

CLXXVII.—*The Osmotic Pressure and Conductivity of Aqueous Solutions of Congo-red, and Reversible Membrane Equilibria.*

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THE object of the work described in this paper was to make a somewhat detailed examination of the molecular state of Congo-red in aqueous solution by means of measurements of osmotic pressure and electrical conductivity, and to investigate the effects of certain electrolytes on these properties.*

Congo-red, the disodium salt of diphenylbisazonaphthylamine-sulphonic acid, is known to possess colloidal properties in aqueous solution. For example, it does not diffuse, or only very slowly, through a membrane of parchment paper, thus rendering the measurement of its osmotic pressure possible with a diaphragm of this sort. W. M. Bayliss (*Proc. Roy. Soc.*, 1909, **81**, B, 345) has made several measurements of the osmotic pressure of Congo-red, his apparatus being a modification of that used by Moore and Roaf (*Bio-Chem. J.*, 1907, **2**, 34). His method consisted in placing Congo-red acid in the osmometer, and adding dilute sodium hydroxide solution to the outside water every twenty-four hours until the latter retained a slight permanent alkalinity. The outside water was then repeatedly changed until no further rise in osmotic pressure occurred. The maximum pressure so obtained was found to vary from 88 to 97 per cent. of the value calculated on the assumption that Congo-red exists in solution as simple, undissociated, and non-aggregated molecules, and from these results Bayliss drew the conclusion that the dye really does exist in solution as simple molecules (M.W. = 696.5). This conclusion, which in itself is rather an unlikely one, will be shown in the course of this paper to be untenable.

Bayliss further showed that the osmotic pressure of Congo-red is lowered by the presence of sodium chloride, sodium hydroxide, and carbon dioxide (carbonic acid).

Since the commencement of the present work † a paper on the osmotic pressure of several colloids has been published by Biltz and von Vegesack (*Zeitsch. physikal. Chem.*, 1909, **68**, 357), among the colloids examined by them being benzopurpurine-4B, a dyestuff

* This work was primarily undertaken as a preliminary study to a similar investigation of the state of soaps in aqueous solution. The osmotic pressures of these solutions are being at present investigated in this laboratory.

† The publication of the present work has been unfortunately delayed for a year, the experiments having been completed in May, 1910.

nearly related to Congo-red in chemical composition. From their experiments they concluded that in the absence of foreign electrolytes benzopurpurine-4B exerts a negligibly small osmotic pressure.* They remarked that during dialysis the solution undergoes a change, the originally bright red solution becoming brownish-black, and they considered this to be simply a mechanical and not a chemical effect, because by heating the dialysed solution (presumably in a glass vessel) the original appearance of the solution was restored. It was assumed by these authors that no hydrolysis could have taken place, because in a particular experiment in which six litres of water were used as outside liquid, no alkali could be detected therein. With regard to the regeneration of the brownish-black dialysed solution by heating, it is quite possible that, even if (partial) hydrolysis had taken place, the alkali taken up from the glass on heating would have been sufficient to reconvert the substance or substances produced by this partial hydrolysis into the original benzopurpurine salt. Thus it is quite easy to convert a dilute solution of the blue Congo-red acid into the bright red solution of the salt by simply boiling the former for some time (less than half an hour) in an ordinary glass beaker.

Again, the fact that no free alkali was detected by Biltz and von Vegesack in the six litres of outside water can easily be accounted for by the large volume of this outside liquid, and the relatively small amount of alkali to be expected.†

It will be shown in the present paper that in this and similar cases hydrolysis, accompanied by the outward diffusion of free alkali, undoubtedly occurs. Indeed, this phenomenon of what we may call "forced membrane-hydrolysis" is an exceedingly interesting and important one, and must always occur to some extent when an electrolyte with a non-dialysing ion (whether kation or anion) is separated by a suitable membrane from pure water.‡

EXPERIMENTAL.

For the osmotic pressure measurements described in the present paper, an osmometer was employed which was a modified form of that described by Roaf (*Quart. J. Physiol.*, 1910, **3**, 75). A short cylinder of glass 2 cm. long and 3.5 cm. in diameter, provided with

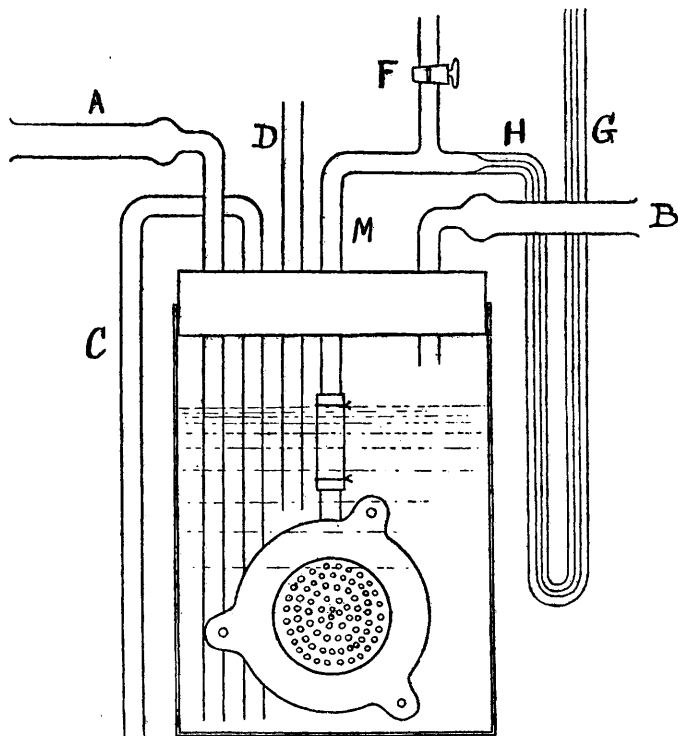
* Some of the erroneous conclusions arrived at by Biltz and von Vegesack have been corrected in a subsequent paper, which will be discussed later (see p. 1575).

† Moreover, unless every precaution was taken to exclude carbon dioxide, a detection of the free alkali which had diffused out by means of phenolphthalein would be very uncertain.

‡ The general theory of the action of membranes in the presence of electrolytes possessing a non-dialysable ion will be discussed in another paper.

a sealed-in side-tube for connexion to the manometer, was closed at either side by parchment-paper diaphragms. These were backed up externally by perforated silver plates, which were tightly pressed against the diaphragms by means of an annular metal framework consisting of two metal rings provided with suitable nuts and screws. A tight joint between the parchment paper and the glass edges of the osmometer vessel was secured by annular rubber washers. The internal volume of the osmometer vessel

FIG. 1.



was 20 c.c. Connexion between the latter and the tube leading to the manometer was effected as shown in Fig. 2, the narrowed end of the manometer-tube projecting into the side-tube of the osmometer vessel, the joint being secured by tightly wired rubber pressure-tubing.

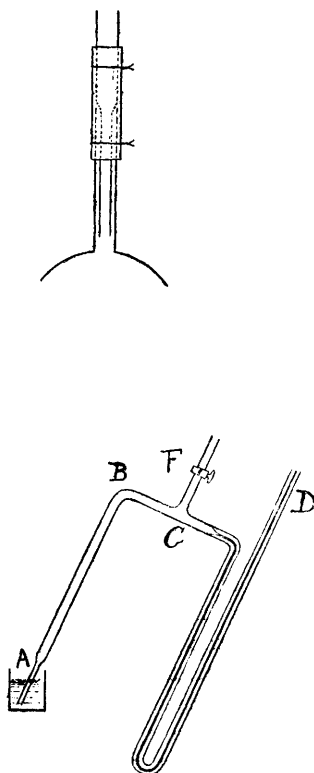
The general arrangement is shown in Fig. 1. The osmometer was suspended in a stout cylindrical glass jar of about 1300 c.c. capacity, closed by a large cork, through which passed the tube *M*

leading to the manometer, two soda-lime tubes, *A* and *B*, by means of which the entrance of carbon dioxide is prevented, and two tubes, *C* and *D* (closed by rubber tubes and screw-clips), for changing the liquid external to the osmometer.

In the manometer *HG* (internal diameter 1 mm.) mercury was employed as manometric fluid. The side-tube *F* was employed in the filling and connecting of the manometer, which was carried out as follows. The osmometer

vessel, together with the pressure-tubing by means of which it is to be connected to the manometer, is completely filled with the solution to be employed. Mercury is then introduced into the dry inverted manometer through the end *A* (see Fig. 2), the mercury collecting at the bend *B*. By now quickly bringing the manometer into its normal position, keeping the end *D* closed by the finger, the mercury is obtained in the two arms of the capillary pressure gauge without any break, sufficient mercury having been introduced so that in the inclined position shown in Fig. 2 one end of the mercury column can be brought to the position *C*. The end *A* being now dipped into the required solution, the latter is drawn into the manometer by suction at *F*. By now bringing *AB* into a vertical position after closing the tap *F* (the end *A* being still immersed in the solution), the excess of mercury can be run out and the levels brought to any desired position in the pressure-gauge by blowing in at *D*, the mercury which

FIG. 2.



is driven over collecting in the vessel *A*, and being replaced by the solution. The manometer and osmometer having been completely filled are joined together in the manner indicated previously. The modelling material "Plasticene" was found to be a very convenient substance for making the cork of the outer cylindrical jar quite air-tight, a thick layer of this material being spread over the whole top of the cork.

After the apparatus has been set up, air, free from carbon dioxide, is drawn for a considerable time through the water in the jar by attaching *B* to a filter pump and *A* to a series of long soda-lime tubes. Throughout all the experiments the presence of carbon dioxide was carefully excluded, for reasons which will appear later.

With regard to the measurement of the osmotic pressure, a correction has obviously to be made for the difference between the level of the outer liquid in the jar and that of the mercury column in the nearer limb of the manometer (the other limb of the manometer was open to the atmosphere). This correction is additive if the water level is below the lower mercury level, and subtractive if the reverse is the case.

Preliminary Series of Measurements of Osmotic Pressure.

The first series of measurements was made with solutions of Grüber's Congo-red, which was found to contain about 30 per cent. of sodium chloride. A litre of distilled water, free from carbon dioxide, formed the external liquid. The same general behaviour was observed in all these experiments. The pressure rose quickly at first, the velocity of increase gradually diminishing. After four to five days the pressure attained a maximum, and then began slowly to decrease. On changing the external water, a further rise in pressure occurred, followed by a slow fall. Succeeding changes of the external water gave rise to the same phenomenon, the increase of pressure becoming, however, rapidly smaller with each successive change of external water. The experiments were carried out at room temperature (17°), and entrance of carbon dioxide carefully prevented. It was found that the original litre of external water contained nearly all the sodium chloride originally present in the Congo-red, the second litre practically removing the whole of the remainder.

The concentration of the Congo-red in the osmometer (after removal of the sodium chloride) was determined by evaporating 10 c.c. of the cell contents at the end of the experiment, and drying to constant weight at 110 – 115° . The following figures give the results of an osmotic pressure measurement. (Experiment I.):

Time (hours).	Difference of mercury levels (mm.).	Time (hours).	Difference of mercury levels (mm.).
24	88.5	282	125.0
73	105.0	306*	124.0
96	107.5	314	125.0
144	108.0	330*	122.0
234*	105.0	356*	123.0
240	119.0	401	121.0
258	123.0	450	115.0

* Indicates a change of the external water.

Final concentration of Congo-red = 0.578 gram per 100 c.c.

Maximum pressure (corrected for difference of levels) = 136 mm. (125 + 11).

For single undissociated molecules the osmotic pressure would be:

$$22.4 \times 760 \times \frac{290}{273} \times \frac{5.78}{696.5} = 140 \text{ mm.}$$

The observed value is therefore 97 per cent. of this calculated value.*

Two other experiments carried out in a similar manner yielded the following final results:

II. Concentration of Congo-red	=	0.387 gram per 100 c.c.
Maximum pressure observed	=	97.0 mm.
Calculated value	=	100.5 mm.
Observed value	=	96.5 per cent. of calculated value.

III. Concentration of Congo-red	=	0.756 gram per 100 c.c.
Maximum pressure observed	=	185.5 mm.
Calculated value	=	196.0 mm.
Observed value	=	95.0 per cent. of calculated value.

These results show a good general agreement with those obtained by Bayliss, in which the Congo-red salt was formed in the osmometer by diffusion of alkali to the blue Congo-red acid. The rise of osmotic pressure observed after each change of external water might be due, as Bayliss supposes, to an increase of "dispersivity" (and therefore presumably of osmotic pressure) of the Congo-red solution, as the sodium chloride diffuses out through the parchment diaphragm, or it might be due to the sodium chloride still present in the osmometer exerting an osmotic pressure before it has time to diffuse out. In order to test the latter explanation, experiments (to be described later) have been made with solutions of pure sodium chloride in the osmometer. The general result of these experiments is that the small amount of sodium chloride remaining in the osmometer, even before the first change of external water, that is, after about seven days, could not cause the rise of pressure observed. This argument assumes an equal concentration of sodium chloride inside the osmometer and in the external water. It will be shown later, however, that after a few days the sodium chloride becomes unequally distributed, that is, its concentration in the external water becomes greater than in the osmometer vessel, thus producing an osmotic pressure opposed to that of the Congo-red. When the external water is changed, this counter-pressure is removed, and so a sensible rise of osmotic pressure

* Throughout this paper, the expression "calculated value" will always refer to that calculated for single undissociated molecules. This method of expressing the results is simply used for the purpose of providing a convenient standard of comparison.

occurs, that is, the true pressure due to the Congo-red present in solution inside the osmometer is observed. That this is probably the true explanation of the phenomenon will appear from the later experiments. It is not necessary, therefore, to suppose that the small concentration of the sodium chloride present in the osmometer exerts any intrinsic action in depressing the osmotic pressure of the Congo-red. This very important matter will be discussed more fully later on.

With regard to the gradual decrease of pressure after a maximum has been attained, this might be attributed to various causes. Sodium chloride might be supposed to be still diffusing slowly from the osmometer, the Congo-red might be undergoing some change of "physical" state, as Biltz and von Vegesack suppose, or a chemical change such as hydrolysis might be occurring. The first hypothesis may be dismissed at once, for the slow fall of pressure occurs long after all the sodium chloride has diffused out of the osmometer.

It may be remarked here that in the foregoing experiments the contents of the osmometer acquire a muddy-brown appearance after about forty-eight hours' dialysis. This appears to be due to the presence of a very fine colloidal suspension. This change in the appearance of the osmometer contents sets in more rapidly at higher temperatures. Moreover, it is found that the muddy-brown solution is unaffected by a small quantity of sodium chloride, whereas a trace of sodium hydroxide suffices to reconvert it into the original bright red solution. We may dismiss, therefore, the hypothesis of Biltz and von Vegesack, and conclude that a slow hydrolysis, accompanied by the diffusion out of sodium hydroxide, and the precipitation in fine, semi-colloidal suspension of some very insoluble substance (perhaps a mixture, or "adsorption-compound," of Congo-red acid, or the acid salt, with the original Congo-red), is occurring. This would give a satisfactory explanation of the gradual fall in osmotic pressure. Were this the case, we should expect to be able to prevent this "membrane-hydrolysis," and so obtain constant osmotic pressures by employing instead of pure water a dilute solution of sodium hydroxide as external liquid. Experiment confirms this conclusion, for the employment of a *N*/800-sodium hydroxide solution suffices entirely to prevent the "precipitation" of an 0.5 per cent. solution of Congo-red at 25°, and enables a perfectly definite and constant osmotic pressure to be obtained. These results enable us also to understand why Bayliss found that carbon dioxide exerted a marked action in lowering the osmotic pressure of Congo-red solutions; for the carbonic acid in the external water will combine with the alkali

that diffuses out, and so hasten and increase the hydrolysis and precipitation in the osmometer. The following experiment will serve to confirm this conclusion. Two similar osmometers were filled with the same 0.5 per cent. solution of Grüber's Congo-red. In the case of one osmometer, entrance of carbon dioxide was prevented as described previously. The other osmometer was simply suspended in an open beaker containing a volume of ordinary distilled water equal to the volume of water, free from carbon dioxide, in the protected outer jar of the first osmometer. The osmotic pressure fell, in one case, from 82.5 mm. to 69.5 mm. in seventeen days, whilst in the other case (unprotected osmometer) it fell in the same time from 85.5 to 53.5 mm.

The gradual hydrolysis of Congo-red on dialysis with continued change of the outer water was conclusively proved as follows: 100 c.c. of a 0.5 per cent. solution of Merck's Congo-red were placed in a parchment dialysing tube, which was suspended in a beaker containing a litre of distilled water. This outer water was changed every day. The original Congo-red (Merck's) was found to contain 16.47 per cent. of sodium, showing the presence of a fairly large amount of foreign electrolytes (sodium chloride and sulphate). After four days' dialysis a portion of the solution was evaporated to dryness, and the sodium content of the dry residue determined. This was found to be 6.5 per cent. (theoretical value for Congo-red, 6.6 per cent.). After three weeks' dialysis the sodium content of the dried residue obtained on evaporation was found to be 6.0 per cent., thus indicating a loss of total sodium due to hydrolysis and diffusion out of sodium hydroxide. In order to obtain a more marked difference, a diluted solution of the muddy-brown liquid obtained after three weeks' dialysis was subjected to further dialysis with daily renewal of the external water. After seven weeks' dialysis the muddy solution in the dialyser had assumed a violet hue, and the dried residue was now found to contain only 1.6 per cent. of sodium. A portion of this solution, still further diluted, on boiling in a glass beaker for some time became clear and bright red, being converted into a solution of the normal Congo-red salt by the alkali taken up from the glass. The above dialysed solution (total concentration about 0.15 gram per 100 c.c., sodium content of dry residue = 1.6 per cent.) gave in an osmometer an osmotic pressure of 40 mm. of water, that is, about 3 mm. of mercury, the measurement being made by substituting a vertical glass tube of narrow bore for the mercury manometer. This result appears to indicate that the first product of the hydrolysis of Congo-red must be mainly a "colloidal" suspension of the sodium hydrogen salt, since, if the free acid were in suspen-

sion, the solution which still contains 1.6 per cent. of sodium in its solid residue ought to have shown a much higher osmotic pressure than that observed.

A similar experiment with Kahlbaum's benzopurpurine-4B yielded the following results:

Initial sodium content of Kahlbaum's preparation	=	22.45 per cent. Na.
Sodium content of dried residue after six days' dialysis	=	6.0 per cent. Na.
Sodium content of dried residue after five weeks' dialysis	=	3.3 per cent. Na.*
Theoretical value for benzopurpurine	=	6.3 per cent. Na.

* The solution in the osmometer after five weeks' dialysis contained approximately 1 gram per 100 c.c.

These results show that benzopurpurine suffers a much more rapid membrane-hydrolysis than Congo-red. This accounts for the fact that Biltz and von Vegesack found a solution of benzopurpurine (of initial concentration 1 per cent.) after three weeks' dialysis to show only a small osmotic pressure. The following experiment confirms this conclusion. A solution of benzopurpurine gave an osmotic pressure of 67.5 mm. of mercury, which fell to 6 mm. in fourteen days (final concentration about 0.25 per cent.), whilst with a Congo-red solution of final concentration 0.17 per cent. the pressure fell from 48 mm. to 34 mm. in nineteen days.

Second Series of Osmotic Pressure Measurements.

In the previous experiments the maximum pressures observed corresponded with solutions which had suffered some degree of hydrolysis, and were in consequence muddy-brown in appearance. In order to see whether this hydrolysis had sensibly vitiated the results, a series of experiments with solutions of the same initial concentrations of Grüber's Congo-red was carried out as follows. After the preliminary dialysis in the osmometer against pure water to remove the sodium chloride, a dilute solution of sodium hydroxide* was used as external liquid in order to throw back the hydrolysis. This solution was then replaced by a more dilute solution of alkali, and so on, pure water being finally employed. In this way one may hope to obtain the maximum osmotic pressure against pure water without sensible disturbance, due either to hydrolysis or to presence of foreign electrolytes. The results are shown in the following table:

* Prepared from metal and pure water free from carbonic acid.

Room temperature 17°.

	Osmom. I.	Osmom. II.	Osmom. III.
Initial concentration (gram per 100 c.c.) (Congo-red + NaCl)	0.5	0.75	1.00
	mm. Hg.	mm. Hg.	mm. Hg.
Max. reading after removal of NaCl	82.0	132.0	166.5
Reading after 24 hours with <i>N</i> /500-NaOH	52.0	90.0	132.0
" " " <i>N</i> /1000-NaOH	57.0	104.0	143.0
" " " <i>N</i> /2000-NaOH	68.0	117.0	155.0
" " " <i>N</i> /4000-NaOH	79.0	128.0	165.0
" " " <i>N</i> /8000-NaOH	81.0	131.0	167.0
" " " <i>N</i> /16,000-NaOH	84.0	137.0	169.0
" " " pure water.....	86.0	137.5	171.0
Correction for difference of levels	+ 3.0	+ 7.5	+ 0.7
Final osmotic pressure	89.0	145.0	171.7
Final concentration (gram per 100 c.c.) ..	0.357	0.564	0.700
"Calculated value" of osmotic pressure	92.7	146.4	179.0
Observed as percentage of calculated value	96.0%	99.0%	96.5%

These results satisfactorily substantiate those previously obtained, and show that at these concentrations and temperatures the osmotic pressure of a pure non-hydrolysed Congo-red solution approaches closely to that calculated for single undissociated molecules.

Third Series of Osmotic Pressure Measurements.

In these experiments, which were carried out according to Bayliss' method for the sake of comparison, the osmometer contained initially a solution (that is, colloidal solution) of pure Congo-red acid (prepared by dialysis of a mixture of Congo-red and hydrochloric acid). In the first experiment *N*/175-sodium hydroxide solution was employed initially as external liquid, in order to convert the Congo-red acid in the osmometer into the Congo-red (sodium) salt. After ten days this was replaced by *N*/1000-sodium hydroxide, which solution was replaced by *N*/2000-sodium hydroxide after twenty-four hours. The alkali solution was then regularly replaced by a more dilute one every twenty-four hours, and finally by pure water. The volume of outer liquid was 1 litre, as in the previous experiments, the temperature 17°. The following table shows the result of one experiment:

Concentration of alkali.	Difference of mercury levels.	Concentration of alkali.	Difference of mercury levels.
<i>N</i> /175	31.0 mm.	<i>N</i> /8000	116.5 mm.
<i>N</i> /1000	76.0 "	<i>N</i> /16,000	122.0 "
<i>N</i> /2000	98.0 "	<i>N</i> /32,000	121.5 "
<i>N</i> /4000	113.0 "	Water	121.0 "

The correction for difference of water and mercury levels was -2 mm., so that the maximum osmotic pressure observed was

120 mm. The final concentration of Congo-red being 0.491 gram per 100 c.c., the calculated value=124 mm. Hence, observed value=97 per cent. of calculated value.

Two other similar experiments gave the following final results :

Final conc. of Congo-red.	Observed pressure.	Calculated value.	Percentage.
0.423 gram/100 c.c.	106.0 mm. Hg.	109.5 mm.	97.0
0.346 ,,	88.7 ,,	90.0 ,,	98.5

These results show a good general agreement with those obtained by the other method.

Fourth Series of Osmotic Pressure Measurements.

These experiments were made with much more concentrated solutions of Congo-red. No alkali was used in the outside water. The latter was changed daily until no more sodium chloride diffused out, this requiring four to five days in the relatively more dilute solutions, and about eight days in the more concentrated solutions. As in the first series of measurements, the pressure rose to a maximum, and then slowly decreased, this maximum being reached after the sodium chloride was all (or practically all) removed. The final results are shown in the following table:

Final concentration.	Max. pressure observed.	Calculated value.	Percentage.
1.276 per cent.	310 mm.	331.8 mm.	93.4
2.617 ,,	603 ,,	676.0 ,,	89.0
5.016 ,,	1139 ,,	1304.0 ,,	87.3
6.033 ,,	1363 ,,	1567.0 ,,	86.9

In the last experiment in this series the form of manometer described by Adie (*Trans.*, 1891, **59**, 234) was employed. These results point perhaps to an increasing "aggregation" of the Congo-red in the stronger solutions, the osmotic pressure per molecule of Congo-red decreasing with increase of concentration.

Behaviour of Sodium Chloride and Sodium Hydroxide alone in the Osmometer.

A detailed examination of this behaviour was made, the results of which need only be briefly referred to. With the sodium chloride solutions (5*N*, *N*/10, *N*/100, *N*/1000) the pressure developed initially falls rapidly at first, afterwards very slowly. This is due to the fact that even when the salt has practically all diffused out, the hydrostatic pressure so established only falls slowly, owing to the slow rate at which water flows through the parchment diaphragm under small differences of pressure. A rapid rise, followed by a rapid fall of pressure, was observed at each change of the external water. This effect is very different from the otherwise somewhat similar one observed in the case of Congo-red solutions

which are being freed from sodium chloride by dialysis. In the present case the rapid rise and succeeding rapid fall of pressure are undoubtedly due to the temporary osmotic pressure caused by the salt still present in the osmometer. In the case of the Congo-red solutions referred to, the rise of pressure on change of the external water is followed by a slow fall of pressure, the latter being due, as shown above, to the progressive membrane-hydrolysis; whilst the rise is due to the removal of the counter-pressure caused by the greater concentration of the salt in the external water.

A concentrated solution of sodium hydroxide appears to pass through the membrane very rapidly, whilst weak solutions do so very much more slowly. The conclusion of Roaf (*loc. cit.*) that diffusible electrolytes do not show any temporary osmotic pressure effects with parchment membranes is therefore incorrect. The following tables will give some idea of the nature of the results observable with the osmometers used in this work.

5 <i>N</i> -Sodium chloride.		<i>N</i> /10-Sodium chloride.	
Time from filling.	Pressure.	Time from filling.	Pressure.
7½ hours	169·0 mm.	18½ hours	56·7 mm.
*48 "	42·0 "	68 "	24·0 "
53 "	54·5 "	*116 "	17·0 "
* 4 days	33·5 "	140 "	26·0 "
4 " 8 hours	35·0 "	*188 "	20·0 "
5 "	27·5 "	194 "	19·5 "
* 6 "	20·0 "		
6 " 8 hours	20·0 "		
7 "	18·0 "		
10 "	11·0 "		
<i>N</i> /2-Sodium hydroxide.		<i>N</i> /1000-Sodium hydroxide.	
0 hour	25·5 mm.	0 hours	23·0 mm.
*18 hours	2·5 "	3 "	36·0 "
49 "	36·4 "	*22 "	24·0 "
*90 "	20·0 "	28 "	21·0 "
95 "	35·4 "	70 "	14·5 "
*118 "	26·5 "	119 "	9·9 "
124 "	26·0 "		
160 "	17·0 "		

* Indicates change of outside liquid.

Electrical Conductivity of Congo-red Solutions.

In order to prepare Congo-red solutions for measurements of electrical conductivity, it was necessary to free them as far as possible from sodium chloride by dialysis, and at the same time to avoid any appreciable hydrolysis. For this purpose the hot saturated solution of Congo-red was placed in an osmometer vessel, the side-tube of which was closed by a rubber-tube and screw-clip. The osmometer vessel was then immersed in distilled water, free from carbon dioxide, which was changed daily for nineteen days.

By thus dialysing strong solutions at room temperature, and preventing dilution of the solutions during dialysis caused by osmotic flowing in of water, any appreciable hydrolysis was prevented, as it was found that hydrolysis increased the more dilute the solutions were. The contents of the osmometer at the end of the dialysis were diluted with "conductivity" water until the solid which had separated in the osmometer on cooling was all dissolved. Small portions of this solution, when diluted further with water, showed a clear red colour, indicating the absence of any appreciable hydrolysis.

The following table shows the results of the first series of measurements (temp. 25°). The more dilute solutions were obtained by diluting the dialysed solution with conductivity water.

Concentration of Congo-red.		Specific conductivity.	Molar conductivity.
Grams/100 c.c.	Mols./litre.		
2.667	1/26.1	4.161×10^{-3}	108.5
1.333	1/52.2	2.284×10^{-3}	119.2
0.667	1/104.4	1.249×10^{-3}	130.4
0.333	1/208.8	0.700×10^{-3}	146.2
0.1667	1/417.6	0.390×10^{-3}	163.1
0.0833	1/835.2	0.210×10^{-3}	175.7
0.0417	1/1670.4	0.110×10^{-3}	183.5
	<i>N</i> /50-NaCl	2.328×10^{-3}	116.4

For comparison are given the specific and molar conductivities of a *N*/50-sodium chloride solution at the same temperature. A portion of the strongest of the above dialysed solutions of Congo-red was evaporated to dryness, and the chlorine content of the dried residue determined. The analysis showed that the solution was not quite free from sodium chloride, containing 0.005 gram per 100 c.c. (that is, *N*/1170 with respect to sodium chloride).

A second solution of Congo-red was therefore dialysed in a similar manner for fourteen days, but in order to hasten the removal of the sodium chloride, a small air-space was left in the osmometer vessel, thus permitting the contents of the latter to be mixed by shaking. In this case the solution of Congo-red so obtained was found to be practically free from sodium chloride. Conductivity measurements with solutions prepared from this solution by dilution with conductivity water gave the following results (25°):

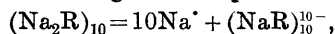
Concentration of Congo-red.		Specific conductivity.	Molar conductivity.
Grams/100 c.c.	Mols./litre.		
2.485	1/28	3.664×10^{-3}	102.6
1.2425	1/56	2.046×10^{-3}	114.6
0.6212	1/112	1.125×10^{-3}	126.0
0.3106	1/224	0.626×10^{-3}	140.2
0.1553	1/448	0.352×10^{-3}	157.7
0.0776	1/896	0.189×10^{-3}	169.3
0.0388	1/1792	0.102×10^{-3}	182.8
	<i>N</i> /50-NaCl	2.328×10^{-3}	116.4

As a further check, two measurements of osmotic pressure were made at room temperature (17°) with the dialysed solution used in the above conductivity measurements. The results may be briefly stated as follows:

Experiment 1 :—Final concentration of solution	=	0.572 gram per 100 c.c.
Maximum pressure (reached in 4 days)	=	140.0 mm. Hg
Calculated value	=	148.7 " "
Observed value as percentage of calculated value	=	94
Experiment 2 :—Final concentration of solution	=	0.257 gram per 100 c.c.
Maximum pressure (reached in 3 days)	=	62.5 mm. Hg.
Calculated value	=	66.7 " "
Observed value as percentage of calculated value	=	94

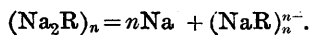
The conductivity-measurements show that Congo-red in aqueous solution behaves as a highly dissociated salt, although the observed value of the osmotic pressure indicates apparently no ionisation. At first sight one would be inclined to explain this somewhat anomalous result by the assumption of simultaneous "aggregation" and ionisation of the aggregated complex.

Consider, for example, a solution containing 1 gram of Congo-red in 100 c.c. The foregoing measurements show that at 17° the observed osmotic pressure of such a solution is somewhat less than 260 mm. (the value calculated for simple undissociated molecules). Suppose now that the solution contained only undissociated aggregates of the formula $(\text{Na}_2\text{R})_{10}$. This would lead to an osmotic pressure of only 26 mm. for the above-mentioned solution. If, however, we were to assume a complete "first stage" ionisation of these complexes according to the equation:



the osmotic pressure would be 11×26 , or 286 mm.

In the same way the assumption of aggregates of the formula $(\text{Na}_2\text{R})_{20}$ ionising in a similar manner would lead to an osmotic pressure of 273 mm. The assumption of the existence of such aggregates ionising (partially) in the manner indicated would therefore suffice to account for the osmotic pressures observed, and might perhaps serve to explain the non-diffusibility of Congo-red through parchment paper; but it is difficult to reconcile such an assumption with the high values obtained for the molar conductivities. Consider, for example, the 1/112-molar solution, the molar conductivity (referred to the formula Na_2R) of which at 25° is 126. The observed osmotic pressure of this solution indicates a van't Hoff factor (referred to the formula Na_2R) of practically unity. Let us suppose for a moment that this solution contained complexes of the (average) formula $(\text{Na}_2\text{R})_n$, very largely dissociated as indicated by the equation:



Then the molar conductivity referred to Na_2R would be approxi-

mately equal to the conductivity of 1 gram-molecule Na^+ -ion plus $1/n$ th the conductivity of 1 gram-molecule $(\text{NaR})_n^-$ -ion. Now the latter term cannot exceed the conductivity of 1 gram-molecule $(\text{NaR})^-$ -ion, which we may put at about 30—40 at 25° ; so that the molar conductivity of the solution cannot certainly exceed at 25° $50 + 40$, that is, 90, whilst the observed value is 126. If therefore the observed conductivities correspond with solutions practically free from sodium chloride and not to any appreciable extent hydrolysed, it does not seem possible to reconcile them with any such explanation of the osmotic pressure results as that suggested above. On the other hand, the assumption that we are dealing with a simple salt Na_2R , dissociating into Na^+ -ions and R^{2-} -ions, although perhaps reconcilable with the measurements of conductivity, leads to degrees of dissociation and total molecular concentrations which are not in harmony with the observed values of the osmotic pressure. It must not be forgotten in this connexion that the value of the osmotic pressure (if it correspond with a true state of osmotic equilibrium) can be thermodynamically related to the vapour-pressure of the solution, which latter is a function of the total molecular concentration of the solution.

The explanation of this apparent contradiction probably lies in the fact that we are not dealing here with an ordinary case of osmotic equilibrium. The dissociated sodium ions can themselves rapidly pass through the parchment membrane, and are only prevented from doing so by the potential difference set up across the membrane.* True equilibrium can only be attained, as shown in the present paper, when a certain concentration of Na^+ - and $(\text{OH})^-$ -ions is produced on the other side of the membrane. Under these conditions it is natural to expect that the Na^+ -ions do not contribute in the ordinary way to the osmotic pressure.

The Lowering of the Osmotic Pressure of Congo-red Solutions caused by Solutions of Sodium Chloride and Sodium Hydroxide.

The experiments with pure sodium hydroxide (prepared from metal and water free from carbon dioxide) will first be described. In these experiments it was found that in presence of a definite concentration of sodium hydroxide, provided this concentration was not below a certain critical value, an absolutely constant osmotic pressure was obtained. Evidently in these cases the alkali concentration was sufficient to arrest the membrane-hydrolysis.†

* These questions are fully dealt with in another paper.

† It will be shown in another paper that this experimental result is in full accord with the theory of the action of membranes in presence of electrolytes possessing a non-dialysing ion.

Below a certain concentration of alkali, the critical value being about $N/1200$ -sodium hydroxide in the case of a 0.4 per cent. Congo-red solution, the pressure gradually decreased, and the previously clear red solution in the osmometer became muddy, showing that hydrolysis was occurring.

The procedure in the first experiment with sodium hydroxide was as follows. The osmometer was filled with a "solution" of Congo-red acid of concentration approximately the same as that used in the third series of experiments on osmotic pressure. This was converted completely into Congo-red salt by means of an $N/90$ -solution of pure sodium hydroxide put outside the osmometer (all entrance of carbon dioxide was, of course, prevented as described previously), the volume of this solution being 1 litre. When the conversion was complete, the alkali solution was replaced by a litre of $N/100$ -sodium hydroxide solution, when a constant pressure was attained after two to three days. The concentration of the alkali solution was then reduced to $N/200$, the pressure thereupon rising, and finally becoming quite constant after a few days. The alkali concentration was successively reduced in the way described, until finally pure water was used, which was changed from time to time until the maximum pressure was attained. The results of an experiment at 25° are shown below:

Concentration of alkali.	Osmotic pressure.
$N/100$	37.0 mm. Hg
$N/200$	57.7 " "
$N/400$	76.3 " "
$N/800$	96.0 " "
$N/1200$	104.6 " "
Pure water	114.5 " " (max. pressure).

In this experiment the final concentration of the Congo-red was 0.395 gram per 100 c.c., for which the calculated value of the osmotic pressure at 25° is 105.5 mm. The observed value in this case exceeds the "calculated value" by about 8 per cent. It is possible that this excess may be partly due to a little alkali still present inside the osmometer, as the maximum pressure was attained about six hours after the $N/1200$ -solution had been replaced by pure water.

A second experiment was made at 25° under similar conditions, but after a constant pressure was obtained with $N/800$ -sodium hydroxide solution, some sodium chloride was added to the outside liquid so as to make it $N/800$ -sodium hydroxide + $N/500$ -sodium chloride. The pressure decreased from the value obtained with $N/800$ -sodium hydroxide alone, and again became constant. Constant pressures were similarly obtained with $N/1600$ -sodium hydroxide + $N/1000$ -sodium chloride, and with $N/3200$ -sodium

hydroxide + $N/2000$ -sodium chloride. After this the concentration of the mixed solution outside the osmometer was decreased daily until it reached the value $N/25,600$ -sodium hydroxide + $N/16,000$ -sodium chloride, when pure water, free from carbon dioxide, was substituted for it, the (final) concentration of the Congo-red solution in the osmometer being determined when the maximum pressure had been obtained and all sodium hydroxide and sodium chloride had been removed. The results of this second experiment are shown in the accompanying table (temperature 25°).

Concentration.	Osmotic pressure. mm. Hg.	Concentration.	Osmotic pressure. mm. Hg.
$N/100$ -NaOH	33.0	$N/800$ -NaOH + $N/500$ -NaCl	61.0
$N/200$ -NaOH	53.0	$N/1600$ -NaOH + $N/1000$ NaCl	79.0
$N/400$ -NaOH	73.0	$N/3200$ -NaOH + $N/2000$ -NaCl	91.0
$N/800$ -NaOH	93.0	Pure water (max. pressure)	107.3

In this experiment the concentration of the Congo-red solution was 0.383 gram per 100 c.c., for which the calculated value of the osmotic pressure at 25° is 102 mm. The observed (maximum) value is therefore about 5 per cent. higher than that calculated for simple undissociated molecules.

It may be remarked that a constant osmotic pressure (that is, absence of membrane-hydrolysis) is obtained with $N/3200$ -sodium hydroxide + $N/2000$ -sodium chloride, although hydrolysis occurs in presence of $N/1600$ -sodium hydroxide alone, which shows that sodium chloride assists the sodium hydroxide in preventing hydrolysis, in spite of the fact (as will be shown presently) that even a fairly strong solution of sodium chloride alone cannot prevent hydrolysis occurring. It may also be remarked that the lowering effect of a mixture of sodium chloride and sodium hydroxide appears to be about the same as that of an equimolecular solution of pure sodium hydroxide. Thus $N/800$ -sodium hydroxide + $N/500$ -sodium chloride, which corresponds with a pressure of 61 mm., is approximately equimolecular with $N/300$ -sodium hydroxide, for which the pressure has very nearly the same value, as may be seen by interpolation from the data given above.

The effect of sodium hydroxide on a more concentrated solution of Congo-red was next investigated. In this experiment the osmometer was filled with a strong solution of Grüber's Congo-red, as used in the fourth series of osmotic pressure measurements. A litre of distilled water, free from carbon dioxide, was placed outside the osmometer, and changed every day for fourteen days, when all the sodium chloride was removed and a maximum pressure of 722 mm. Hg developed. Up to this point the experiment was carried out at room temperature (17°). The pure water was now

replaced by a litre of $N/25$ -sodium hydroxide, and the apparatus suspended in a thermostat at 25° . The pressure quickly dropped, becoming constant at 261 mm. in three days. As in the previous experiments, the constant pressures corresponding with more dilute solutions of sodium hydroxide were determined, and finally the alkali was replaced by pure water, which was changed daily until the maximum pressure was attained. The results are shown in the following table:

Concentration of sodium hydroxide.	Pressure.
$N/25$	261.0 mm.
$N/50$	405.0 "
$N/100$	551.0 "
$N/200$	636.0 "
$N/400$	687.0 "
Pure water (max. pressure)	763.6 "

The final concentration of the Congo-red was 3.10 grams per 100 c.c., for which the calculated value of the osmotic pressure at 25° is 828 mm., the observed value at 25° being thus 92 per cent. of the calculated value. The calculated value at 17° is 806 mm., the observed value (722 mm.) being in this case 89.6 per cent. of the calculated value. The osmotic pressure per molecule of the Congo-red solution appears therefore to increase more rapidly with increase of temperature than would correspond with the simple gas law. The effect of sodium chloride solutions alone on the osmotic pressure of Congo-red was next investigated. It was found, for example, that a normal solution of sodium chloride placed outside the osmometer was sufficient to reduce the osmotic pressure of a saturated solution of Grüber's Congo-red to zero. Under similar conditions a 0.5 per cent. solution of Grüber's Congo-red showed zero pressure, some of the Congo-red salt being precipitated in the osmometer. Another portion of this 0.5 per cent. solution contained in an osmometer immersed in $N/25$ -sodium chloride solution showed a pressure of only 60 mm. of water (about 4.4 mm. Hg), no precipitation occurring in this case.

It was not possible to carry out exact measurements with sodium chloride as in the case of sodium hydroxide, for the pressures always fell slowly after attaining a maximum, owing to progressive hydrolysis.

The following is an example of many similar experiments. An osmometer was filled with a 2.5 per cent. solution of Grüber's Congo-red, and immersed in a litre of pure water, free from carbon dioxide, at room temperature (17°). The greater portion of the sodium chloride was removed by dialysis, which was continued for two days, with three changes of water. The pressure at this point having risen to 203 mm., the outside water was replaced by a

N/25-sodium chloride solution, the pressure immediately falling and becoming fairly steady at 82 mm. in three to four days. The concentration of the outside sodium chloride solution was then successively reduced, and the approximately steady values of the pressure noted. Finally, pure water was used, and changed daily until all salt was dialysed out and the maximum pressure obtained. The results are given below (temperature 17°).

Concentration of sodium chloride.	Pressure.
<i>N</i> /25	82 mm.
<i>N</i> /50	131 "
<i>N</i> /100	191 "
<i>N</i> /200	259 "
Pure water (max. pressure)	365 "

This solution contained 1.713 grams of Congo-red per 100 c.c., for which the calculated value of the pressure at 17° is 446 mm. The observed maximum pressure is only 82 per cent. of this, which is to be accounted for by hydrolysis occurring in the osmometer, the dialysis having lasted thirty-one days in all.

Cause of the Lowering of Pressure produced by Sodium Chloride and Sodium Hydroxide.

If the remarkable effects produced by sodium chloride and sodium hydroxide on the osmotic pressure of a Congo-red solution were due solely, as Bayliss supposes, to an increased aggregation of the Congo-red, we might expect this to show itself in a greatly reduced conductivity. Experiment shows, however, that on mixing a solution of pure Congo-red with enough sodium chloride solution to effect an enormous lowering in the measured osmotic pressure, the conductivity of the mixed solution only shows the usual small deviation from the additive law to be expected in a mixture of two strong electrolytes which slightly influence each other's dissociation. The following data illustrate this, the values given being the conductances (not the specific conductivities), as measured in a certain conductivity cell at 25° (in reciprocal ohms).

Concentration of Congo-red solution = 2.485 per cent.	
10 c.c. of Congo-red solution + 10 c.c. of conductivity water	2.651×10^{-2}
10 c.c. of <i>N</i> /50-sodium chloride + 10 c.c. of conductivity water.....	1.565×10^{-2}
10 c.c. of Congo-red solution + 10 c.c. of <i>N</i> /50-sodium chloride.....	3.940×10^{-2}

It will be seen that the last value is only slightly less than the sum of the two others. From the table given above it will also be seen that a *N*/50-sodium chloride solution lowers the osmotic pressure of a Congo-red solution (containing 1.7 grams of Congo-red per 100 c.c.) from 365 mm. to 131 mm.

Before discussing another possible explanation of the effects produced by sodium chloride and sodium hydroxide on the osmotic

pressure, an account will be given of experiments which have revealed a new and hitherto quite unsuspected phenomenon. It will be shown that when Congo-red is present on one side of a parchment diaphragm, sodium chloride (that is, total Cl^- -ion) does not distribute itself in equal concentration on both sides, although the membrane of parchment paper is perfectly permeable to both Na^+ and Cl^- .

In the first experiment, 5 grams of Merck's Congo-red dissolved in 200 c.c. of distilled water, free from carbon dioxide, were placed inside a parchment dialysing tube suspended in a large beaker containing a litre of pure distilled water. By means of three changes of water the greater portion of the admixed salts was removed, the dialysis being continued for twenty-four hours. Then the outer water was replaced by a litre of $N/10$ -sodium chloride solution. After thirteen days the chlorine content of the inside and outside liquids was determined. This was done by withdrawing 50 c.c. of each by means of a pipette, evaporating to dryness, heating the residues over a Bunsen flame for about a quarter of an hour (by which procedure the Congo-red from the inner liquid was charred, the residue from the outer liquid being subjected to the same process so as to have similar conditions), extracting with hot water, and estimating as silver chloride by precipitation in the usual way. The results were as follows:

Outer liquid: 5.106 grams of sodium chloride per litre.

Inner liquid: 4.478 " " " " "

The concentration of the Congo-red in the inner solution was determined, and found to be 1.074 per cent. At the same time the conductances of a given cell (at 25°) filled with each of the two solutions were determined, with the following results:

Liquid used.	Conductance of cell (in mhos).
Inner	12.99×10^{-2}
Outer	12.50×10^{-2}

The foregoing experiment shows that after thirteen days' dialysis the concentration of the sodium chloride in the outer solution is undoubtedly greater than that in the inner solution containing the Congo-red. In order to obviate the natural objection that in the above experiment the sodium chloride might have been still diffusing into the inner solution, two further experiments were made, in which the concentration of the sodium chloride was initially higher in the inner solution (containing the Congo-red). In the first experiment, a mixture of approximately 2.8 grams of pure Congo-red and 7.2 grams of sodium chloride dissolved in 200 c.c. of conductivity water was placed inside the dialyser and a litre of pure water outside. After six days' dialysis the inner

liquid contained 5.136 grams of sodium chloride per litre, and the outer liquid 5.728 grams. The concentration of Congo-red in the inner liquid was 0.917 gram per 100 c.c.

In the next experiment the inner solution consisted initially of about 2.1 grams of pure Congo-red and 3.9 grams of sodium chloride, dissolved in 200 c.c. of pure water, the outer liquid being, as before, a litre of pure water. After five days' dialysis the inner liquid contained 2.53 grams of sodium chloride per litre, and the outer liquid 3.03 grams. The concentration of Congo-red in the inner liquid was 0.875 gram per 100 c.c.

These results show that we are dealing with a reversible equilibrium, and that the equilibrium state corresponds with a greater concentration of sodium chloride on the opposite side of the membrane to the Congo-red. Now this unequal distribution will clearly set up, in the osmotic pressure experiments, a counter osmotic pressure, which will make the observed osmotic pressure lower than that corresponding with the Congo-red.

The following calculation will serve to indicate the amount of this counter pressure. Consider the first of the three experiments given above. The osmotic pressure (at 0°) of the outer solution of sodium chloride will be $22.4 \times 760 \times \frac{5.106}{58.5} \times i_1$ mm. Hg, where i_1 = van't Hoff's factor for a solution of this concentration. The osmotic pressure due to the sodium chloride concentration in the inner solution will be similarly $22.4 \times 760 \times \frac{4.478}{58.5} \times i_2$. Putting $i_1 = i_2 = 1.85$, we get for the counter osmotic pressure at 17° (in mm. Hg):

$$22.4 \times 760 \times \frac{290}{273} \times \frac{0.628}{58.5} \times 1.85,$$

or 359 mm. Now the osmotic pressure at 17° of the Congo-red solution present in this experiment (containing 1.074 per cent. of Congo-red) would, according to the data given previously, amount to about 290 mm. Hence the approximately $N/12$ -solution of sodium chloride of this experiment would more than suffice to annul the osmotic pressure of the 1.074 per cent. Congo-red solution.

These considerations show that the unequal concentrations of an electrolyte (such as sodium chloride) with a common ion, which exist at equilibrium on either side of the membrane in presence of Congo-red on one side only, are sufficient to explain the apparent lowering effect of these electrolytes on the osmotic pressure of a solution of Congo-red. The results obtained by Bayliss with Congo-red and by Biltz and Vegesack with benzopurpurine are now intelligible, without any special assumptions as to the effect of the

electrolytes on the aggregation of the molecules of the electrolytic colloid. It is, of course, possible that some such aggregation may occur, but the results of the present paper show that the unequal distribution discovered in the present work is a sufficient explanation.

This unequal distribution can be easily shown to be thermodynamically necessary.* Suppose we have on one side of a membrane a solution of the salt NaX, the membrane being supposed permeable to Na', but impermeable to X' and to undissociated NaX. Let now a solution of sodium chloride be put on the other side of the membrane, the latter being supposed permeable to Cl' and sodium chloride. The initial state can be represented as follows:



Na'- and Cl'-ions will now begin to diffuse from (2) to (1). Equilibrium will be attained when the work gained by the isothermal reversible transport of δn gram-mol. Cl'-ion from (2) to (1) is equal to the work required for the isothermal reversible transport of δn gram-mol. Na'-ion from (2) to (1), that is:

$$\delta n \cdot RT \log \frac{[\text{Na}']_1}{[\text{Na}']_2} = \delta n \cdot RT \log \frac{[\text{Cl}']_2}{[\text{Cl}']_1}$$

where the square brackets indicate molar concentrations at equilibrium.

Hence:

$$\frac{[\text{Na}']_1}{[\text{Na}']_2} = \frac{[\text{Cl}']_2}{[\text{Cl}']_1}$$

Now since at equilibrium we have in (1) both NaX and sodium chloride, in general $[\text{Na}']_1 > [\text{Cl}']_1$. Also from the above $[\text{Cl}']_2 = [\text{Na}']_1 \times [\text{Cl}']_1$. Hence $[\text{Cl}']_2 > [\text{Cl}']_1$. For highly dissociated solutions we shall therefore find the total "chlorine" concentration in (2) greater than that in (1). This has actually been shown to be the case in the experiments described in this paper.

Since the conclusion of this work a further paper has been published by Biltz and von Vegesack (*Zeitsch. physikal. Chem.*, 1910, **73**, 481), in which, contrary to their previous work, they find that both benzopurpurine and Congo-red exhibit considerable osmotic pressures. From measurements made in presence of other electrolytes they obtain values for the osmotic pressure which lead them to the conclusion that the true value for the molecular weight of Congo-red in aqueous solution is in the neighbourhood of 2000. It may be pointed out, however, that this conclusion is not binding,

* In a paper which is ready for publication, the thermodynamical theory of such 'membrane equilibria' will be more fully considered.

for, as shown in the present paper, the other electrolytes present will be unequally distributed on either side of the membrane, and so give rise to a counter osmotic pressure. The experimentally observed pressure will therefore be lower, and the value calculated for the molecular weight higher, than that which would be obtained if no foreign electrolytes were present.

Summary of Results.

(1) It has been shown that Congo-red gives against pure water an osmotic pressure agreeing approximately with that which would be obtained if it existed in aqueous solution as single undissociated molecules. The values found agree as to order of magnitude with those recorded by Bayliss (*loc. cit.*).

(2) Conductivity measurements show that Congo-red is a highly dissociated electrolyte.

(3) From the results of the conductivity measurements it is concluded that Congo-red exists in aqueous solution very largely as a simple and considerably dissociated salt.

(4) On dialysis, both Congo-red and benzopurpurine-4B undergo a peculiar "membrane-hydrolysis," sodium ions in company with hydroxyl ions diffusing out, and the free acids, acid salts, or some other insoluble phase being gradually aggregated or thrown out of solution inside the dialyser or osmometer. This "membrane-hydrolysis" can be prevented by solutions of alkali hydroxide above a certain definite concentration, the latter varying with the temperature and the concentration of the dye-salt. The gradual fall of osmotic pressure observed with pure water as external liquid is due to the membrane-hydrolysis.

(5) A consideration of the results given under (1) to (4) show that the osmotic pressures observed do not correspond with an ordinary state of osmotic equilibrium, inasmuch as the otherwise diffusible sodium ions are arrested in their diffusion across the membrane by potential differences, and do actually pass slowly through, to a certain extent, in company with hydroxyl ions.

(6) The osmotic pressure of a solution of Congo-red is lowered by sodium hydroxide and sodium chloride, the fall of pressure increasing with the concentration of these electrolytes.

(7) Sodium chloride distributes itself in unequal concentrations on either side of the parchment paper membrane when Congo-red is present on one side. A reversible ionic equilibrium is produced, the concentration of the sodium chloride at equilibrium being higher on the side of the membrane opposite to that in which the Congo-red is present.

(8) This highly important and interesting "membrane-equili-

brium" has been shown to be thermodynamically necessary, and is part of a group of very general and important phenomena.

(9) The counter osmotic pressure caused by the unequal distribution of sodium chloride (or sodium hydroxide) referred to in (7) is probably to a very large extent sufficient to account for the effects described under (6).

(10) Measurements of the osmotic pressures exhibited by an electrolyte with a non-dialysable ion in presence of other electrolytes cannot be used to draw conclusions concerning the molecular state in solution of the former, unless account be taken of the unequal distribution referred to in (7) and (9).

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