

XXVI. THE USE OF LITMUS PAPER AS A QUANTITATIVE INDICATOR OF REACTION.

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In dealing with nearly neutral solutions of feebly dissociated electrolytes, especially when they are highly coloured, it is frequently convenient to gauge reaction by the use of litmus paper, and so avoid either electric measurement or the introduction of a fluid indicator into a comparatively large volume of fluid. And it has been found that if suitable precautions are taken quite reliable information can be obtained as to the H^+ ion concentration of such fluids in this way. The apparently contradictory results obtained in such cases by using different commercial samples of litmus paper, immersing the paper for a short or a long time in the fluid, using one drop on a piece of paper or a small piece of paper in a considerable volume of fluid, and so on, are capable of simple interpretation.

The precautions to be observed are all those which apply to the use of litmus solution with the addition of those necessitated by the fact that it is applied to paper. As an example of the former class the "neutral salt effect" may be mentioned: the "time factor" is typical of the latter.

EXPERIMENTAL.

The following solutions were used:

Ammonia-ammonium chloride mixtures. A solution of ammonium chloride and ammonia was prepared so as to be approximately 3 N with respect to the former constituent and $3/32$ N with respect to the latter. Dilutions with boiled distilled water were made $\times 2$, $\times 4$, $\times 16$, $\times 32$. According to Fels [1904] these solutions should have the same P_H^+ value at $18^\circ C$. The values obtained on examination in the hydrogen cell using a calomel electrode were 8.14, 8.14, 8.13, 8.14 respectively, while 8.12 was that obtained for the undiluted material.

Acetic acid-sodium acetate mixtures. A solution of commercial sodium acetate 0.61 N, containing acetic acid corresponding to 0.61/64 N was diluted $\times 1$, $\times 2$, $\times 8$ and $\times 16$. These solutions should have the same P_H^+ value very closely. The values found were 6.25, 6.20, 6.27, 6.23.

Neutral phosphate solution. This was a mixture of 68.8 c.c. of Sørensen's N/15 Na_2HPO_4 solution, and 131.2 c.c. of his N/15 KH_2PO_4 solution. The P_H^+ value determined was 7.05. The figure at present accepted for absolute neutrality is $P_H^+ = 7.07$ [Sørensen, 1909].

Solutions of serum globulin and Witte peptone were also examined, but they presented, in addition to the phenomena observed with solutions free from proteins and their primary disintegration products, others of a more complex and different order. For this reason it was decided to deal with them more fully in a separate communication and cite here only one or two typical cases.

Litmus solution. The reaction between litmus solution and the solution with which it is mixed is practically instantaneous. In saline solutions it gives an indication of the reaction of the solution subject only to a correction for the "neutral salt effect": for it is well known that, for solutions of the same H⁺ ion concentration, indicators in the presence of larger quantities of neutral salt give slightly different colours from those which they give when little salt is present [Sørensen, 1912].

The litmus tincture used was itself apparently neutral, or very nearly so, since it gave almost exactly the same tint when added in the same quantity to equal volumes of boiled distilled water and neutral phosphate solution.

The ammonia-ammonium chloride mixtures all gave precisely the same tint as far as could be observed with litmus solution, with the exception of the $\times 32$ dilution, 100 c.c. of which required 0.02 c.c. N/10 NaOH to bring the colour to the same tint as that of the others. The acetic acid-sodium acetate mixtures, on the other hand, gave progressively pinker solutions with litmus tincture as dilution increased. From this observation I think it is legitimate to draw the conclusion that the neutral salt effect is observable in all the acetic acid-acetate mixtures, and only in one case in the ammonia-ammonium chloride mixtures. This is probably due to the fact that the P_H^+ value of the latter mixture is near the alkaline limit of the sensitive range of litmus.

"Equilibrium tint." If a small piece of litmus paper be introduced into a comparatively large volume of any of these solutions it will, after a lapse of a longer or shorter time, assume a definite tint which undergoes no further change. This it is proposed to call the "equilibrium tint" of the paper used in the solution under consideration.

"*Reaction inertia.*" A solution whose H^+ ion concentration is but little affected by the addition of H^+ or OH^- ions, say in the form of hydrochloric acid or caustic soda, can be said to have a greater "reaction inertia" than pure water or a dilute solution of a completely dissociated electrolyte, whose H^+ ion concentration would be profoundly affected by such an addition.

The above acetic acid-sodium acetate mixtures, although of the same absolute reaction ($P_H^+ = 6.2$), differ very much in reaction inertia, as is in fact patent from their composition. The number of c.c. of N/10 NaOH solution required to bring 100 c.c. of any one solution from $P_H^+ = 6.2$ to $P_H^+ = 8.1$ may be for the purpose of this note considered as a numerical expression of this property. A 50 c.c. sample of each dilution being taken and equal quantities of litmus tincture added to each they presented different colours but had $P_H^+ = 6.2$ in each case as described above. A 50 c.c. sample of an ammonia-ammonium chloride mixture to which an equal quantity of litmus tincture was added furnished a standard tint $P_H^+ = 8.1$. The acetic acid-acetate samples were then titrated with N/10 NaOH to this standard. The results are tabulated:

Acetic acid-sodium acetate mixture.

Dilution	Reaction inertia
1	8
2	4
8	1
16	0.5

In a similar manner titrations of the ammonia-ammonium chloride mixtures were undertaken from $P_H^+ = 8.1$ to $P_H^+ = 6.2$ using N/10 HCl, and from these results a similar table may be prepared. The standard pink tint ($P_H^+ = 6.2$) was that of the undiluted acetic acid-acetate mixture.

Ammonia-ammonium chloride mixture.

Dilution	Reaction inertia
1	90
2	45
4	22
16	6
32	3

With N/15 phosphate mixtures it was found that 20 c.c. of a solution $P_H^+ = 8.1$ required 0.9 c.c. N HCl to bring the reaction to $P_H^+ = 6.2$. The "reaction inertia" for N/15 phosphate solution may then be taken as $0.9 \times 5 \times 10 = 45$.

OBSERVATIONS WITH LITMUS PAPER.

Four commercial varieties of litmus paper were examined, each providing a "red" and a "blue" paper. Three brands were glazed with the litmus applied to one side only, and one soft and absorbent.

Of the three that were glazed, No. 1 was dyed heavily and apparently loaded with a material of considerable "reaction inertia"; No. 2 was not so heavily dyed though the grain of the paper was similar; No. 3 was faintly dyed on a perfectly smooth paper, presenting a homogeneous appearance. The unglazed paper was labelled No. 4.

Colour tint of paper after 24 hours immersion. Small pieces of each paper about 25 mm.² area were immersed for 24 hours in 240 c.c. of ammonia-ammonium chloride solution of each dilution. At the end of that time all the pieces of paper were of the same tint.

Only one difference could be observed. The heavily loaded paper, No. 1 (blue) was more deeply dyed in the more concentrated solutions than in those more dilute. The adsorption equilibrium seems to shift in the direction of greater adsorption of dye with increasing concentration of salt at constant reaction. A special experiment ratified this conclusion. Pieces of paper No. 1 blue after prolonged immersion in solutions $P_{\text{H}}^{+} = 8.1$ containing 15, 7.5, 3.8, 1.9, 0.5 per cent. NaCl gave up progressively more dye to the solution as dilution increased, though in similar solutions of $P_{\text{H}}^{+} = 6.1$ no such change could be definitely observed.

Paper No. 1 red immersed in N/15 phosphate solutions $P_{\text{H}}^{+} = 4.5, 7.1, 9.2$ respectively gave up very markedly increased quantities of litmus to the solution with increasing alkalinity. In parallel experiments the litmus found in the alkaline solution was 5-6 times that found in the acid solution.

This, in conjunction with many similar observations in this connection, has led me to the conclusion that in similar solutions the adsorption equilibrium changes in the direction of diminished adsorption of litmus by paper in solutions of increasing alkalinity.

In the acetic acid-sodium acetate solutions similarly examined small pieces of each paper gave the same tint after 24 hours immersion, only in one and the same dilution. Passing from one dilution to another it was found that with increasing dilution just that change could be noticed in the equilibrium tint which, as far as could be judged corresponded to the variation in tint with litmus tincture due to the "neutral salt effect" described above.

Observation of rate of change of colour. In the above experiments the

time taken for a piece of litmus paper to acquire its equilibrium tint was seen to vary very considerably. The same paper changed more rapidly in a solution of greater reaction inertia than in one of less reaction inertia in all cases, and, as one would expect, a paper took longer to reach its equilibrium tint when the colour change from its original to its final tint was great. For instance a red paper (No. 1 red) took several hours to come to its equilibrium tint in the most dilute alkaline solution, while a blue paper did so in a few minutes as it was already almost at that tint.

Observation of effect of exposure to the air. It will have been noticed that the two main test mixtures chosen each have a volatile constituent. Pieces of paper that have been immersed for sufficient length of time to assume the equilibrium tint, change that tint rapidly on exposure to the air in such a case. The reddening of blue paper due to the volatilisation of ammonia is naturally more rapid than the blueing of red paper due to acetic acid evaporation.

“Amphoteric solutions.” Dealing with approximately neutral solutions a distinction is frequently made between a solution that is “merely neutral” and one that is “amphoteric.” Milk, urine, and solutions of phosphates are regarded as typical examples of the latter class and they are said to “turn blue litmus paper red, and red litmus paper blue.” What is actually seen may be better described as the “reddening of blue litmus paper and the blueing of red litmus paper.” The “merely neutral” solution is one of low reaction inertia. A drop placed on paper wets it but only to a very small extent alters its reaction. The colour of the paper therefore remains unchanged. The “amphoteric” solution, on the other hand, has a high reaction inertia. It usually contains a salt of a feebly dissociated acid or base and, being neutral or nearly so, alters the reaction of litmus paper with which it is brought into contact in the direction of neutrality.

It would naturally be expected that small pieces of paper, red and blue, would, when immersed in a large volume of an amphoteric solution, ultimately assume the same tint. During the first few hours of observation it is seen that the blue paper is becoming progressively redder, and the red paper bluer, but before equilibrium can be attained certain disturbing influences may be encountered. For instance if protein be present the colour of the paper will be interfered with. And, again, since the range of reaction through which the litmus changes is different in the two cases, the losses of litmus to the solution will not be the same. A blue and a red sample of the quickly reacting paper No. 4 were seen to assume very nearly the same tint in a large volume of milk in 24 hours.

Preliminary observations in protein solutions. In solutions of protein, peptone and the like the use of litmus paper has, as far as my experience goes, always indicated the solution to be more alkaline than it really is. For instance an acid 4 per cent. solution of Witte peptone containing 1 per cent. of sodium chloride, and 1 c.c. per cent. of normal hydrochloric acid was found by electric measurement to have $P_H^+ = 6.5$. In a tintometer arranged to compensate for the colour of the Witte peptone [Walpole, 1910] and using neutral red as indicator, the P_H^+ value was made out to be the same as that of a phosphate mixture $P = 6.8^1$. A neutral N/15 phosphate solution was prepared ($P_H^+ = 7.07$) and a comparison instituted between this solution and the acid Witte peptone solution above. It will have been noticed that neither contains a volatile constituent.

Three papers were taken (No. 4 red, No. 2 blue and No. 3 red) and one slip of each paper dropped into a large volume of each fluid. They were removed at intervals, laid on white paper and examined in diffused daylight.

The unglazed, quickly-reacting paper (No. 4 red) gave with the acid peptone solution a bluer tinge almost at once than the corresponding paper in the phosphate solution and this relation continued indefinitely. The blue paper No. 2 was always bluer in the acid peptone solution: the red paper No. 3 lost most of its colour in the phosphate solution before the progressively bluer paper in the acid Witte peptone solution could match it.

In another case a faintly alkaline 4 per cent. solution of Witte peptone of reaction $P_H^+ = 7.3$ gave with papers No. 2 (red and blue), No. 3 (red and blue), No. 4 (red and blue) the same equilibrium tint, as nearly as could be judged, as any of the ammonia-ammonium chloride dilutions examined, which had $P_H^+ = 8.1$ in each case.

EXAMPLES.

By neglecting to consider these secondary influences it is possible to obtain some bewildering results. For instance we may take two alkaline solutions having the same reaction, $P_H^+ = 8.1$; A is a solution of ammonium chloride and ammonia, 3N with respect to the former and 3/32 N with respect to the latter; B is this solution diluted 32 times and the "reaction inertia" of A is about 30 times that of B; C is a neutral phosphate solution;

¹ In the same apparatus using litmus tincture as indicator no real match could be obtained but the indications were that the peptone solution was more alkaline than a phosphate mixture $P_H^+ = 9$.

and *D* is pure water. Red litmus paper No. 1 dipped into the solutions, removed, and examined shows at once a bright blue colour with *A*, red with *B*, a bluish tinge with *C* and red with *D*. Blue paper No. 4 shows blue rapidly turning red with *A*, blue slowly turning not quite so red with *B*, red with *C*, and blue with *D*; while blue paper No. 2 shows with *A* and *B* blue very slowly turning red, and with *C* and *D* slight change towards red, less marked in the latter case.

The following interpretations suggest themselves. With red paper No. 1 the concentrated solution *A* neutralises the acid red material of the heavily loaded paper, and in spite of the glazing shows a pronounced blue colour at once; with *B* the reaction of the absorbed fluid is determined more by the paper than by the substances originally dissolved in the water, and the evaporation of the free ammonia has been almost complete long before any great effect would have been observed even if the paper had been bathed in an excess of solution. Solution *C* gives the neutral tint appearing blue on a red paper, while distilled water leaves the paper unaffected.

With blue paper No. 4 the attainment of equilibrium is very much more rapid because the paper is not glazed, and the rate of evaporation of ammonia is also accelerated though to nothing like the same extent. Blue paper No. 2 has a smaller "reaction inertia" than No. 1, besides it is already blue. It differs from No. 4 in that it is glazed. The instances cited above are exaggerated and, in the main, their interpretation obvious; but these factors operating in a minor degree may lead to incorrect conclusions.

SUMMARY.

Except only in so far as the colour is influenced by the presence of neutral salts, the reaction of a solution is indicated by the colour of a piece of litmus paper which has been immersed in an excess of the fluid until no further change can be observed. The correction for neutral salt effect is a small one and is of the same order as that applying to litmus solution when used in the same way.

When the *quantity of solution used is limited in amount*, as when a drop of solution is placed on the paper and the effect observed, the following secondary influences determine the colour changes seen—

(1) Gradual attainment of reaction equilibrium between the litmus on the paper and the solution; more rapid with unglazed paper than with paper that is glazed.

(2) The actual effect of the reaction of the material in the paper on the

limited amount of solution used. This will, with the same paper, be most marked with a solution of lowest "reaction inertia." It is obviously fallacious to test the reaction of tap water by watching the effect of one drop of water on a heavily dyed paper.

(3) The "reaction inertia" of the paper. A thick heavily loaded paper has more effect in modifying the reaction of a drop or two of a solution than a thin paper lightly dyed with a litmus solution of low reaction inertia.

(4) The effect of exposure to the air. The evaporation or oxidation of some constituent of the solution affecting its reaction, will naturally produce a corresponding effect.

(5) The liberation of indicator from the paper, which is greater in the absence of neutral electrolytes and is more marked in alkaline than in acid solutions.

Determination of reaction of nearly neutral solutions of *proteins* and *their decomposition products* by means of litmus paper is very difficult. Such cases have not been dealt with fully here, but will form the subject of a later communication. The general effect is to indicate that the solution is more alkaline than it really is.

The same phenomena may be observed with litmus tincture to a much more marked degree. For this reason it has been frequently described as useless in such cases.

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