

The Preparation of Permanently Non-acid Formalin for Preserving Calcareous Specimens.

By

W. R. G. Atkins, O.B.E., Sc.D, F.I.C.,

Head of the Department of General Physiology at the Plymouth Laboratory.

ATTENTION was directed by the Plankton Committee to the desirability of using neutral formalin for preserving calcareous and other specimens likely to be injured by the acidity usually found in the ordinary preparation.

Chamberlain, in *Methods of Plant Histology*, 1915 Ed., recommends the distillation of the formaldehyde solution after addition of sodium bicarbonate. The solution thus obtained is said to develop acidity on standing.

Lee, in the *Microtomists' Vade-mecum*, 1921 Ed., recommends neutralising "by saturation with magnesium carbonate or sodium carbonate." Of the two magnesium carbonate may appear preferable, since it only dissolves appreciably as acidity is developed and does not render the liquid very strongly alkaline, though it may reach pH10, whereas the sodium salt in slight excess gives a much more pronounced alkaline reaction, which is likely to damage the soft parts of specimens since it makes the skin very slimy. Against the use of the magnesium carbonate must be set the fact that being a solid it is liable to settle out and is not attacked more readily than the calcareous plankton. Sodium bicarbonate added to the acid formalin in excess has the advantages that it acts readily and uniformly, since it is in solution, and it gives no undue alkalinity, the value being about pH8.2, which is close to that of sea water. On the other hand, additional carbon dioxide, set free in neutralising the formalin, remains in solution and the alkalinity tends to fall below pH8. Thus there will be a slight solvent action upon the calcium carbonate of the organisms.

It appears that this can be obviated by the use of borax. This in M/20 solution is no more alkaline than pH9.24, which is unlikely to cause trouble through being too alkaline. Neither concentration nor dilution within wide limits alter this appreciably. The neutralisation of a little formic or other acid merely results in the liberation of boric acid and slightly lowers the pH value, which, however, only reaches pH8.2 when

approximately half the boric acid has been set free. It is not, therefore, till this, the bicarbonate, stage has been passed that there will be any appreciable amount of hydrogen ion to attack the specimens. About five to ten grams of borax per litre should be added, or considerably less may be required. It need not be weighed out, but the resulting solution should be tested by adding phenolphthalein, when the appearance of a good red colour denotes a reaction of pH8.5-9.0. The tint may be compared with that given by the borax alone in water. With thymol blue a slaty blue colour is shown at pH9; this is a good region in which to have the reaction of the formalin. Strong formalin should be diluted in the test tube before adding the indicator.

That the quantities of acid found in fresh and stored formalin are by no means negligible may be seen by titrating with sodium hydroxide. A sample of 30 per cent formaldehyde was found to contain 0.51 grams of acid per litre, calculated as formic acid. The carboy 40 per cent formaldehyde was at pH2.8. Dilute "5 per cent formalin," viz. that percentage of the laboratory stock 30 per cent formaldehyde, after being stored with fish for fifteen years was at pH5.0, and its titratable acid amounted to 1.26 grams per litre. This solution had a considerable buffer action while a precipitate appeared in the solution, probably an organic product derived from the fish. The acid of the "5 per cent formalin" should only be 0.025 grams calculating from its original strength and the dilution.

By the help of indicators formalin may be observed to produce acid in quite a short time. About two litres of 30 per cent formaldehyde were neutralised and brought to slight alkalinity, pH8.0, by means of sodium hydroxide. One portion stood in the warmth and light of a south window, the other under the bench in darkness. The condition after various times is shown below.

Time in days from Aug. 9.	In dark. pH.	In sunlight and warmth. pH.
0	8.0	8.0
20	7.0	5.9
56	6.4	5.2

The acidity of the latter is accordingly more than ten times as great as that of the former. This appears to prove that light as well as heat has been concerned in effecting the change, for were the mean temperature of the insulated solution as much as 10° C. above that of the other, it would only result in the rate of production of acid being doubled or trebled, and the temperature difference is certainly an overestimate.

This development of free acidity is in contrast to the behaviour of 5 per cent formalin rendered alkaline by borax, so that it was at pH8.8-9.0.

After insolation for thirty-six days from August 29th, it was at pH8.95, showing that it had remained constant in reaction, nor was there any appreciable change in a further period of six months. Since 5 per cent formalin was found to have developed about 1.26 grams of acid in fifteen years and borax neutralises almost one-fourth of its weight of formic acid it is evident that an amount of 5 grams per litre would avoid acidity for over this period, or longer with a solution kept in the dark.

The distillation of 40 per cent formaldehyde was also tried to test the acidity of the product. When the liquid at pH2.8 was distilled through a Liebig condenser the distillate was also at pH2.8.

On adding sodium bicarbonate in excess, so that the liquid was at about pH8.2, and distilling, the first portions that came over were at pH5, the greater bulk at pH2.8. The residue in the flask was at pH5.

Similarly formalin distilled with excess of sodium hydroxide became acid and gave an acid distillate. With magnesium carbonate in excess and renewed when dissolved the liquid in the flask was maintained at pH8-9, but even then the distillate was very uniformly at pH4.4, due in part probably to carbonic acid.

In this connection attention may be drawn to the results obtained by R. K. S. Lim (Proc. Physiol. Soc. in *J. Physiol.*, **53**, ciii, 1920), who showed that fixation in formalin of mucous membranes bearing goblet cells, resulted in an abnormal appearance through discharge of the mucinogen. With formalin neutralised by means of sodium hydroxide this does not occur.

SUMMARY.

Formalin, which is permanently non-acid and only slightly alkaline, close to pH9, may be prepared by the addition of borax till a good red colour is shown with phenolphthalein, or a slaty blue with thymol blue, when added to the diluted formalin.

Distillation of formalin from solid magnesium carbonate gives an acid product, which is at pH4.4.

Formalin neutralised with sodium hydroxide becomes acid on standing, the change being more rapid in sunlight than in the dark. A reaction of pH5.2 was reached in fifty-six days in light, the initial value being pH8.0. Commercial formalin, "40 per cent," may be as acid as pH2.8.