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THE USE OF PARAFORMALDEHYDE AS A BIOLOGICAL PRESERVATIVE[1]

By Clarence M. Taube

Paraformaldehyde, $HO \cdot (CH_2O)_n \cdot H$, is a snow-white, solid polymer of formaldehyde, $(CH_2O)_n$. It is the most important of the several commercial polymers of formaldehyde, and is produced in powder and flake forms which contain 90-98 percent of formaldehyde (Walker, 1953). It is used mostly for the manufacture of resins.

"Paraform" is a synonym for paraformaldehyde, and an aqueous solution of formaldehyde is often called "formalin." Dissolving the polymer in water produces formalin that is identical to that obtained with gaseous formaldehyde (Walker, 1944 and 1953). Paraform has several potential advantages over concentrated formalin as stock for preservative solutions; however, its restricted solubility has impeded this use. Various compounds were tested as catalysts to increase its solubility, and several proved effective. The preserving qualities of the resultant solutions were evaluated.

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[1] Contribution from Dingell-Johnson Project F-27-R, Michigan.

Materials and methods

Chemicals employed in the experiments were: powdered paraformaldehyde, 95 percent $(CH_2O)_3$; potassium hydroxide (85 percent); sodium hydroxide (97.8 percent); borax (99.5 percent sodium tetraborate). The kinds of water used were distilled, tap, and those from lakes and streams. The solutions were prepared in 1-quart glass jars graduated in ounces. The preserving qualities of the solutions were tested with various kinds of fish that occur in Michigan, and with snails, *Triodopsis multilineata.*

An aqueous solution of 37 percent by weight of formaldehyde is commonly referred to as 100 percent formalin. Usually the goal in the tests on solubility was to produce a 20 percent solution, that is, one in which the mixture was equivalent to 2 parts of saturated formalin with 8 parts of water. A final concentration of 10 percent (or 1:9) was attained by the addition of organisms and water. Thirty-seven grams of 95 percent paraformaldehyde are required for a pint of 20 percent formalin. From 37 to 39 grams were used in the tests.

The steps of preparation were as follows: the paraform was put into a 32-ounce jar, followed by the catalyst; water up to the 16-ounce mark was added immediately, and then the mixture was shaken until all the solids were in suspension; momentary shaking at intervals continued until dissolution was considered complete.

Organisms were placed in the solutions any time following preparation. The addition consisted of a pint of fish, snails, and more water to a total volume of one quart.

Over 100 tests were made to develop methods for dissolving the polymer quickly and to evaluate the preserving qualities of paraform-formalin prepared with catalysts.

Preparation of test solutions

Several pilot tests showed that both borax and potassium hydroxide greatly increased the solubility of paraformaldehyde. With boiling water, pint quantities of 20 percent formalin were made in 3 minutes from paraform with 11 grams of a household brand of borax, and within 1 minute with 8 grams of KOH pellets.

Experiments were conducted with cool water also. The paraform dissolved readily (in 6 and 12 minutes, respectively) when 8 grams or 4 grams of KOH was used with water of about 70° F., but these solutions darkened fish excessively. Two grams of KOH provided a satisfactory solution. With this amount of catalyst and 70-degree water, the polymer dissolved in about 30 minutes.

The utility of borax as a catalyst was tested further with crystals of reagent grade. In the preparation of a pint of 20 percent formalin with approximately 11 grams (3 teaspoons) of borax and boiling water, the paraform dissolved in less than 2 minutes. Smaller amounts of borax were but slightly less effective. Only a few more seconds were required with 2 teaspoons or 1 teaspoon. Solutions made with the smaller amounts darkened fish less but were not buffered as well. This compound is much less effective in cool water. Preparation of a pint of 20 percent formalin with 3 teaspoons of borax, distilled water at 76° F., and frequent agitation required over 5 hours.

Sometime after this investigation was begun, a method of preparing formalin by dissolving paraformaldehyde in boiling water with sodium hydroxide was published (Taub, 1962). Besides KOH and borax, I tested the hydroxides of sodium, ammonia, and calcium as catalysts; of the latter chemicals, only sodium hydroxide was satisfactory. Potassium hydroxide and NaOH are equally effective, and 2 grams of either may be used with cool or boiling water. With boiling water, though, a quantity of either base as small as that recommended by Taub (op. cit.) for NaOH (5 grains or 0.324 gram) will provide a pint of 20 percent solution within a minute.

Other observations on the tests for solubility were these:

(1) Agitation, as well as higher temperatures, quickened solution.

(2) Paraform can be dissolved in rather cool water; pint quantities of 20 percent formalin were prepared with 38-degree water and 2 grams of either KOH or NaOH within 100 minutes.

(3) Precipitates were formed in the solutions made with paraform. More precipitate appeared when KOH or NaOH was used with undistilled

water, and the amount was related to the hardness of the water. Analysis of the precipitate from one of these solutions showed that it consisted mostly of calcium hydroxide.

(4) The alkalinity of the water apparently did not affect the solubility of the polymer; the methyl orange alkalinities of the test waters ranged from zero to 320 p.p.m. It is reported,[2] however, that when 37 percent formalin is prepared from paraform at boiling temperature the polymer dissolves much faster at extremes of pH than at intermediate levels, being slowest around 5.

The catalysts can also be used to make concentrated solutions. Preparation of pint quantities of 100 percent formalin was tried with 184 grams of paraform and hot distilled water. The catalyst for one test consisted of 5 grams of KOH pellets, and in another test it was 28 grams of borax. The paraform dissolved after 3-8 minutes of boiling over a Bunsen burner. The formaldehyde content was determined by the sodium sulfite method (Walker, 1953) to be 30-32 percent rather than the expected 37 percent. Some of the missing formaldehyde probably was combined in precipitated by-products, and another portion surely had evaporated.

These precautions apply to making preservative solutions with paraformaldehyde: (1) Addition of the water should immediately follow the addition of the catalyst, to avoid undesirable reaction products. (2) Paraformaldehyde is poisonous and therefore must be handled with

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[2] "Celanese Paraformaldehyde," Product Bulletin S-16-5 (1961), Celanese Chemical Co., 522 Fifth Ave., New York.

reasonable respect. When it is used to produce concentrated solutions, the boiling should be done under a hood equipped with an exhaust fan.

Preservation of fish

As mentioned before, formalin made with paraform, 8 or 4 grams of KOH or NaOH, and cool water darkened fish excessively; other effects were an increased slipperiness of fish and the impartation of transluscence to small specimens. Two grams of either base proved satisfactory, although some darkening occurred when the solutions were used soon after they were mixed. However, when 2 grams of KOH or NaOH was employed with boiling water, preserved fish were not darkened and looked exactly like those kept in conventional formalin. Discoloration also was prevented when cool-water preparations stood for several days before use. The acidic nature of these mixtures evidently accounted for the different effect; the other solutions were basic. The acidification process (Canizzaro reaction) in formalin is said to involve a reduction and oxidation of formaldehyde, producing formic acid and methyl alcohol, and the reaction is catalyzed by alkalies and heat (Walker, 1953).

The solutions prepared with paraform and 11 grams of borax darkened fish to about the same degree as those made for immediate use with 2 grams of KOH or NaOH and cool water.

Fish have been kept in the test preservatives for as long as two years. Those in solutions prepared by the recommended formulas are judged to be good specimens for reference or study purposes. Several of the collections that had been held from six months to a year in paraform-formalin were deformalinized with water and then transferred to 70 percent ethyl alcohol and 37 percent isopropyl alcohol. These specimens are preserved as well as those from control solutions of ordinary formalin that were placed in alcohols at the same time.

The pH of the solutions were determined repeatedly to learn if the catalysts would also serve as buffers. Snails usually were preserved with fish to afford ready evidence of decalcification, which is an undesirable feature of acidic preservatives. Miller (1952) discussed unfavorable effects of acidity that result from long-term preservation in formalin, and presented several methods of alkalinization.

Figure 1 illustrates the pH characteristics of three types of formalin. The formulas for the quart volumes of 10 percent concentrations were: (1) 39 grams of paraform, 11 grams of borax, and boiling water; (2) 39 grams of paraform, 2 grams of KOH, and cool water; (3) 95 cc of 100 percent formalin plus water. The solutions were prepared with lake water and contained from 8 to 14 volumetric ounces of fish. The fish were added on the day of mixture, except that in some borax preparations the additions were made later. Testing for pH commenced the day after fish were introduced.

It will be seen that the pH of KOH-paraform solutions tended to be slightly higher after 5 and 7 weeks than after 3 weeks; the pH of some of these preparations declined to an acidic value, followed by

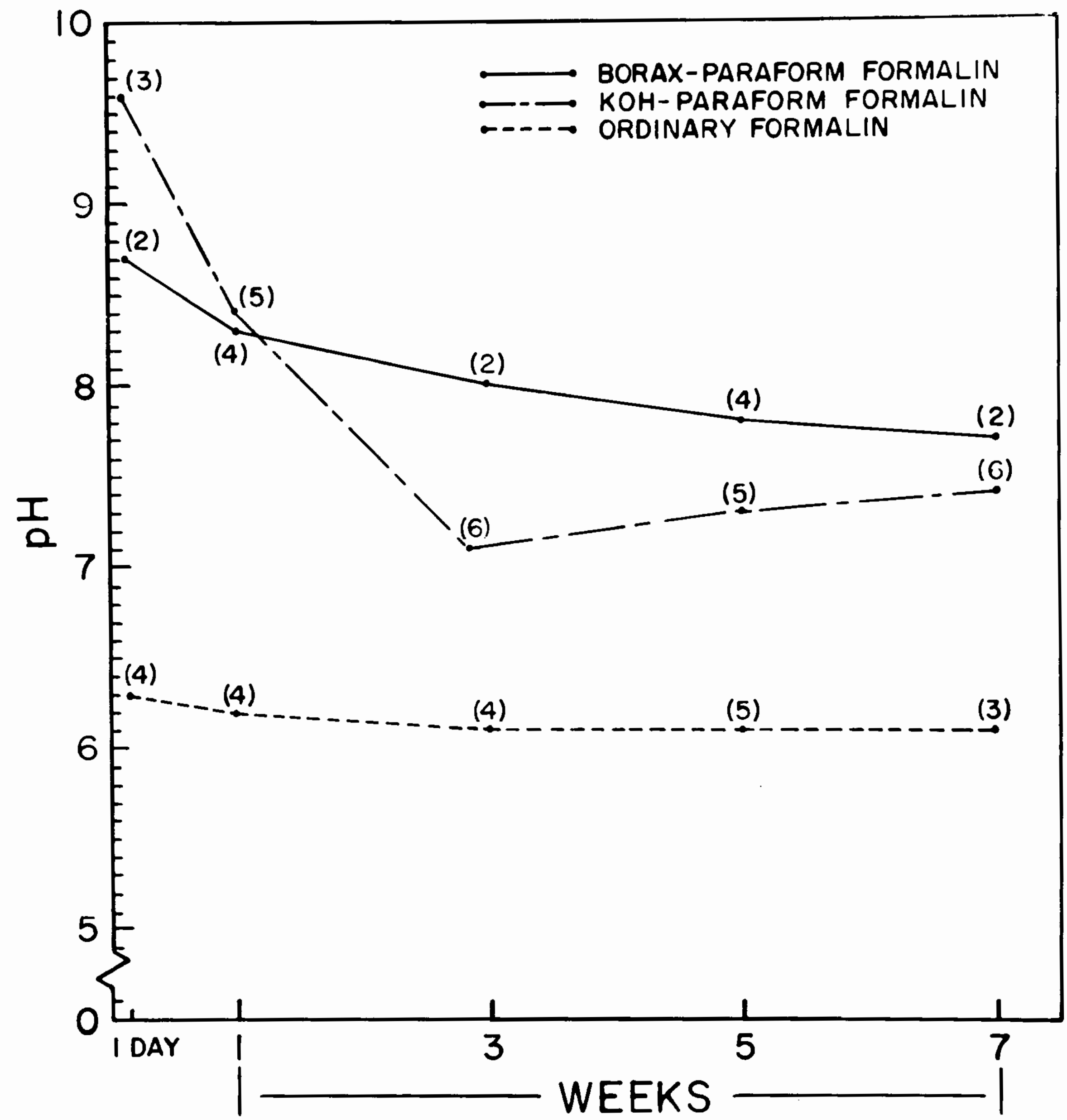

Figure 1. --Progressive, mean pH of 10 percent formalin solutions. Figures in parentheses are the number of samples represented in the means.

return to a basic value. Several of the solutions made from concentrated formalin showed a similar but less pronounced fluctuation. Further, corrosion eventually occurred in snail shells in solutions that evidently were continuously basic. These phenomena are unexplained.

Firmness and natural colors in snails were retained generally as follows: in solutions made from concentrated formalin, 4 weeks; in KOH-paraform formalin, 6 weeks; in borax-paraform formalin, 12 weeks. Beyond these times the shells were progressively softened and whitened. It is reasonable to suppose that the progress of decalcification is similar in fish. Because effective buffering is not maintained, specimens should not stay in any formalin a long time when decalcification is to be avoided.

Utility of paraform

Solutions made from paraform probably can be used on any organism that is commonly preserved with formalin. Although 100 percent formalin usually is the more convenient stock material, the polymer would sometimes be more advantageous. Some situations in which paraform may be preferred are these: when economy in bulk and weight of supplies is highly important, as in collecting expeditions to remote regions; at field stations which lack nearby commercial sources of formalin; when large amounts of stock are to be transported far, and when any amount is shipped by air (to economize on freight costs, simplify packing, and reduce hazard of breakage).

The cost of concentrated formalin is less, but the difference is apt to be trivial when the polymer is definitely preferable. At local prices of $2.90 per gallon for 100 percent formalin and $1.40 per pound for paraformaldehyde, the cost of the respective materials in a quart of 10 percent solution is 7 cents and 12 cents.

Paraform-formalin may be made for field use by the method usually employed for the tests; that is, a pint of 20 percent solution was prepared in a quart jar. Adding a pint of specimens and water reduced the concentration to 10 percent. Volumetric measurement of the chemicals will be sufficiently accurate. With 95-percent paraformaldehyde, 10 1/2 level teaspoons (37 grams) of the powder are required for a pint of 20 percent solution. The other volume-to-weight equivalents are a slightly rounded 1/4 teaspoon of KOH or NaOH pellets for 2 grams, and 3 level teaspoons of borax crystals for 11 grams. An alternate method is preparation of concentrated formalin that is used like the commercial concentrate.

Acknowledgments

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Paraformaldehyde or paraform, $HO \cdot (CH_2O)_n \cdot H$, is a snow-white, solid polymer of formaldehyde, $(CH_2O)_n$. It would sometimes be preferable to concentrated aqueous formaldehyde for making preservative solutions; however, its limited solubility has restrained such usage. Methods for dissolving the polymer quickly were developed with catalysts, and the preserving qualities of the solutions were tested on fish. Potassium hydroxide, sodium hydroxide, and borax (sodium tetraborate) proved to be effective catalysts.

The formaldehyde content of 100 percent formalin is 37 percent by weight. With paraform of 95 percent purity, 37 grams (10 1/2 level teaspoons) of the powder are required to prepare a pint of 2:8 or 20 percent formalin. Two grams, or a slightly rounded 1/4 teaspoon, of KOH or NaOH pellets are added, followed immediately by sufficient water to bring the total volume to one pint. The water may be cool or boiling, distilled or undistilled. With boiling water, as little as 5 grains (0. 324 gram) of KOH or NaOH is sufficient. A satisfactory solution can also be made with boiling water and reagent-grade borax--11 grams (3 level teaspoons) of crystals for a pint of 20 percent formalin.

When boiling water was used with any of these formulas, the paraform dissolved in about a minute. When water at 70° F. was used with 2 grams of KOH or NaOH, the polymer dissolved in 30 minutes; at 38°, 100 minutes were required. Occasional agitation assisted dissolution. The rate of solubility apparently was not influenced by the alkalinity of the water; methyl orange alkalinities of the test waters ranged between 0-320 p. p. m. Some precipitate was formed in all the solutions. More precipitate appeared when KOH or NaOH was used with undistilled water, and the amount was proportional to the hardness of the water.

Adding a pint volume of organisms and water to a pint of 2:8 or 20 percent formalin dilutes it to 1:9, or 10 percent, the concentration commonly used for fish and other organisms.

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[1] Contribution from Dingell-Johnson Project F-27-R, Michigan.

Solutions made with borax darkened fish to some degree, as did those made with KOH and NaOH and cool water when used soon after mixing. These preparations were moderately alkaline, and borax gave the best buffering effect. Darkening did not occur when boiling water was employed with KOH or NaOH, or when cool-water preparations made with these catalysts stood for several days before use. These solutions were acidic.

Paraform also was used to prepare concentrated formalin. Assuming that all of the polymer will enter and remain in solution, 184 grams of 95 percent paraformaldehyde are required for a pint of 100 percent formalin (37 percent formaldehyde). For one test, 5 grams of KOH was combined with 184 grams of paraform in a graduated glass container, followed by the addition of boiling distilled water to the pint mark. The procedure for another test was identical to the first except for substitution of 28 grams of borax for KOH. The mixtures were boiled until the solids were dissolved (3-8 minutes). The solutions contained 30-32 percent of formaldehyde, rather than the expected 37 percent. Some of the missing formaldehyde probably was combined in precipitated by-products, and another portion surely had evaporated.

These precautions apply to the use of paraformaldehyde for preservative solutions: (1) Addition of the water should immediately follow the addition of the catalyst, to avoid undesirable reaction products. (2) Paraformaldehyde is poisonous and therefore must be handled with reasonable respect. When it is used to produce concentrated solutions, the boiling should be done under a hood equipped with an exhaust fan.

Although the polymer has no obvious advantage over concentrated formalin in ordinary situations, it would be advantageous when economy in weight and bulk is desired, or when hazards of shipment are involved.

The cost of concentrated formalin is less, but the difference is apt to be trivial when the polymer is definitely preferable. At local prices of \$2.90 per gallon for 100 percent formalin and \$1.40 per pound for paraformaldehyde, the cost of the respective materials in a quart of 10 percent solution is 7 cents and 12 cents.