Colorimetric Assay for Carboxymethyloxysuccinate, a New Detergent Builder

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ABSTRACT

A spectrophotometric assay was developed for the quantitative determination of carboxymethyloxysuccinate, a new detergent builder. The method was based on the color produced with β-naphthol in 92.5% (w/w) sulfuric acid. As little as 1.0 μg of carboxymethyloxysuccinate can be measured. Determination of the builder in primary sewage effluent and river water, and interference studies were also reported.

INTRODUCTION

In recent years national concern for protecting the environment has been extended to the problem of accelerated eutrophication due to the input of man's wastes into receiving bodies of water. Many factors contribute to an increase in algal growth, but the most prominent target of criticism at the present time is phosphates. Although phosphates emanate from many sources, one that is considered controllable is the phosphate builders or water softeners in synthetic detergent products. For this reason the state and federal agencies have urged the soap and detergent industry to search for new builders that are innocuous or readily biodegradable to nontoxic end products when discharged into waste treatment systems.

A promising ether polycarboxylate called trisodium carboxymethyloxysuccinate (CMOS) has been synthesized as a possible substitute for phosphates (1). Before the biodegradation and ultimate bioconsequences of CMOS can be determined, an analytical method capable of detecting the builder in microgram quantities must be developed. In addition, the procedure must be relatively specific so that determinations can be made in the presence of river water and sewage effluents. A colorimetric assay, which fulfills these requirements, is described in this study. The method was developed from a quantitative test for glycolic acid based upon its reaction with β-naphthol in sulfuric acid (2). The present paper also reports a detailed investigation of the reaction and its interferences.

MATERIALS AND METHODS

Trisodium Carboxymethyloxysuccinate Pentahydrate ($C_6H_5O_7Na_3 \cdot 5H_2O$)

All analyses were reported in terms of the anhydrous trisodium salt (Fig. 1), formula weight 258. The abbreviation "CMOS" used throughout this paper also refers to the anhydrous salt.

Sulfuric Acid Reagents

A solution of 92.5% (w/w) sulfuric acid was prepared by adding 100 ml concentrated sulfuric acid (95.5-96.5%) to 7 ml distilled water. The final volume at room temperature was 102 ml. An 80% (w/w) solution was prepared with 100 ml concentrated acid and 37 ml distilled water. After cooling the acid solution to room temperature, its volume was ca. 131 ml.

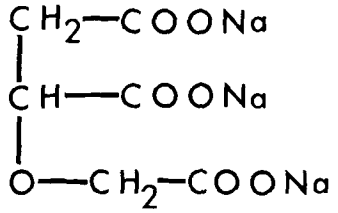

FIG. 1. Trisodium carboxymethyloxysuccinate.

β-Naphthol Reagent

Reagent grade β-naphthol was used without further purification. β-Naphthol (50 mg) was added to 50 ml 92.5% sulfuric acid contained in a 100 ml volumetric flask. After complete dissolution of the β-naphthol crystals the solution was diluted to 100 ml with 92.5% sulfuric acid. A freshly prepared solution was bright yellow in color but became colorless at room temperature in ca. 1 hr. Prolonged exposure to daylight gradually changed the solution from colorless to violet. The reagent, however, was stable for 72 hr if refrigerated and protected from light. Before the reagent was used in the analysis, the approximate volume needed was transferred into a beaker and allowed to come to room temperature. This portion was also protected from light until ready for use. Any surplus reagent was discarded.

Pretreatment Procedure

Samples from sewage effluents and river waters were briefly centrifuged, Millipore-filtered through a 0.22 μ membrane and acidified with concentrated HCl to an approximate normality of 1.0. The solutions were then maintained at room temperature for 30 min and again centrifuged, if necessary. One milliliter of each supernatant solution was transferred to a 12 ml heavy duty conical centrifuge tube and heated in a boiling water bath for 30 min. After the tubes were cooled to room temperature, 0.3 ml 0.5% phenylhydrazine or 2,4-dinitrophenylhydrazine in 2N HCl was added to each sample; the tubes were then reheated in a water bath (100 C) for 30 min. At the end of this heating period each solution at room temperature received 0.5 ml 10% $BaCl_2$ solution. The tubes were allowed to stand at room temperature for 30 min, after which 0.15 ml concentrated NH_4OH (mixed immediately after delivery) and 6.0 ml absolute ethanol were added to each sample. The solutions were stored at 4 C for ca. 20 hr to insure complete precipitation. Following the overnight

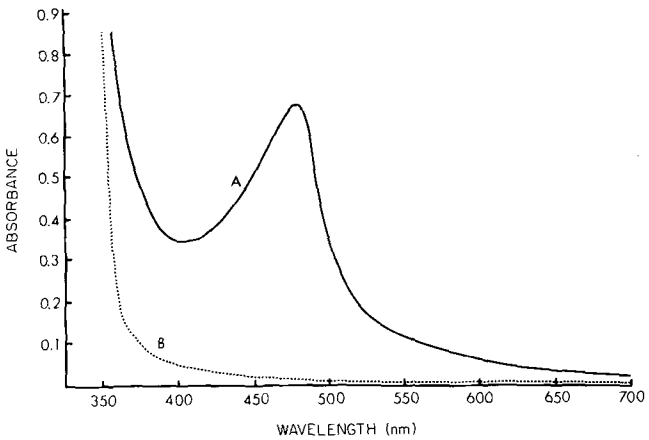

FIG. 2. Absorption spectra. (A) Reaction color for CMOS (20 μg); (B) blank. Recorded against distilled water.

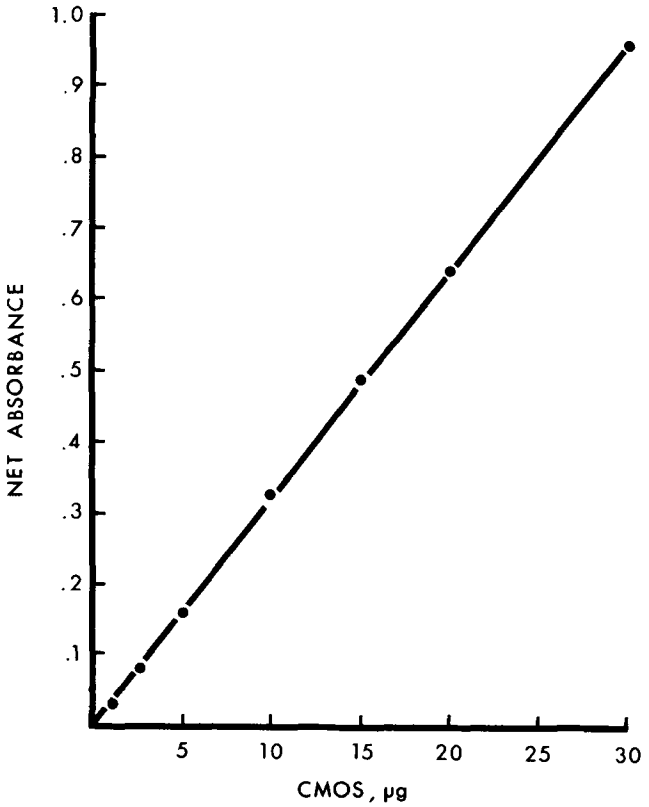

FIG. 3. Calibration curve for trisodium carboxymethyloxysuccinate. Samples containing various amounts of trisodium carboxymethyloxysuccinate in distilled water were assayed according to prescribed method. All indicated points were corrected for a reagent blank.

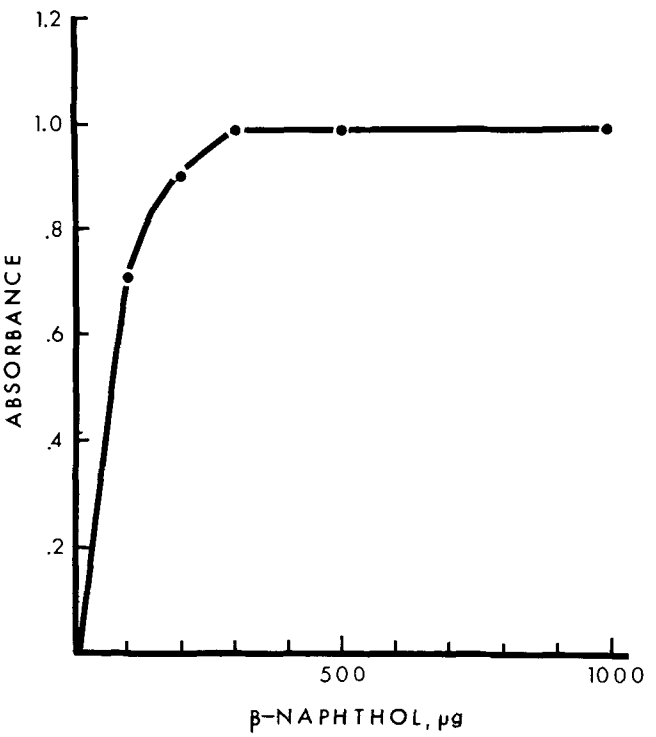

FIG. 4. Effect of β-naphthol concentration on absorbance.

storage the tubes were centrifuged for 15 min at 7000 x g and the resulting clear supernatant fluids were removed and discarded with the aid of a Pasteur pipette. The precipitates were baked in an oven at 130 C for 30 min to remove all traces of moisture. These samples received 1 ml β-naphthol reagent and were subsequently treated according to the prescribed method.

Assay Procedure

The solutions to be analyzed contained not more than 30 μg CMOS and were acidified with concentrated HCl (0.1 ml HCl to 1 ml sample). The samples, preferably 1 ml or less, were introduced into 18 x 150 mm test tubes and evaporated to complete dryness in an oven maintained at 125-130 C. One milliliter β-naphthol reagent was added to each sample, followed by vigorous mixing on a vortex mixer to insure complete dissolution of the residue. The test tubes were capped with glass marbles and then heated in a boiling water bath for 60 min (water bath must not be boiled vigorously, for turbidity may occur if droplets of water from condensation fall into the reaction mixture). After the heating period the tubes were cooled to room temperature and all the water droplets near the rim of the test tube were carefully removed with a paper tissue. Three milliliters 80% sulfuric acid was added to each sample, followed by mixing on a vortex mixer until the solution was homogeneous. The samples were allowed to stand at room temperature for 20 min before the absorbance of each solution was determined at 480 nm with distilled water as a reference.

RESULTS AND DISCUSSION

Investigation of Assay Conditions

A solution containing 30 μg CMOS was treated according to the prescribed method. The sample displayed maximum absorbance after 20 min, following the dilution of the reaction mixture; the absorbancy reading was stable for several hours. The color produced, which had a maximum absorption at 480 nm (Fig. 2), was also found to follow Beer's law within a range of 1 to 30 μg CMOS (Fig. 3). The average slope of the curve (calculated by least squares) was 0.0321 with a SD of .0003. The SD of a single observation was 0.0129. The absorbance value of a distilled water blank was usually less than 0.04 when prepared with a completely colorless β-naphthol reagent. The use of a tinted reagent increased the blank reading and affected the sensitivity of the assay. Although a calibration curve to 30 μg CMOS can be obtained with as little as 300 μg β-naphthol in the reaction mixture (Fig. 4), a concentration of 500 μg is recommended for most samples. This amount of β-naphthol produces a small blank reading and prevents the reagent from becoming limiting due to the presence of other reactive compounds.

The heating time required at 100 C for maximum color development is illustrated in Figure 5. The reaction was quite rapid during the initial 15 min and appeared completed after 30 min for 20 μg CMOS; however an additional 30 min of heating was needed for the 30 μg concentration. Higher concentrations of CMOS will require a longer incubation period.

The color intensity as a function of the sulfuric acid concentration during the reaction of CMOS and β-naphthol is presented in Table I. Evaporated samples of CMOS were treated with various acid solutions ranging from 70 to 96% (concentrated). These concentrations were calculated on a weight to weight basis of acid to total water. As indicated, maximum absorbance occurred with the 92.5% acid reagent. The data suggest that the ratio of acid to water during the reaction is critical, since acid levels of 90 and 96% greatly reduced the absorbance. This delicate balance between acid and water is apparently needed for cleavage of the ether linkage and for the subsequent hydrolysis of the products. The 70 and 80% acid solutions displayed very low absorbancy readings due to the insolubility of β-naphthol.

The final acid content also affected the color intensity. The sample exhibited maximum absorbance when the reaction mixture was diluted with 80% sulfuric acid or to final acid concentration of ca. 83% (Table II).

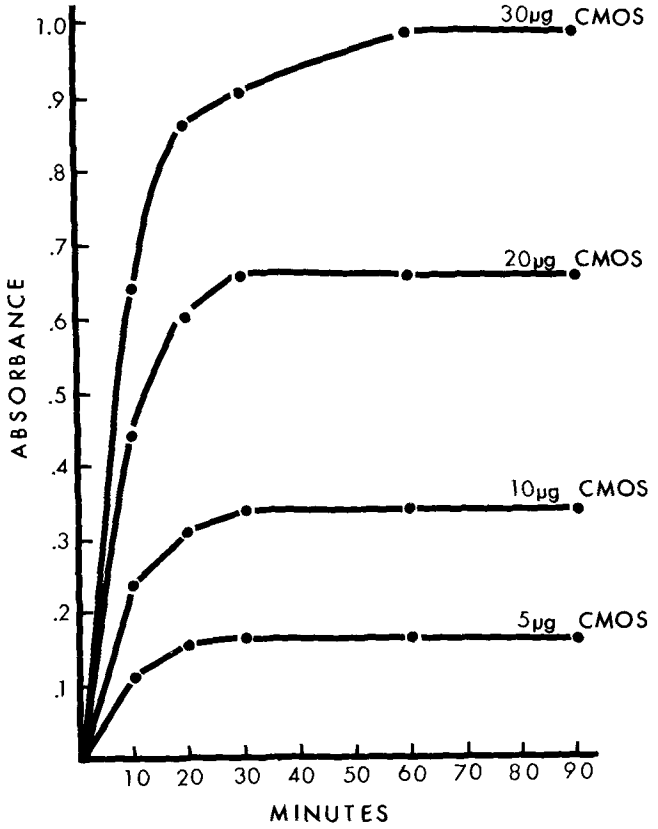

FIG. 5. Effect of heating time on absorbance.

To insure maximum sensitivity and precision it is advisable to use the same manufacturer and grade of sulfuric acid throughout the entire investigation.

Recovery of CMOS from Primary Sewage Effluent and River Water

Solutions of CMOS, ranging from 1 to 30 ppm, were prepared with primary sewage effluent obtained from the sewage treatment plant (Ridgewood, N.J.) and river water from the Passaic River downstream from the Ridgewood sewage installation. The samples were centrifuged, Millipore-filtered through a 0.22 μ membrane, and acidified with concentrated HCl to normality of 1.0. One milliliter samples, containing the indicated concentrations of CMOS, were placed into 18 x 150 mm test tubes, evaporated to dryness and assayed according to the prescribed method. As indicated in Table III, the CMOS recovery values were almost identical in both the sewage effluent and river water

TABLE I

Effect of Acid Concentration during Reaction

% H_2SO_4 in reaction	Net absorbance	Color
96.0	.55	Yellow-green
92.5	.73	Yellow-green
90.0	.49	Yellow-green
85.0	.19	Yellow
80.0	.04	Yellow
70.0	.00	--

[a]Samples of trisodium carboxymethyloxysuccinate (30 µg) were heated in 1 ml of various concentrations of sulfuric acid containing 500 µg β-naphthol at 100 C for 60 min. After the heating period the volume of each reaction mixture was adjusted to 4 ml with distilled water.

TABLE II

Effect of Final Acid Concentration

% H_2SO_4 diluents	Net absorbance	Final % H_2SO_4
96.0	.87	95.5
92.5	.91	92.5
90.0	.91	90.8
85.0	.93	87.1
80.0	.98	83.3
70.0	.76	76.1
Distilled water	.73	35.5

[a]Samples of trisodium carboxymethyloxysuccinate (30 µg) were heated in 1 ml of 92.5% H_2SO_4/β-naphthol (500 µg) reagent at 100 C for 60 min. After the heating period each reaction mixture received 3 ml of the above diluents.

samples; the mean absolute recovery values (least squares slopes) were 97 and 96.4%, respectively. The recovery of similar amounts from the same samples, which were treated as described under the pretreatment procedure in Materials and Methods, was 94.5% for the primary sewage and 93.7% for river water. In none of the above cases was the relative standard deviation of the slope greater than 1%. The tabulated recovery values, which were calculated from the standard slope (Fig. 3), are the average of triplicate assays. The estimate of the standard deviation for a single observation was 0.37 µg for the untreated samples and 0.41 µg for the pretreated solutions. The major loss of recovery in each calibration curve may be due to some adsorption of the CMOS onto the particulate matter present in the sample.

The data also reveal that the control blanks contribute some error, and it is apparent that the error will vary with the source of the sample. The interference, however, was significantly reduced when the solutions were pretreated.

TABLE III

Recovery of Trisodium Carboxymethyloxysuccinate from Primary Sewage Effluent and River Water

Trisodium carboxymethyloxysuccinate added, µg	Trisodium carboxymethyloxysuccinate recovered, µg			
	Primary sewage effluent	River water	Pretreated primary sewage effluent[a]	Pretreated river water[a]
0	1.83[b]		0.91[b]	
0		1.53[b]		0.89[b]
1.0	0.93	0.91	0.89	0.86
2.5	2.59	2.59	2.40	2.38
5.0	4.98	5.03	4.78	4.76
10.0	9.86	9.85	9.55	9.64
15.0	14.81	14.79	14.17	14.27
20.0	19.75	19.72	18.95	18.95
30.0	29.06	29.10	28.35	28.32

[a]Samples were pretreated according to the procedure described in Materials and Methods.

[b]Controls were corrected for the reagent blank. All other tabulated values were corrected for the appropriate control blank.

TABLE IV

Interference of Biological Compounds

Compounds	Net absorbance	Equivalent to μg trisodium carboxymethyloxysuccinate	Error, %	Color
Benzoic acid	0.003			
Citric acid	0.005			
Trisodium carboxymethyloxysuccinate	0.660	20.00		Yellow-green
Dextrose	0.010	0.30	1.5	Green
Fumaric acid	0.004			
Gluconic acid	0.005			
Glycolic acid	0.008	0.24	1.2	Pale yellow
Lactic acid	0.010	0.30	1.5	Pale yellow
Malic acid	0.006			
Oxalic acid	0.004			
Oxaloacetic acid	0.004			
Succinic acid	0.003			
Tartaric acid	0.031	0.94	4.7	Blue-green

[a]Each test solution (1 ml) containing 20 μg of the sample compound was treated according to the prescribed method.

Although this treatment proved to be quite effective (ca. 50% reduction in the control value) with the present test solutions, the procedure should not be interpreted as a cure for all interferences; but, where it is applicable—that is, for samples which may contain significant concentrations of carbohydrates and aldehydes—excellent recoveries of CMOS can be expected. If greater accuracy is desired or an excessively high blank reading is obtained, a control containing no CMOS must be available to establish a valid blank response.

The amount of interference produced by the untreated samples employed in this investigation was not considered excessive, and therefore it was concluded that the present assay should prove useful for detecting CMOS in most sewage effluents and receiving bodies of water. This conclusion is further strengthened by a biodegradation study conducted by David Jenkins (personal communication; report published by College of Engineering, School of Public Health, University of California, Berkeley, SERL No. 72-10), in which the method was successfully employed in determining ppm quantities of CMOS in effluents from septic tanks, percolation fields, soil columns and oxidation ponds.

Interference Studies

The results of an investigation to determine which compounds may interfere with detection of CMOS are summarized in Table IV. These materials were chosen as the most likely candidates, since each compound may be present in significant quantities in biological samples.

Preliminary experiments revealed that the addition of HCl and evaporation steps not only permitted the use of very dilute samples and an initial acid concentration of 92.5% for maximum sensitivity, but also provided additional specificity to the method by reducing interferences from glycolic and lactic acids, and dextrose. Glycolic acid, when heated in high concentrations of sulfuric acid, yields formaldehyde (3-5), whereas lactic acid forms acetaldehyde (3,4,6). These end products react with β-naphthol to form interfering colors (2). Dextrose under similar conditions interferes mostly by reacting with β-naphthol through the formation of a furfural (7-10) and partly by charring due to the sulfuric acid. The evaporation procedure destroys glycolic and lactic acids by converting them to lactides (11), while dextrose (or carbohydrates) in the presence of boiling HCl is decomposed to formic and levulinic acids (10). Since these reactions are slow, a prolonged evaporation period of ca. 2-3 hr is recommended. If the sample contains large quantities of carbohydrates or interfering nonvolatile aldehydes, CMOS may be separated from the solution as the insoluble barium salt by the pretreatment procedure described in the Materials and Methods. In essence, the barium CMOS is quantitatively precipitated by ethanol in the presence of phenylhydrazine, while the osazones (if formed) remain in the alcoholic supernatant solution. Tartaric acid, which also interferes with the assay, unfortunately has chemical characteristics very similar to those of CMOS; therefore it cannot be destroyed or separated from a CMOS sample by simple means. Tartaric acid also decomposes in hot sulfuric acid to yield glycolaldehyde (3,5). This compound is apparently responsible for the interference. Under the prescribed assay conditions, glycolic, lactic, tartaric and dextrose equal in weight to CMOS, contributed only 1.2, 1.5, 4.7 and 1.5% errors, respectively. These small errors could be tolerated in most instances. Of particular significance was the lack of interference from the Krebs cycle intermediates, citric, fumaric, malic, oxaloacetic and succinic acids.

Formaldehyde and acetaldehyde were also included in this experiment but displayed no absorbances, since both compounds were removed during the evaporation step.

Investigation of the Reaction

Aliphatic ethers are split fairly readily when heated in

TABLE V

Reaction of Aldehydes and Aldehyde-Producing Compounds with β-Naphthol

Test solutions, .000968M	Net absorbance	Equivalent to μg trisodium carboxymethyloxysuccinate	Color
Trisodium carboxymethyloxysuccinate	0.66	20.0	Yellow-green
Glycolic acid	0.68	20.5	Yellow-green
Acetaldehyde	0.09	2.7	Pale yellow
Butyraldehyde	0.003	---	
Formaldehyde	0.68	20.5	Yellow-green
Propionaldehyde	0.003	---	

[a]Concentrated H_2SO_4/β-naphthol reagent (1 ml) was added to 0.08 ml of each test solution and treated according to the prescribed method.

concentrated sulfuric acid to form alcohols and alkyl hydrogen sulfates (12-14), a reversal of the method by which ethers are formed. The alkyl hydrogen sulfates in addition can be converted to alcohols by hydrolysis (15,16). Therefore it was reasonable to assume that CMOS in the presence of high concentrations of sulfuric acid at 100 C would yield a mixture of malic and glycolic acids. Since glycolic acid under similar conditions can be measured quantitatively with β-naphthol (2), it became apparent that this method could be adapted for determining CMOS. This procedure is based upon the production of formaldehyde and its ability to condense with β-naphthol to form a highly colored diarylmethane. The condensation process is generally recognized as the Baeyer reaction (17-20). The results appeared to have substantiated the proposed reactions since CMOS, glycolic acid and formaldehyde, on an equal molar basis, displayed similar absorbances and colors (Table V). These colored products also exhibited identical spectra from 350 to 700 nm with maxima at 480 nm (Fig. 2). In addition, the reaction was relatively specific for formaldehyde since acetaldehyde was the only other aldehyde tested which gave a significant response. These data are in accordance with the results obtained in the glycolate study (2).

In the above experiment the evaporation step was omitted due to the volatility of the test compounds. The liquid samples, therefore, were treated with a concentrated sulfuric acid-β-naphthol (500 μg) reagent in order to maintain an initial acid concentration of 92.5% for maximum sensitivity.

The chromotropic acid test described by MacFayden (21) was used to provide further evidence that CMOS yields formaldehyde quantitatively. This reaction is also quite specific for formaldehyde or formaldehyde-producing compounds (22,23). Under these conditions CMOS and a formaldehyde control again displayed similar absorbancy readings and colors (Table VI).

An investigation was also initiated to prove the existence of the second end product, malic acid. Accordingly, various concentrations of CMOS were treated under the prescribed conditions; however the reaction mixtures after the incubation period were diluted with 80% sulfuric acid to a final volume of 50 ml. The fluorescence of each sample was determined at an excitation and emission wavelength of 375 and 441 nm, respectively. The final dilution volume and wavelengths were those employed by Christian and Moody (24) for the fluorometric determination of malic acid with β-naphthol. The procedure is based upon a reaction in which malic acid and β-naphthol when heated in sulfuric acid produce a blue fluorescence. There is evidence that 5,6 benzocoumarin is the fluorescing product (25). Fluorescence of the CMOS samples was maximum at the excitation and emission wavelengths reported by Christian and Moody, and a relatively linear calibration curve was obtained with 1-30 μg CMOS. Although these data indicate that this method may be an alternate procedure for the determination of CMOS, poor precision (estimated total error of a single reading was ca. 8.7%) made the assay unacceptable. However the investigation did provide evidence that some malic acid was formed.

The colored product described in the present method appears to be formed via the same mechanism previously reported for the determination of glycolic acid with β-naphthol (2). The reaction therefore involves the hydrolysis of CMOS by sulfuric acid to yield formaldehyde with glycolic acid as an intermediate; formaldehyde in turn reacts with β-naphthol ortho to the hydroxy group forming a methylene bis naphthol. This colorless product is then oxidized by sulfuric acid to a yellow-green *o*-quinoidal (26) or cyclic keto ether compound (27). Analogous reactions employing 2,7-dihydroxynaphthalene (28,29) or chromotropic acid (21,30) proved not as sensitive as the present assay.

TABLE VI

Chromotropic Acid Assay for Formaldehyde

Test solutions, .000388M	Net absorbance	Color
Trisodium carboxymethyloxysuccinate	0.170	Violet-pink
Formaldehyde	0.174	Violet-pink

[a]The test solutions (0.1 ml) were heated in 1 ml 92.5% H_2SO_4/ chromotropic acid (2 mg) reagent at 100 C for 60 min. After the heating period each reaction mixture received 3 ml 80% H_2SO_4 and the absorbance of each sample was determined at 570 nm.

[b]The amount of trisodium carboxymethyloxysuccinate employed was equivalent to 10 μg.

REFERENCES

1. Lamberti, V., M.D. Konort and I. Weil, Lever Brothers Co., U.S. Patent 3,692,685 (1972).
2. Viccaro, J.P., and E.L. Ambye, Microchem. J. 17:710 (1972).
3. Denigès, G., Ann. Chim. Phys. 18:149 (1910).
4. Denigès, G., Bull. Soc. Chim. Fr. 5:647 (1910).
5. Eegriwe, E., Z. Anal. Chem. 89:121 (1932).
6. Eegriwe, E., Z. Anal. Chem. 95:323 (1933).
7. Bredereck, H., Chem. Ber. 65B:1110 (1932).
8. Dische, Z., in "Methods in Carbohydrate Chemistry," Vol. 1, Edited by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, p. 478.
9. Feigl, F., "Spot Tests in Organic Analysis," Seventh edition, Elsevier Press, New York, 1966, p. 337, 425.
10. Litwack, G., "Experimental Biochemistry," John Wiley & Sons, New York, 1966, p. 19.
11. Ray, E.R., "Organic Chemistry," Lippincott, New York, 1947, p. 236.
12. Ray, E.R., Ibid., Lippincott, New York, 1947, p. 207.
13. Patai, S., "The Chemistry of the Ether Linkage," Interscience Publishers, New York, 1967, p. 26.
14. Jacques, D., and J.A. Leisten, J. Chem. Soc. (London) 1964:2683.
15. Ray, E.R., "Organic Chemistry," Lippincott, New York, 1947, p. 76.
16. Fieser, L.F., and M. Fieser, "Organic Chemistry," Third edition, Reinhold, New York, 1956, p. 130.
17. Fieser, L.F., and M. Fieser, Ibid., Third edition, Reinhold, New York, 1956, p. 867.
18. Migrdichian, V., "Organic Synthesis," Vol. 1, Reinhold, New York, 1957, p. 235.
19. "The Merck Index," Eighth edition, Merck, Rahway, N.J., 1968, p. 1140.
20. Walker, J.F., "Formaldehyde," Third edition, Reinhold, New York, 1967, p. 305.
21. MacFayden, F., J. Biol. Chem. 158:107 (1945).
22. Hoffpauir, C.L., G.W. Buckaloo and J.D. Guthrie, Ind. Eng. Chem. Anal. Ed. 15:605 (1943).
23. Feigl, F., "Spot Tests in Organic Analysis," Seventh edition, Elsevier Press, New York, 1966, p. 435.
24. Christian, G.D., and J.R. Moody, Anal. Chim. Acta 41:269 (1968).
25. Leininger, E., and S. Katz, Anal. Chem. 21:1375 (1949).
26. Kohn, M., and A. Ostersetzer, J. Chem. Soc. (London) 1919:114, 501.
27. Sěstanj, K.A., Ark. Kem. 23:80 (1951).
28. Eegriwe, E., Z. Anal. Chem. 89:121 (1932).
29. Calkins, V.P., Ind. Eng. Chem. Anal. Ed. 15:762 (1943).
30. Snell, F.D., and C.T. Snell, "Colorimetric Methods of Analysis," Third edition, Vol. 3A, Van Nostrand, New York, 1953, p. 249.

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