A SPOT TEST FOR DISTINGUISHING FORMALIN FROM ALCOHOL SOLUTIONS

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ABSTRACT—Formalin and alcohol are routinely used as preservatives for biological specimens in museum collections and biological and medical laboratories. In some cases the identity of the preservative fluid that a specimen is contained in is not known, but must be discovered before the specimens can be transferred to an appropriate permanent preservative.

The traditional method for distinguishing between formalin and alcohol solutions has been to sniff the preservative. Because formaldehyde is irritating, toxic, and probably carcinogenic, a safer test for distinguishing formalin from alcohol was developed.

The test employs strips of filter paper containing an acid/base indicator and a mixture of sodium sulphite and sodium metabisulphite. These test strips respond rapidly to aqueous formalin solutions containing more than about 1.5% formalin by changing colour from yellow to red. They will not respond to the low levels of formalin that are present in most alcoholic preservative fluids from prior fixation in formalin, and hence will rapidly distinguish formalin solutions from alcohol solutions.

The test strips are inexpensive and simple to make and to use.

Introduction

Formalin and alcohol are routinely used as preservatives for biological specimens in museum collections and medical and biological laboratories. Frequently specimens are initially fixed in a 5% or 10% solution of formalin in water, then washed in water and transferred to 40 to 75% ethanol or isopropanol. Per cent formalin is used in this paper to mean per cent, volume by volume, of standard 37 weight per cent (40 volume per cent) formaldehyde solution in the specified solvent; per cent of alcohol means per cent, volume by volume, of alcohol in water. For recent, staff-collected specimens, the kind of preservative the specimens are currently stored in is known, but in the case of specimens donated by other museums, collectors, or biological investigators, or held in old, uncatalogued lots, the identity of the preservative is often not known. The nature of the preservative must be discovered before the specimens can be transferred to the appropriate permanent preservative.

The time-honored method for distinguishing between formalin, isopropanol, and ethanol solutions has been to sniff the preservative. This test has several disadvantages:

1) The acuity of the nose declines after sniffing several samples, until one is unable to identify any further samples.

2) Formalin and isopropanol, and even ethanol to some people, are disagreeable to smell. Formaldehyde irritates the nose and eyes and causes them to water. If the specimens are poorly preserved, or are in strong formalin, the test will be even more disagreeable.

3) Formalin and isopropanol are toxic. The inhalation of formaldehyde and isopropanol can be expected to stress the body and to impose a load on the liver. Some people develop allergic responses to formalin. Inhalation of formaldehyde causes irritation of the mucous membranes and can cause cough, dysphagia, bronchitis, pneumonia, edema or spasm of the larynx, and, uncommonly, pulmonary edema (Gosselin, et al., 1976). Perera and Petito (1982) reviewed the scientific literature pertaining to the possible carcinogenicity of formaldehyde and concluded that for practical purposes formaldehyde should be considered to be carcinogenic. More recent studies have supported this conclusion (F. Perera, pers. comm., 1985).

For the above reasons it is desirable to reduce exposure to these substances to a minimum. However, for an alternative to the olfactory test to be effective it must satisfy a number of requirements. Criteria for an acceptable test include:

1) It must clearly distinguish between formalin and alcohol solutions.

2) Alcohol solutions containing trace amounts of formaldehyde must not be confused with formalin solutions.

3) It must be non-toxic and non-irritating.

4) It must be simple to use. Most users would not bother with complicated tests. The test should be immediately understandable to technicians, students, and volunteers with no background in chemistry.

5) It must be quick. Slow test methods would interfere

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with efficient work flow in the lab.

6) It must not adversely affect the specimens or require use of a large amount of the preservative.

7) It must be inexpensive. Most museums have modest budgets.

Method

The requirements for speed and simplicity are satisfied by the use of a test employing suitably treated strips of paper that change colour on exposure to formalin.

There are numerous methods available for both the qualitative and quantitative analysis of formaldehyde (Walker, 1975). The method chosen for adaptation to strip testing was the sodium sulphite (Na_2SO_3) method of quantitative analysis. This method is based on the reaction of formaldehyde with sodium sulphite to form the formaldehyde-bisulphite addition product and sodium hydroxide:

$$CH_2O_{(aq)} + Na_2SO_3 + H_2O \rightarrow CH_2(NaSO_3)OH + NaOH$$

The hydroxide produced can be detected by an acid/base indicator. This reaction is rapid at room temperature, requires only solid reagents, is relatively specific for formaldehyde, and hence is well suited to being used as a basis for a paper strip test for formalin.

Two factors influenced the choice of the indicator to be used: 1) the pH transition range must be beyond the pH ranges encountered in buffered preservative fluids yet be within the range that the test reaction can produce; 2) The colour change must be readily discernible, preferably even for colour-blind people. Both of these requirements are satisfied by 5-(4-nitrophenylazo)salicylic acid, also known as either Alizarin Yellow R or Mordant Orange 1. Its pH transition range is 10.1 to 12.0 and the colour change from the acid to the base form is yellow to red (Weast, 1976).

The use of sodium sulphite as the test reagent produced test strips that were too sensitive. They gave a full response to solutions of less than 0.5% formalin in 50% isopropanol. Since many preservative fluids that are essentially alcohol solutions contain varying amounts of

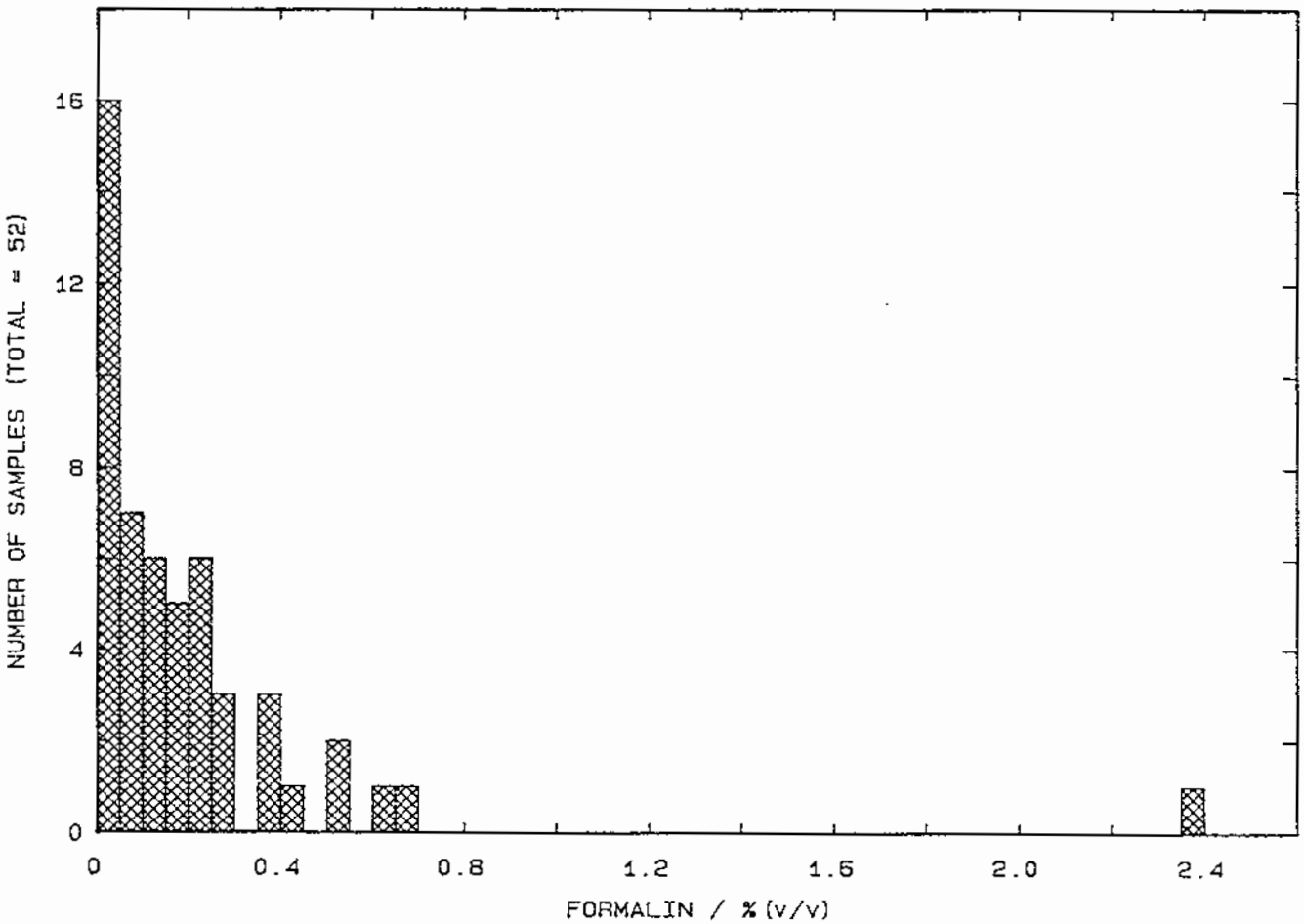

Fig. 1. The distribution of formalin concentration in 52 random samples from the National Museum of Natural Sciences Ichthyology Collection.

Table 1 Approximate ranges of sensitivity for test strips made with a 0.70 M Na_2SO_3 and 0.15 M $Na_2S_2O_5$ solution

Solvent	Percentage Formalin Concentration That Shows:	
	Negligible Response	Full Response
Water	0.5	1.4
50% Isopropanol	0.5	2
75% Ethanol	1.5	4

residual formaldehyde from the fixing process, these strips could not distinguish what was essentially a formalin solution from what was essentially an alcohol solution. To determine what level of formalin concentration should be used to differentiate a formalin solution from an alcohol solution, the formaldehyde concentration in 52 samples chosen at random from the National Museum of Natural Sciences Ichthyology Collection was determined. The results are given in the form of a histogram in Figure 1. The highest concentration, 2.38%, was found to be a formalin in water solution containing no alcohol. Consequently, all specimens in alcohol solutions contained less than 0.7% formalin and hence, 1% would be a reasonable level to distinguish between formalin and alcohol solutions.

The sensitivity of the papers can be most easily varied by replacing some of the sodium sulphite with sodium metabisulphite ($Na_2S_2O_5$). A formulation using 0.7 M Na_2SO_3 and 0.15 M $Na_2S_2O_5$ provided the range of sensitivity shown in Table 1.

Materials

1) Filter paper sheets 46 × 57 cm, medium porosity
2) 5-(4-nitrophenylazo)salicylic acid
3) Sodium sulphite
4) Sodium metabisulphite
5) Ethanol

All materials are available from laboratory suppliers.

Procedure

Producing the test strips simply involves impregnating sheets of filter paper—first with the indicator solution and secondly with a solution of the test reagents. Following impregnation, individual strips are cut from the sheets. Figure 2 is a schematic illustration of a filter paper sheet as it will appear following the second impregnation step, that is, when being unrolled during step 7 of this procedure. Exact dimensions are not important, but those given here were found to give satisfactory results. The procedure is as follows:

1) Cut filter paper sheets into 11 × 57 cm sheets.

2) Tightly roll a single 11 × 57 cm sheet lengthwise onto a section of glass tubing. Tubing having a diameter of 2 to 3 cm is a convenient size for this. Apply transparent tape to completely cover the bottom 5 cm of filter paper.

3) Place the taped end of the roll into a solution containing 0.5 g of the indicator 5-(4-nitrophenylazo)salicylic acid per 100 ml of 95% ethanol.

4) When the indicator has risen 4 to 5 cm in the paper remove the tape and unroll the sheet. Set the sheet aside to dry. To ensure consistency among the finished test strips it is important that, while it is drying, the sheet should be in a position such that no area of the sheet touches another area of the sheet.

5) When dry, roll the sheet again, in the same manner as was done in Step 2, and tape over the bottom 2 cm.

6) Dip the roll into the test reagent solution until the solution has risen about 2 cm in the paper. The test reagent solution is prepared by dissolving 8.8 g Na_2SO_3 and 2.9 g $Na_2S_2O_5$ in 100 ml water. This solution should be prepared the same day as the strips are made.

7) Unroll and spread the sheet and place it in an oven at 50°C until dry (approximately 30 minutes). Again, as in step 4, it is important to ensure that no part of the sheet is contacting another part of the sheet while it is drying.

8) Cut the sheet to make the finished test strips approximately 11 cm × 0.5 cm.

9) Store the finished strips in a well-sealed container together with a desiccant. Indicating "Drierite" is a convenient and effective desiccant for this purpose.

Interferences

Aldehydes other than formaldehyde will react to some extent with these test strips. However this is not considered a problem since higher aldehydes might be expected to exist as impurities in formalin solutions, but not in any significant amount as impurities in alcohol.

Ketones show, to a limited extent, a reaction with the test strips. Volume per cent solutions of acetone in water gave the following responses:

5%—negligible
10%—trace response (response $\cong$ 0.6% formalin)
20%—¼ response (response $\cong$ 1% formalin)

The responses, in addition to being minor even at high acetone concentrations, appear more muted and more transient than equivalent responses to formalin solutions.

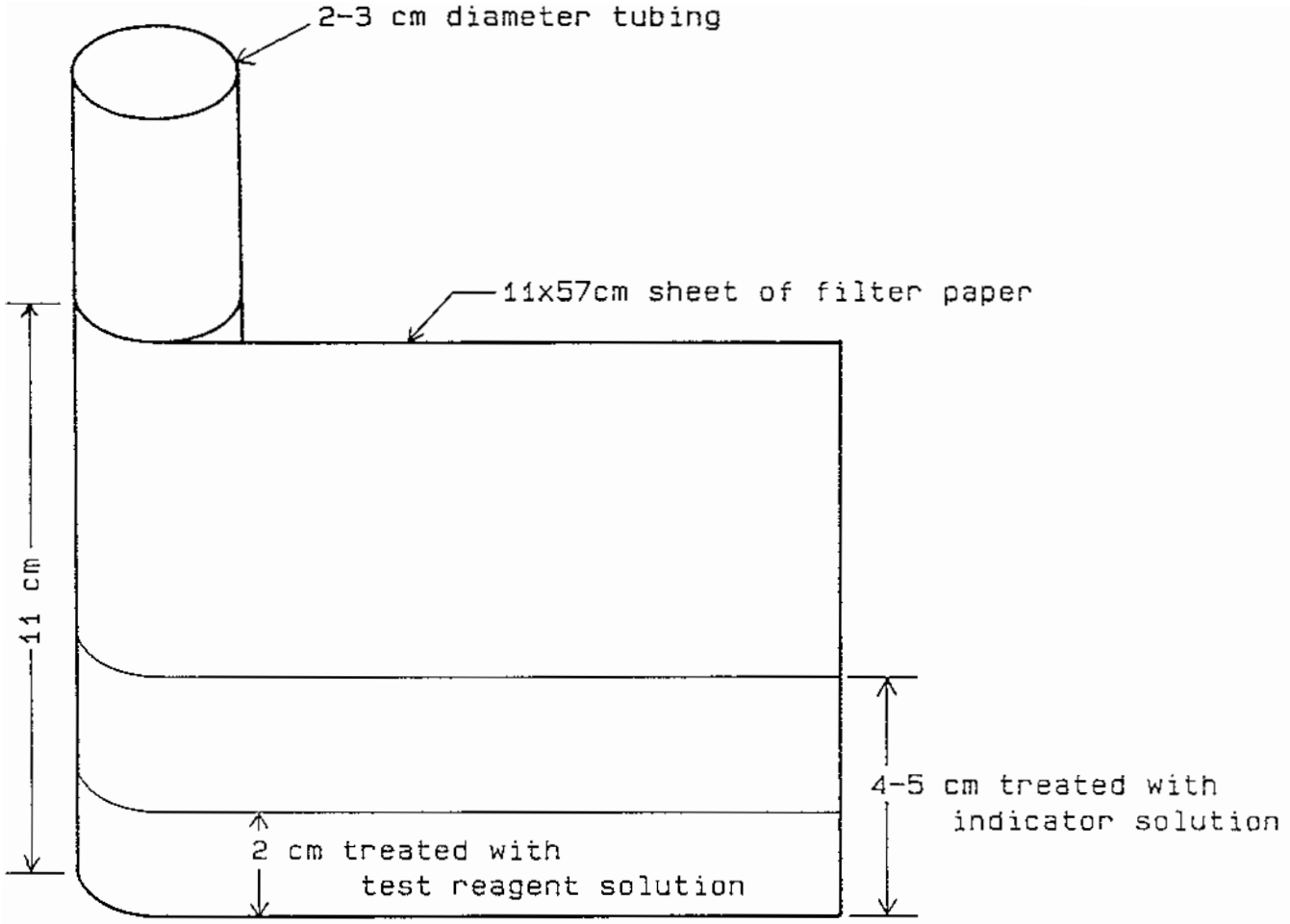

Fig. 2. A schematic illustration of a filter paper sheet after impregnation with the indicator and the test reagent solutions.

Since this test measures formaldehyde indirectly through a change in pH, pH and total titratable acidity or alkalinity may affect or even nullify the validity of the response.

The total quantity of acid or alkali present is more significant than pH in its effect on responses. The test strips were found to be capable of distinguishing between solutions that were 5% and 0% formalin in water throughout the pH range of 1.3 to 12.4. This rather wide range applies only when strong acids or alkalies are the sole sources of titratable acidity or alkalinity.

Because acidity and alkalinity encountered in preservative solutions will more likely be due to the presence of weak acids and bases, the effect on response is more a result of the quantity of acid or base present rather than the pH of the solution.

For example, 6 g/l of sodium carbonate added to a 0.5% solution of formalin in water resulted in a pH of 11.2 but did not noticeably affect the response of the test strips. The addition of 150 g/l to a 0.5% solution of formalin in water resulted in a pH of 11.3, a difference of only 0.1, but changed the response of the test strips from negligible to three-quarters full. Fortunately, buffers are not added in such high concentrations, and are generally not as alkaline as sodium carbonate. These facts suggest that the presence of a buffer of the type, and in the quantity, likely to be employed in preservative solutions will not interfere with the response of the test strips. The response of the test strips to the following published formulations (McAllister, 1965) for buffered fixative solutions was tested.

10% formalin + 2.6 g/l borax
14% formalin + 14 g/l hexamine
16% formalin + 3% concentrated ammonium hydroxide
5% formalin + excess calcium carbonate
40 g/l paraformaldehyde + 10 g/l sodium carbonate

The test strips gave immediate positive responses to all of these solutions.

It is clear from the discussion above that the effect of alkalinity in a solution being tested is to make the strips more sensitive to formaldehyde than they would be otherwise. In solutions that are extremely alkaline, the treated part of the test strips may undergo a complete colour change even in the absence of formaldehyde. It is for this reason that a section of the test strip is treated with indicator only and not with the other test reagents. A test that shows a colour change only in the section of the test strip treated with indicator alone indicates that the solution being tested is strongly basic but contains a negligible (by definition) amount of formaldehyde. An example of a solution that would show this response is a 1% (w/v) solution of trisodium phosphate in water. Extremely alkaline solutions may cause both the reagent plus indicator and indicator alone sections of the test strips to undergo a colour change. If this occurs a sample of the solution must be made less alkaline before the strip test can be employed.

The effect of excess acidity is to reduce or, in extreme cases, to eliminate the response of the test strip to formaldehyde. In order to determine whether or not acidity levels in preservative solutions are high enough to be of concern, 52 samples of nominal 45% isopropanol preservative chosen at random from the National Museum of Natural Sciences Ichthyology Collection were titrated with N/10 NaOH to a phenolphthalein endpoint. The results, expressed in milliequivalents per litre (meq/l), are given in Figure 3. The highest value found was 31.4 meq/l. The response of the test strips was checked in solutions containing 5% formalin and 100 meq/l hydrochloric acid. The test strips gave an immediate response to this solution if water was the solvent, but in 70% ethanol 5 minutes was required for the full response to develop. The same solution in 50% isopropanol produced a full response in 30 seconds. The times given here were recorded for strips stored over a desiccant. Test strips in equilibrium with a higher relative humidity were faster to respond, but as discussed later the test strips have a greatly reduced shelf life when stored at higher relative humidities, and hence,

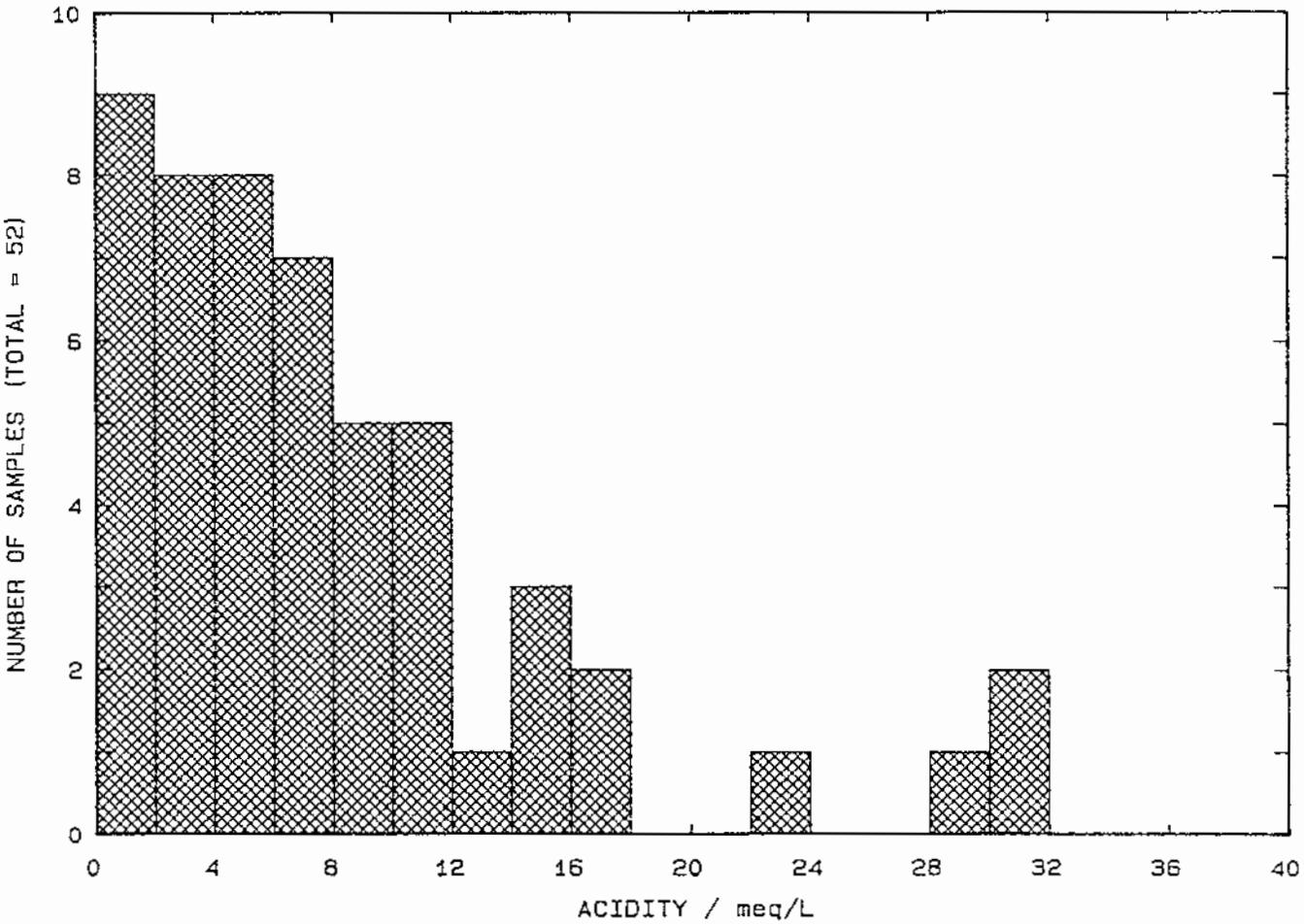

Fig. 3. The distribution of acidity in 52 random samples from the National Museum of Natural Sciences Ichthyology Collection. Determined by titration to the phenolphthalein end point.

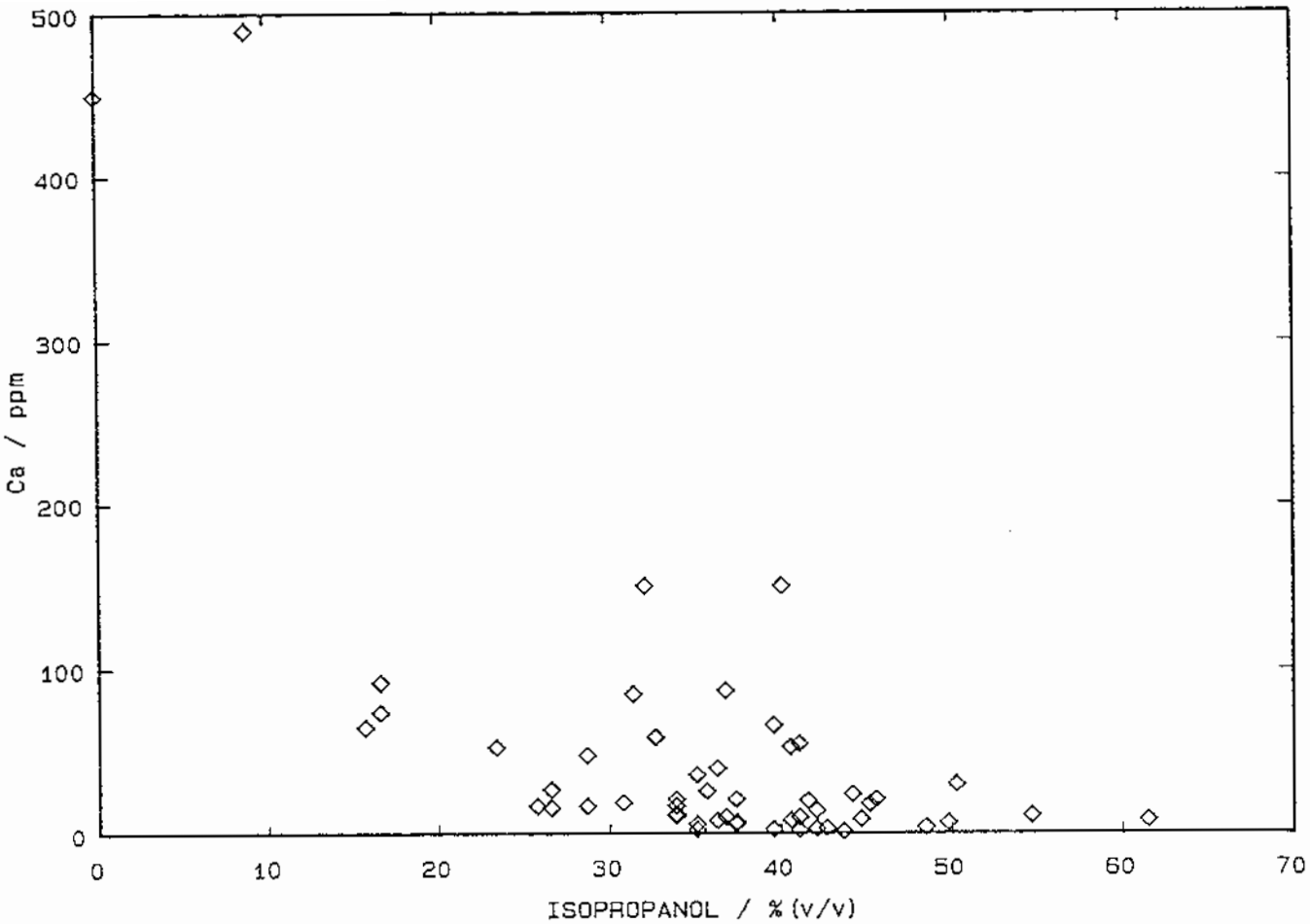

Fig. 4. The distribution of dissolved calcium concentration as a function of isopropanol concentration in 52 random samples from the National Museum of Natural Sciences Ichthyology Collection.

should be stored, and probably would be used, in a desiccated state.

Cations that form insoluble sulphites constitute another possible interference. Calcium and iron are the only such cations that would be expected to be present in preservative solutions in significant quantities. The 52 solutions used for other tests were analysed for calcium and iron by atomic absorption spectroscopy. Iron was present only as a trace impurity. The median iron concentration was 55 ppb and the maximum was 2.5 ppm, consequently iron is unlikely to be a cause of interference.

Calcium, on the other hand, was present in significant amounts. The calcium concentrations are shown plotted against isopropanol concentration (from density determinations) in Figure 4.

It is clear that calcium concentration declines with increasing alcohol concentration. This is fortunate since, as with level of acidity, high calcium concentrations are most significant as an interference when alcohol concentrations are high. For example, 1500 ppm Ca added to a 5% solution of formalin in water had no effect on the response of test strips. On the other hand, 200 ppm Ca added to a solution containing 5% formalin in 75% ethanol changed the time for full response from 15 seconds to 2.5 minutes. In general, calcium is not likely present in sufficient concentrations to seriously affect the response of test strips to purely aqueous solutions of formalin. For example, a 5% formalin in water solution stirred vigorously for three weeks with a large excess of powdered calcium carbonate continued to give a full and immediate response. Calcium is probably a significant interference in some cases when the solution being tested contains both formalin and alcohol, but a meaningful assessment of its significance as an interference cannot be made without data from more analyses performed on a wider variety of preservative fluids.

To summarize, interferences are unlikely to result in a 5% or 10% solution of formalin in water with or without

If the yellow portion of the test strip above the 2 cm

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buffers ever failing to give a full response to the test strips. In the case of alcoholic solutions, interferences will sometimes cause the response to be greatly delayed, but in all cases so far investigated, a response eventually will be generated if the concentration of formalin is 5% or greater.

Shelf Life

Sodium sulphite, in the presence of atmospheric moisture, is oxidized to sodium sulphate. This fact limits the shelf life of test strips exposed to the atmosphere. Test strips stored at 85% relative humidity gave unsatisfactory responses after 15 days. The shelf life for test strips stored in the absence of atmospheric moisture is as yet undetermined. Test strips stored in a jar together with indicating "Drierite" have shown no deterioration for, at the time of writing, 14 months. It is probable that under these conditions there is practically no limit to shelf life. Significantly, aging of the test strips at any relative humidity does not change the sensitivity of the strips, but simply mutes the colour response. This fact makes it possible to check the viability of a supply of test strips simply by immersing one of the strips in a stock formalin solution to determine whether or not the response is readily detectable.

Use

Test strips should be stored in a well-sealed container together with a small amount of a desiccant. To use, insert the yellow end of a strip into the solution to be tested. The time that the strip is in the solution should be kept as brief as possible. Less than a second is required to wet the strip. Responses in the various solutions that are likely to be encountered are given in Table 1.

Solutions not containing alcohol will usually show the appropriate response within 10 seconds. Depending on the quantity of interferences present, solutions containing 75% ethanol may require as long as 5 minutes for the appropriate response to develop. In many cases this will permit the distinction between aqueous formalin solutions and alcohol solutions containing residual formalin.

If the yellow portion of the test strip above the 2 cm sulphite-treated portion turns red then the solution is too strongly alkaline (pH>12) to be tested without prior neutralization.

Discussion and Conclusions

A simple and inexpensive paper test strip has been developed that will distinguish between formalin-based fixative solutions and alcohol solutions containing traces of residual formalin. This distinction is readily apparent within seconds of wetting the test strip. The test was not developed to provide a quantitative determination of the amount of formalin in alcohol. The results obtained during testing do, however, suggest that semi-quantitative results for the quantity of formalin in alcoholic solutions might be obtainable if the type and concentration of the alcohol present is known. The further development of this test to make it capable of rendering semi-quantitative results reliably and within a short time (less than one minute) would require solutions to the combined problems of limited solubility and interference from acidity and dissolved calcium. These problems can only be solved when data on the alcohol, formalin, acidity, and calcium concentrations of fluid preservatives in a variety of different collections is available.

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