205	20	185	21.3

From these numbers a curve was constructed from which the osmotic pressure corresponding to any given conductivity could be obtained. From the previous conductivity measurements of solutions of known dilution, the dilutions corresponding to any given conductivity were known.

In Table III a series of such values are recorded.

Table III.

Conductivity in recip. ohms $\times 10^6$ .	Dilution in litres.	Osmotic pressure in mm. Hg.	"Molecular" osmotic pressure.	Percentage of "molecular" found.
6772	14	1220	1328	90
5506	18	1020	1032	98
3782	28	625	664	94.5
2086	56	326	332	98
1154	112	176	166	106
662	224	106	83	127
367	448	45	41.5	108
201	896	24	20.8	114

All at 25° C.

In fig. 1 a curve is given correlating conductivity in recip. ohms  $\times 10^6$  as abscissæ with osmotic pressure in mm. Hg as ordinates and in fig. 2 a curve with concentration in millimols. per litre as abscissæ and osmotic pressure in mm. Hg as ordinates.

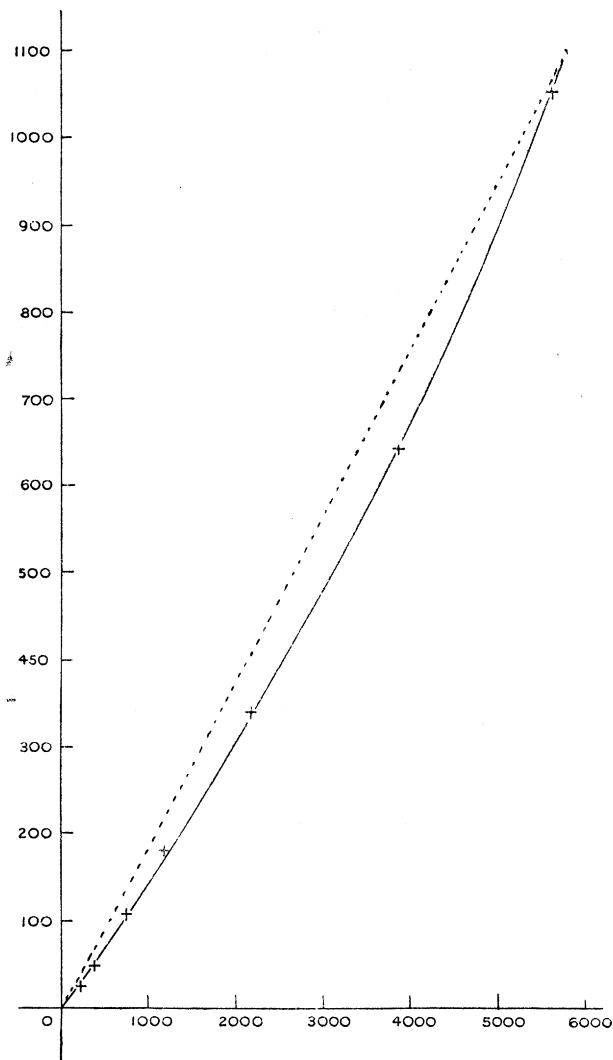


FIG. 1.

It will be noticed that the curve of fig. 1 is slightly convex to the axis of abscissæ, whereas that of fig. 2 is practically a straight line, although some of the points marked are somewhat divergent, as would not be unexpected in measurements of so much difficulty as direct determinations of osmotic pressure.

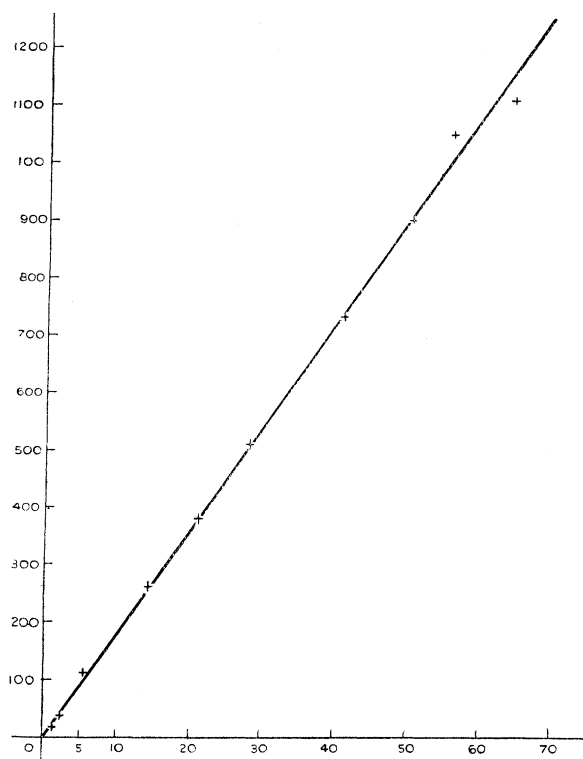


FIG. 2.

The apparent tendency of the numbers in the last column of Table III towards a maximum at 224 litres dilution is merely accidental, since other experiments do not show the phenomenon. Table IV gives some additional values.

Table IV.

Dilution in litres.	Osmotic pressure obtained.	"Molecular" osmotic pressure.	Percentage of "molecular" found.
15.5	1136	1200	94.8
17.8	1040	1044	99
19.55	902	950	95
24.2	726	768	94.7
35.55	508	523	97
47.2	386	394	98
73.5	253	251	101
110	178	169	105
203	107	92	111
445	40	42	96

The results of an application of v. d. Waals' formula to the present case have been worked out by Mr. Hardy, who informs me that, if such a formula

applies, the osmotic pressure should be a linear function of the conductivity. Fig. 1 shows that this is not the case; the departure, however, although unquestionable, is not very great. The explanation may perhaps lie in the fact that the part played by the non-dissociated molecules in the production of osmotic pressure, and which would be relatively greater in the more concentrated solutions, is not taken into account. That these molecules do take part is shown by the fact that the increase of osmotic pressure for a given rise in conductivity shows a steady increase from the more dilute solutions upwards. For example, at 500 litres dilution, an increase of 50 recip. megohms in conductivity is associated with a rise in osmotic pressure of about 5.5 mm. Hg. At 20 litres dilution the same increase in conductivity gives a rise in osmotic pressure of 14 mm. Hg and there is a fairly regular transition from one to the other. At higher concentrations the rise per 50 recip. megohms falls to 10 again, but obvious precipitation commences to appear. The curve representing these ratios has an S-shape and is given in fig. 3, since it may turn out to be of some significance.

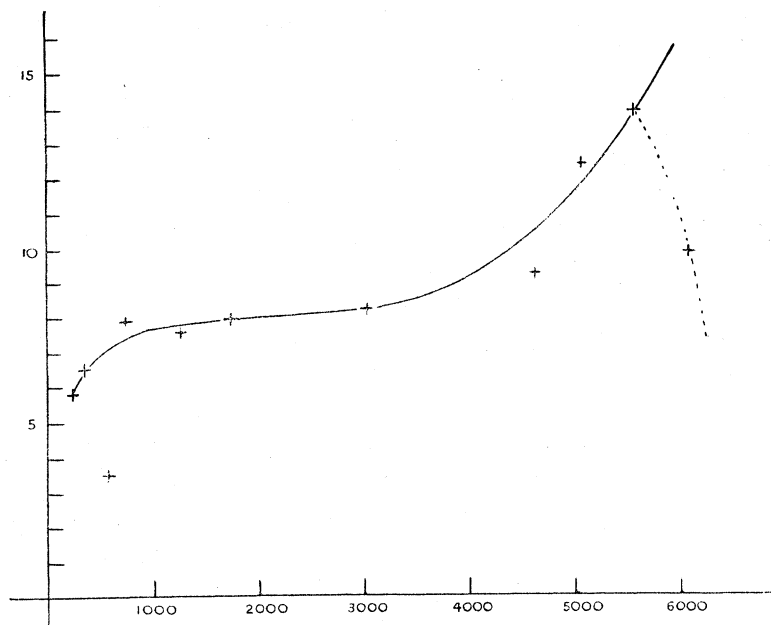


FIG. 3.

*Ordinates.*—Rise of osmotic pressure in mm. Hg for each 50 recip. megohms in conductivity.

*Abscissæ.*—Dilution in litres.

It may perhaps be possible ultimately to arrive at some knowledge of the relative parts played by the dissociated and non-dissociated molecules

on the lines of these facts; the data at present available seem scarcely sufficient.

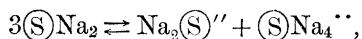
Why the osmotic pressure is a linear function of the molar concentration, although the dye is dissociated and all the ions are osmotically active, it is impossible to explain. It can only be suggested that the dissociation is not normal, but that complex ions are formed. Whether these are of the nature of chemical compounds, like the ferrocyanic ion, or whether they are associations similar to those of colloidal solutions, requires further investigation to decide.

*Electrolysis of Congo Red.*

It was hoped that some light might be thrown on the nature of the ionisation of the dye by electrolysis of its solutions.

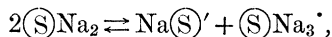
When the experiment is made in a U-tube containing the dye solution at the bend and above it, in both limbs, distilled water, and a current passed through platinum electrodes immersed in the water, the phenomena are complex and difficult to interpret. In the cathodic limb the boundary surface remains sharp, but a second sharp meniscus travels slowly towards the anode, so that behind it on the cathodic side the solution is pale red and in front of it deep red. The water on this side remains colourless. In the anodic limb there is also a sharp meniscus separating a lower deep red solution from a paler one above, but there is no definite boundary between this pale solution and the water above it. From the upper part of this indefinite boundary pale pink streamers pass through the water to the anode. When they reach this they become blue. I am inclined to regard these streamers as dissociated anions; when discharged at the anode the blue insoluble free acid is formed. I am unable to express an opinion as to whether the existence of a pale red instead of a colourless solution area travelling from the cathode means that  $\text{Na}^+$  ions were not present.

Other experiments were made by arranging three flasks in series, connected by glass syphon tubes and provided with platinum electrodes in the two end flasks. A current of about 2 milliampères was passed for five days, by which time it was calculated that sufficient current had passed to decompose the whole of the salt present. The amounts of dye-acid and of sodium were then estimated in the anodic and cathodic flasks, the sodium by the usual method of conversion to sulphate and the former by subtracting the sodium values from the total solids. It was found that the proportion of  $\text{Na}^+$  to anion in the positive flask was 1 to 1.9, and in the negative flask 4 to 1. If the proportion in the former had been 1 to 1, the dissociation equation might be

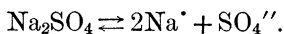


where  $\textcircled{\text{S}}$  stands for the non-diffusible anion. It is probable that there is an uncertainty about the concentration of the dye-acid in the positive flask owing to precipitation.

But this equation, even if otherwise acceptable, goes too far, since the osmotic pressure of the dissociated salt would be *less* than that of the non-dissociated. If it were



the experimental facts would be satisfied as far as the osmotic pressure is concerned. There is difficulty, however, with the electrical conductivity. We have seen that the molar conductivity at infinite dilution is practically the same as that of sodium sulphate, if we allow for the slower migration rate of the large organic anion. Now one dissociated molecule of this salt carries four times as many charges as one of Congo red, according to the last equation, if sodium sulphate dissociates thus:—



Moreover, if we suppose that at infinite dilution Congo red is completely and normally dissociated, an osmotic pressure considerably exceeding the “molecular” as dilution increases would be given. This is so only to a very small degree, if at all, as we have seen. On the other hand, if the dissociation were of the type suggested, with complex ions, it seems impossible to explain the facts illustrated by the following case: at 28 litres dilution the molar conductivity of a Congo-red solution is half that at infinite dilution, whereas, on the supposition of complex ions, it would have only one-quarter the conductivity which it has at infinite dilution, even if it were completely dissociated at the higher concentration, a very unlikely assumption.

If, however, it were allowable to assume that the complex ions could retain the combined charges of their components, thus:—



the matter could be explained.

A similar difficulty was met with by Brailsford Robertson\* in his investigation on the conductivity of the potassium salt of caseinogen. Under certain conditions the addition of potassium chloride to such salts causes no diminution of ionisation. The conclusion drawn is that potassium caseinogenate does not form  $\text{K}'$  ions. But, at the same time, it is pointed out that, in order to explain other experimental facts, it must be supposed that the complex ions are able to transport an increased number of atomic charges.

\* ‘Journ. Phys. Chem.,’ 1910, vol. 14, pp. 556 and 557.

There is one more hypothesis which might be made on the ground of the colloidal behaviour of the anions in the case of Congo red. These large organic ions may aggregate while retaining the combined charges of their components. This seems to imply, however, so great a density of the charge on the surface of the aggregates as to be improbable.\* It could not, in any case, apply to the  $\text{Na}^+$  ions, which cannot be assumed to form aggregates of this kind. If, however, we suppose that the non-dissociated molecules, as well as the anions, are so far aggregated as to give an osmotic pressure too small to be detected by a mercury manometer, so that the  $\text{Na}^+$  ions alone are osmotically active, the osmotic pressure at a dilution such that the dissociation is 50 per cent. would be accounted for, but not when the dilution is greater than this, while the difficulty as to the large charge on the aggregated anions remains. The total charge of the anions must, of course, be equal and opposite to that on the cations.

The following experiment gives evidence, so far as it goes, against the formation of complex ions. When solutions of Congo red are exposed to electrolysis in a parchment-paper cup so that the anode is inside and the cathode outside, cations are driven out, as we have already seen. These ions are not coloured, as seems would be the case if they contained acid components. The outer fluid becomes strongly alkaline but remains colourless. It may be, perhaps, that under the action of a current the naturally present complex ions are caused to dissociate further, although the natural conclusion would be that the  $\text{Na}^+$  ions were formed in the solution already.† It is of interest to note that, in this experiment, when the current is reversed, the escaped cations are driven back and that, after this has taken place, the current practically ceases to pass, owing to the inability of the anion to pass through the membrane. The apparatus is, in fact, a kind of rectifier.

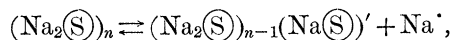
On the whole it is evident that there is something abnormal in the form of the dissociation of salts, one of whose ions is colloidal. It may tentatively be suggested that aggregation of molecules may play some part in the mode of ionisation, so that this may be a function of the surface of the aggregates.

The fact that the observed values of the osmotic pressure are less than

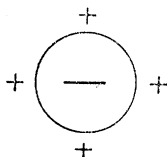
\* It is pointed out to me by Mr. Hardy that the abnormally high velocity of the negative ion shows, either that resistance to movement decreases with increase of volume, or that the charge is increased. It might be argued that, as the normal velocity would be about  $28 \times 10^{-5}$  cm. per second, the charge is  $57/28$  (*vide ante*), or twice as great as that carried by an ordinary ion.

† This experiment cannot, perhaps, be regarded as offering very strong evidence against the presence of complex cations, since, if only a small fraction of the dye were dissociated with formation of  $\text{Na}^+$  ions, these would pass through the membrane, and their place inside be filled by fresh dissociation, according to the law of mass action.

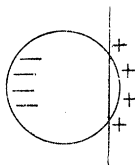
the "molecular" may be due to the presence of complex positive ions which are unable to pass the membrane. Thus, the undissociated molecules having only a low solubility, if complexes are formed, an aggregate consisting of an internal phase of particles, which ionise at the surface, *e.g.* :—



might be produced owing to the solubility of the undissociated molecules being exceeded. In this way a typical condenser system is formed :—



At the membrane-face these particles would, by osmotic forces, be orientated thus :—



and so contribute to the electromotive force discussed in the following section.

Although Congo red is aggregated by foreign electrolytes there is some uncertainty as to whether there is any such association of its molecules in pure solutions. I stated in my previous paper on the osmotic pressure of Congo red that the ultra-microscope is unable to resolve such solutions. The path of the beam of light merely appears hazy. This haze may be the expression of aggregates beyond the limits of resolving power of the instrument used or it may be due to the individual molecules, or to anions, which must be of very considerable dimensions.

#### *The Electromotive Force at the Membrane.*

If, in the dissociation of Congo-red solutions, complex or associated ions are produced, both anion and cation containing the organic acid grouping, it seems to follow that the membrane would be impermeable to both ions, so that no complication would arise as to the part played by the diffusible ion, since there is none.

But, whatever may be the precise nature of the ions in question, the following facts show that the cation is in point of fact diffusible and kept within the membrane by electrostatic constraint alone.

Lacqueur and Sackur pointed out\* that, if the  $\text{Na}^+$  ions in the case of

\* 'Beitr. chem. Physiol. und Pathol. Hofmeister,' 1903, vol. 3, p. 203.

sodium salts of caseinogen were kept within the membrane of an osmometer by electrostatic forces, this membrane itself would be the seat of a considerable potential difference.

Hardy\* has investigated the question mathematically from different points of view, (1) by consideration of the force acting upon the diffusible ions due to the potential difference between the two oppositely charged sides of the Helmholtz double layer as being equal and opposite to the osmotic pressure of these ions, and (2) by consideration of the work done in moving electricity from the lower potential to the higher. This latter is the method used by Nernst in the calculation of metallic electrode potentials and is based on Helmholtz' theory of contact potential. The same equation is arrived at by both methods :

$$E = RT/q \cdot \log \text{nat } c_2/c_1,$$

where  $E$  is the potential difference between the two sides of the membrane,  $R$  the gas constant,  $T$  absolute temperature,  $q$  the charge on 1 gram. equivalent of the ion concerned,  $c_2$  the concentration of this ion inside the membrane, and  $c_1$  its concentration outside the membrane. Expressed in volts  $RT/q = 0.0247$ .

This equation, as will be noticed at once, has a striking similarity to the well-known one of Nernst expressing the potential at a metallic electrode, and may in a sense be regarded as an illustration of the way such a potential is produced. In any case it shows how a permanent electromotive force is produced by means of a membrane permeable to one ion only. It is quite different from that concerning the electromotive force of contact of two solutions of different concentration. Here the different rates of migration of the anions and cations are the cause of the potential difference, which only lasts until diffusion has put an end to concentration differences.

In attempting to apply this equation to actual experiment, I was struck by a result which follows from the above equation as also from that of Nernst on concentration batteries, but which seems to have escaped the notice of writers on the subject. If the outer fluid in the osmometer is distilled water,  $c_1$ , the denominator of the fraction, becomes zero, and the potential difference therefore infinite. The same applies to the concentration battery when the one solution is water. If the original paper of Nernst† be consulted, it will be found that this fact had not escaped his notice. He also points out that, theoretically, any diffusion into a vacuum should take place with infinite velocity; in ordinary cases this would only last for an infinitesimally short time. In actual practice, moreover, there is never an

\* Forthcoming monograph on "Colloids."

† 'Zeits. f. physik. Chem.,' 1889, vol. 4, p. 139.

actual "vacuum" for electrolytic diffusion, since water itself is ionised and always contains impurities. For this reason, measurements are not reliable when  $c_1$  is less than  $m/1000$ .

The side of the membrane in contact with the more dilute solution will be charged positively to the inner side, owing to the movement of the diffusible positive ion under osmotic forces in this direction until these forces are counterbalanced by electrostatic attraction of the negative ion.

In the experimental testing of the equation for the potential difference between the two sides of the membrane I have met with some difficulty. The first experiments were made with an osmometer of the pattern described by Roaf,\* constructed of glass and ebonite only. A T-tube in the course of that leading to the manometer was connected with a tube containing Congo-red solution of the higher concentration, as contained in the osmometer, but made stiff with gelatin in order to withstand the pressure. In later experiments, it was found unnecessary to use the gelatin. The osmometer was immersed in the more dilute solution, from which a syphon tube dipped into Congo-red solution of the same concentration as that within the osmometer. Into this solution the tube of an Ostwald calomel electrode was immersed, while a second similar electrode was in connection with the content of the osmometer, with or without the gelatin protection. The system was, therefore, symmetrical, with the exception of the presence of the parchment-paper membrane between the two solutions of the dye on the one side. The electromotive force was measured by compensation with a potentiometer in the usual way, using a capillary electrometer as indicator.

It was found that the sign of the potential difference was as deduced from theoretical considerations, viz., the side of the membrane in contact with the more dilute solution was positive to the other side, or, as the stronger solution was always inside the osmometer, the outside was positive to the inside. But values corresponding to those calculated from the equation given above were obtained, as a rule, only immediately after the apparatus was put together. In one such case, solutions of 30 and 80 litres dilution gave an electromotive force of 0.025 volt against the calculated one of 0.022 volt. This value soon fell to 0.0136 volt, although the manometer had not perceptibly fallen. On the next day, however, it had risen again to 0.0165 volt.

In another experiment, in which the relative ionic concentration of the two solutions was determined, by conductivity measurements, to be  $m/32$  and  $m/150$  respectively, the E.M.F. found was 0.025 volt, instead of 0.022 volt calculated. With more dilute outer solution, the E.M.F. observed

\* 'Quart. Journ. Exp. Physiol.,' 1910, vol. 3, p. 80.

was 0.068 volt instead of 0.088 calculated, but in this case the concentration of the outer solution was too low for accurate measurements, and the reading was not made until some time after setting up.

The reason why the E.M.F. steadily falls for a time after setting up the apparatus is, I think, to be explained by the fact that it is almost impossible to prevent a small diffusion of water through the membrane, owing to the high osmotic pressure within. The difference of concentration must be considerable in order to obtain sufficiently high potential differences for accurate measurement. The dilute layer next the membrane will only slowly diffuse into the rest of the solution and as long as it is contact with the membrane, the conditions are not those corresponding to the concentration of the main bulk. It seems then that the values obtained before the water has had time to pass through are the more reliable. When the osmometer was replaced by an open parchment-paper dish, the E.M.F. fell so rapidly that it was impossible to obtain satisfactory readings. In a special osmometer constructed by cementing parchment-paper over the end of a tube of 8 mm. diameter, it was found that, with solutions of conductivities of  $3950 \times 10^{-6}$  and  $1955 \times 10^{-6}$  recip. ohms respectively, an E.M.F. of 0.0149 volt was found, with an osmotic pressure of 288 mm. Hg at  $21^{\circ}$  C. The calculated value was 0.0174 volt, so that 86 per cent. of the theoretical number was obtained.

It occurred to me that, perhaps, by sending a continuous current of the more concentrated solution through the interior of a parchment-paper tube and one of the dilute solution over the outside, more permanent results might be obtained. The figures were:—

Conductivity of the stronger solution .....	$5780 \times 10^{-6}$ recip. ohms.
"          dilute          "          .....	$192 \times 10^{-6}$ "
E.M.F. calculated, 0.083 volt; found, 0.07 volt, or 84 per cent. of theory.	

Since, in these experiments, the osmotic pressure is the sum of that of the ionised and non-ionised salt, while the E.M.F. is due only to the former, it should be possible, by calculation of the osmotic pressure corresponding to the potential difference as measured and subtracting this from the observed osmotic pressure, to determine the relative proportion of the ionised and non-ionised parts of the salt in the osmometer.

It is worth recording that the E.M.F. can be at once abolished by replacing the outer solution by one of sodium chloride of the same ionic concentration, as estimated by electrical conductivity, as the solution on the other side of the membrane. If the solution thus placed outside is more concentrated in Na<sup>+</sup> ion than that inside, the sign of the potential difference is reversed, as would be expected. This reversal lasted longer than would be supposed from

the fact of the diffusibility of the sodium chloride, viz., 18 hours at least. If the outer solution be replaced by water while the sign is reversed, this latter is changed back again to the original direction, but is naturally greater in numerical value owing to the previous diffusion of sodium chloride into the inner solution, which gradually escapes again into the water.

The fact that the E.M.F. given by Congo-red solutions of unequal concentration separated by a membrane can be abolished or reversed by  $\text{Na}^+$  ions does not, it seems to me, warrant the conclusion that the dye solutions necessarily dissociate with the production of  $\text{Na}^+$  ions. If I rightly understand the rationale of the process, the production of the potential difference under discussion is a matter of electric charge of positive sign, so that the precise chemical nature of the carrier of the charge is a matter of indifference. That this point of view is correct is shown by the fact that potassium chloride, as well as sodium chloride, is effective in reducing the E.M.F. A Congo-red solution in dilution of about 21 litres gave, with water outside the membrane, an E.M.F. of 0.05 volt; when the water was replaced by  $m/20$  potassium chloride, this value was reduced to less than 0.001 volt. This being the case,  $c_1$  and  $c_2$  in the equation must apparently be regarded as expressing the concentrations of positive charges. The abolition of E.M.F. is, of course, only a temporary one, since the  $\text{K}^+$  ions diffuse through the membrane and thus produce again a concentration of positive ions greater on the inner side of the membrane. A potential difference due to the greater migration rate of the potassium ion than that of the sodium ion would not, I think, be perceptible under the conditions of the experiment. It is of interest to note that this experiment shows how sodium ions are enabled to escape by interchange with similarly charged ions in the outer fluid.

The possibility that the membrane itself may carry an electric charge must not be neglected, although the concordance of the most reliable of the experimental results given above with the calculated values shows that, if so charged, this charge plays no appreciable part in the production of the E.M.F. Moreover, it would only be very small in the presence of the large electrolyte concentration on both sides. As far as osmotic pressure is concerned, *à priori* considerations, for which I must refer to Mr. Hardy's forthcoming work on "Colloids," show that a charge on a membrane could only affect the time taken to attain equilibrium and would have no effect on the permanent result, unless the permeability of the membrane were affected. The total constraint exerted by the membrane, upon the non-dissociated molecules and anions directly, upon the cations indirectly, would be unaffected.

Since the cation is held within the membrane by electric forces alone, it is

to be expected that, by passing an electric current through the membrane by putting a platinum anode inside the osmometer and a similar cathode in the outer solution, the cations would be enabled to escape. This does in fact happen. The osmotic pressure falls, the solution inside becomes turbid owing to excess of free acid, while the outer fluid becomes alkaline. If the current be stopped and the outer alkaline solution be replaced by water, the osmotic pressure does not rise again until free alkali is added to replace that which has been allowed to diffuse out by the agency of the electric current. If the cation were a complex one containing colour acid it might be supposed that the outer fluid would be coloured in the above experiment. This, in point of fact, was the case to a slight degree. But parchment-paper is not absolutely impermeable to Congo red and the permeability is somewhat increased by the presence of alkali. The depth of colour did not seem to be greater than would have been attained in the absence of the electric current, and in the experiment described in the previous section, made for another purpose, no colour passed through the membrane during the time of the experiment, although considerable amounts of sodium hydroxide were formed.

The degree to which parchment-paper of the thick kind used in my experiments is permeable to the dye is actually very small. In an experiment in which the outer water in the osmometer was not changed for ten weeks the dye contained therein only amounted to 0.0078 per cent.

There is a slight possibility that, in a metallic osmometer, closure of a circuit between the two sides of the membrane may, to a very small extent, enable  $\text{Na}^+$  ions to escape. It seems most probable that polarisation would rapidly annul such a current. As already stated, moreover, no diffusion of alkali can be detected in the absence of an extraneous E.M.F.

The problem dealt with in this section is closely connected with a fundamental difficulty in the Arrhenius theory of electrolytic dissociation. According to the kinetic theory, the dissociated ions with which we are concerned are free from mutual influence upon one another, so that there should be no electrostatic attraction to prevent the diffusible ions from passing freely through the membrane. The origin of the energy required to separate the ions is a matter of some uncertainty. It has been suggested by Larmor\* that "internal potential energy is released owing to the ions entering into relations of closer affinity with the solvent," and by Bousfield and Lowry† that the affinity of the "ionic nucleus" for water is the main source of the energy required.

\* "Wilde Lectures," 'Mem. Manchester Lit. and Phil. Soc.,' 1908, vol. 52, Mem. 10, p. 37.

† 'Trans. Faraday Soc.,' 1907, vol. 3, p. 125.

*The Distribution of Salts between Congo-red Solutions and Water separated by a Membrane.*

The electrical conditions within the membrane are undoubtedly the cause of the peculiar distribution of a foreign salt between the dye inside and the water outside.

The salt used in my experiments was chiefly sodium chloride, on account of the ease with which chlorine can be estimated by Volhard's method. In order to do this in the presence of the dye, it was found sufficient to precipitate the dye acid in a known volume by addition of excess of nitric acid and filtering off the precipitate before the actual Volhard determination. Tests were made in order to see whether any chloride was carried down with the precipitate of the dye acid. This was found not to be the case.

When sodium chloride is added to either the inner or outer solution in an osmometer there is a considerable fall in osmotic pressure, if sufficient time be allowed for equilibrium to be established. This fact was described in my former paper. If the chlorine content of the two solutions, inside and outside the membrane, be estimated at this time, it is invariably found that the outer fluid, from which dye is absent, contains a *larger* percentage of chloride than the inner dye solution. The difference is more marked the greater the concentration of the dye salt in relation to that of the sodium-chloride. Table V gives a few of the measurements made. The numbers are dilutions in litres.

Table V.

Dye.	Chlorine.	
	Inside.	Outside.
30	52	30
30	465	73·6
30	<5500	180
100	32·9	29·5

I regret that my experiments are not sufficiently numerous to enable a complete theory to be given of this partition, but there are two views which must be referred to.

Donnan\* has investigated the question from the thermodynamic point of view, and comes to the conclusion that, as far as the non-dissociated salt is concerned, there must be equal distribution between the two sides of the membrane. This seems quite what would be expected, since there are no

\* In the Press. Prelim. Communication to the Physiological Society, December, 1910.

forces to prevent free diffusion of bodies devoid of electric charge. Moreover, the proportion of non-dissociated to dissociated salt is naturally *greater* within the membrane, owing to the diminished ionisation in the presence of the dye which has an ion ( $\text{Na}^+$ ) in common with the sodium chloride. This proportion will also be greater the greater the concentration of the dye. The result will be that the total concentration of sodium chloride will be less within the membrane, since less will be necessary in order to provide the same amount of non-dissociated salt.

On the other hand, Biltz and v. Vegesack\* maintain that the concentration of diffusible ions becomes equal on both sides of the membrane, diffusible ions including the cations of the dye. Consideration of the case where the dye solution is of high concentration and the sodium chloride dilute shows that this view does not afford a sufficient explanation. Such a case is the third one in Table V. The chlorine in the inner solution was, in fact, too small to be accurately estimated; a faint turbidity only was produced by the addition of silver nitrate. The diffusible ions of the inner solution would have a concentration of at least  $m/30$ , since at the concentration in question the dye is more than 50 per cent. dissociated. The ions of the sodium chloride outside could not have a concentration greater than  $m/90$  if the salt were completely dissociated. The investigators referred to find that the conductivity is equal on both sides of the membrane, as their theory requires, when a relatively large concentration of sodium sulphate is present along with a dilute solution, 0.0007325 molar, of benzo-purpurin within the membrane. I have always found that the conductivity of the inner solution containing the dye is greater than that of the outer one, although, of course, when the proportion of foreign electrolyte is very great, the difference comes very near the limit of detection.

The actual values of the conductivity of the inner solutions found in my experiments afford, as far as they go, confirmation of the theory put forward by Donnan. For example, in the first experiment of Table V, the conductivity of the inner solution was found to be 5920 recip. megohms. That of the dye alone could not be greater than 3935 recip. megohms, so that the ionised sodium chloride must have had at least a conductivity of 1985 recip. megohms, which is that of an  $m/60$  solution at the temperature of the experiment. The amount of chlorine found corresponded to  $m/52$   $\text{NaCl}$ , so that  $(m/52 - m/60 =) m/400$  was present non-ionised. It has been assumed, however, that there was no depression of the ionisation of the dye by the sodium chloride. If there were such an effect, the concentration of the ionised sodium chloride would be greater than  $m/60$ , and therefore that

\* 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 492.

of the non-ionised less than  $m/400$ . The outer solution, if 90 per cent. dissociated, would have a concentration in non-dissociated salt of 10 per cent. of  $m/30$ , or  $m/300$ . If its dissociation had been 87 per cent., the concentration of the non-ionised salt would have been  $m/400$ . The result may then be taken as supporting Donnan's theory.

The marked effect of foreign electrolytes in depressing the osmotic pressure of Congo-red solutions described in my former paper is, for the most part, due to this peculiar distribution of the salt between the two sides of the membrane. In the third experiment of Table V the osmotic pressure actually observed was 400 mm. Hg. That of the dye against water should have been about 620 mm., that is 220 mm. higher than that found. But the osmotic pressure of the  $m/180$  sodium chloride in the outer fluid would be 206 mm., which must be deducted from the 620 mm., leaving 414 mm. instead of 400 mm. observed. The osmotic pressure of the small amount of the sodium chloride within the membrane, being less than 3 mm. Hg, may be neglected. The difference between the observed and calculated values is probably to be accounted for by aggregation of the dye induced by the foreign salt, which would not be great, since the amount of this salt within the membrane was so small.

#### *The Action of Carbon Dioxide.*

It remains to refer to the effect of the presence of carbon dioxide or other acid in the outer fluid of the osmometer.

Various observers have noticed the difficulty in obtaining anything like permanent pressures with colloidal salts, such as those of caseinogen, similar to those dealt with in the present paper. This fact is due to the access of carbon dioxide in the air.

In dealing with colloidal systems, which are essentially unstable, it is not to be expected that really permanent osmotic pressures are to be obtained. If the manometer readings in my experiments did not fall more than by 2 or 3 per cent. of their values in 24 hours I considered it justifiable to assume that equilibrium was established between the two sides of the membrane. Experiments showed that equilibrium between diffusible bodies took place in the form of osmometer used in less than 24 hours. In the experiments of Biltz and v. Vegesack\* on benzo-purpurin the pressure fell from 9.6 to 5 cm. of solution in 38 hours and to 0.73 in 84 hours. That it is possible to obtain practically constant pressures for several days, if carbon dioxide is effectively excluded, is shown by the following manometer readings from one of my

\* 'Zeits. f. physik. Chem.,' 1909, vol. 68, p. 367.

experiments with benzo-purpurin, "conductivity" water being outside and frequently changed before these readings were taken :—

Date.		Date.	
1910.		1910.	
May 25 .....	49	May 31 .....	49
" 27 .....	49	June 3 .....	48
" 30 .....	48.5	" 6 .....	48
In all 288 hours.			

The effect of carbon dioxide was described in my former paper. In order to discover how this effect is produced I have made further experiments as follows:—

A solution of Congo red was placed in an osmometer of Graham's pattern and carbon dioxide passed for some time through the outer water. It was found that the dye became purple-brown in colour and for the most part precipitated. The outer water on evaporation to dryness deposited crystals of sodium carbonate. The explanation of this fact is clearly that when  $H^+$  ions are present in the outer fluid there is no hindrance to the free interchange between these and  $Na^+$  ions through the membrane. Accordingly the latter pass out and combine with  $CO_3^-$  ions when the solution is concentrated by evaporation. The hydrogen ions entering the dye solution cause aggregation of the acid salt formed.

Graham\* noticed that if he placed within a dialyser a solution of the sodium salt of albumen, the whole of the sodium was ultimately found in the outer water in combination with carbon dioxide derived from the air. I find that a similar loss of sodium occurs with the sodium salt of caseinogen.

Biltz and v. Vegesack† state that solutions of Congo red through which carbon dioxide has been passed recover their original state when boiled, even supposing that they have been dialysed. I find that this statement is correct for non-dialysed solutions; as the carbon dioxide is driven off, the dye acid displaces more of it from the sodium carbonate and enters itself into combination with the sodium. If the solution has been dialysed after subjection to the action of carbon dioxide, so that the sodium carbonate has been removed, I find it impossible to regenerate the dye by boiling, provided that this be done in a quartz flask fitted with a reflux condenser also of quartz. The corresponding experiment of Biltz and v. Vegesack‡ was made with a

\* 'Phil. Trans.,' 1861, vol. 151, p. 217.

† 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 487.

‡ 'Zeits. f. physik. Chem.,' 1910, vol. 73, p. 488.

very dilute solution of the dye, and required boiling for two hours with a reflux condenser, presumably of glass, in order to regenerate it. I think it quite possible that sufficient alkali was dissolved from the glass to account for the result.

There is, also, apart from the action of carbon dioxide, a slow aggregation in solutions of Congo red, and especially of benzo-purpurin. This change is reversed by heating. For example, a dilution of the latter dye of 94 litres, which had remained for some time in the osmometer with frequent changes of water free from carbon dioxide, had an osmotic pressure of only 68 mm. Hg, or 35 per cent. of the "molecular." After heating to 86° C. the osmotic pressure rose to 175 mm. Hg, or 88 per cent. of the "molecular."

*The Temperature Coefficient of the Osmotic Pressure of Congo Red.*

Some incidental observations on the effect of temperature seem to show, as far as they go, that the osmotic pressure of this dye is proportional to the absolute temperature. A solution which had an osmotic pressure of 123 mm. Hg at 28.5° C. showed one of 138 mm. Hg at 62° C. Now

$$123 \times \frac{273 + 62}{273 + 28.5} = 137.$$

*Summary of Conclusions.*

1. No hydrolytic dissociation is to be detected in solutions of Congo red, and merely a trace, if any, in sodium caseinogenate.

2. On the other hand, electrolytic dissociation occurs to a large degree. Carefully purified Congo red in dilutions of 28 litres is 50 per cent. ionised; in 500 litres, 80 per cent. Although considerable, however, it is not so great as that of sodium salts of other organic acids of small molecular weight at corresponding dilutions, probably owing to colloidal aggregation in the case of the solutions of the dye salt.

3. The osmotic pressure found experimentally, both by direct measurement and by vapour pressure, is, throughout a wide range of concentration, uniformly between 95 and 100 per cent. of what it would be if no dissociation existed. Since it should be from one and a half to three times this value, according to the concentration, it is plain that there are some abnormal conditions present.

4. The sodium ion being kept within the membrane merely by electrostatic forces, it might be supposed that it is inactive in the production of osmotic pressure. The agreement of vapour pressure with direct determinations is sufficient to show that this is not the case. Moreover, Chicago blue, consisting of a single large non-diffusible anion, like Congo red, but with four

Na<sup>+</sup> ions instead of two, gives *double* the osmotic pressure of the latter at the same concentration, whereas it should be the same, on the view of the non-activity of the diffusible ion. The suggestion is also disproved by theoretical considerations.

5. The curve expressing the ratio of the conductivity of Congo-red solutions to their osmotic pressure is convex to the axis of abscissæ when these are the values of the conductivity. It is a straight line when expressing the relation of osmotic pressure to molar concentration.

6. The value of osmotic pressure per unit increase of conductivity rises with concentration, forming an **S**-shaped curve.

7. Difficulties are pointed out in the hypothesis of formation of complex ions if these are supposed to contain both acid and base components. The possibility of aggregated simple ions carrying the sum of the charges of their components is suggested in order to explain the experimental results.

8. Whatever may be the nature of the cation, that it is diffusible is shown by the fact that the membrane is the seat of an electromotive force. The sign and numerical values of this potential difference agree, within experimental errors, with the equation deduced by Hardy.

9. The distribution of a foreign salt, such as sodium chloride, between the solution of the dye on one side of the membrane and water on the other side is always such that its concentration is greater in the water. Numerically the results agree with Donnan's view of equality of concentration of non-ionised sodium chloride on both sides of the membrane, and not with that of Biltz and v. Vegesack of equality of total diffusible ions. The effect of sodium chloride on the osmotic pressure is chiefly due to this peculiarity of distribution, since its aggregating effect is relatively small.

10. It is impossible to obtain even an approximately constant osmotic pressure in the case of colloidal salts with a diffusible cation if carbon dioxide be allowed access to the outer water of the osmometer. This effect is shown to be due to the fact that the presence of H<sup>+</sup> ions in the outer fluid allows Na<sup>+</sup> to escape by interchange of ions. The final result is the escape of the greater part of the sodium from the interior and precipitation of the acid salt.

11. Congo red appears to obey the gas law so far as the effect of temperature on the osmotic pressure of its solutions is concerned.

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