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Chromatographic Identification and Determination of Organic Acids in Water

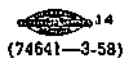
by H. F. MUELLER, T. E. LARSON, and W. J. LENNARZ

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Chromatographic Identification and Determination of Organic Acids in Water

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Analysts in the field of water and water chemistry have for many years faced the problem of quantitative separation and identification of small concentrations of organic compounds in water. Adsorption chromatography can quantitatively separate the various acids for later identification. This technique provides a new method for the separation of anionic synthetic detergents from analytical interferences. It may be considered as a research tool, where B.O.D. and C.O.D. determinations are inadequate, which may be further developed for study of additional metabolic products of biological purification processes. Neither "catch-all" method can approach the sensitivity and accuracy of chromatography.

BIOCHEMICAL oxidation of numerous organic materials in natural waters produces many metabolic products, including significant amounts of organic acids. The presence of organic acids in natural waters and their relationship to water quality are of particular interest to workers in sanitation. The primary object of this study was to determine the nature and quantities of

the organic acids that may appear in natural waters. It was also of interest to identify organic acids that survive the steps in sewage treatment. Such acids would be of concern as natural water contaminants.

Recently numerous studies have pointed to the usefulness of chromatographic separation and identification of many naturally occurring acids (1, 2, 4, 7, 9). The technique described here is the method of Bulen, Varner, and Burrell (1) as modified by Mueller, Buswell, and Larson (6) for quantitative identification of volatile acids. It seemed proper to evaluate this modified column for the separation of other recognized or unrecognized acids in waters. The organic acids in aqueous solution are adsorbed on silicic acid and subsequently eluted from the adsorption column by varying concentrations of butyl alcohol in chloroform. The eluting agents force the organic acids to travel through the column in such a manner that they are selectively adsorbed and separated into bands of pure substances. Though limited in the resolution of some acids, the method provides a favorable approach for the separation and tentative identification of several organic acids.

SAMPLE PREPARATION

The samples are made alkaline, immediately upon collection, to a pH of 8 to 9, so as to retain the organic acids as the nonvolatile salts. After filtration, 1-liter amounts are heat-concentrated to a volume approximating 30 ml. After cooling, the sample concentrate is acidified with hydrochloric acid and clarified by centrifugation when necessary, and the organic acids are removed by the continuous ether extraction method of Neish (5). These acids in the undissociated state are readily extracted by the ether, while the excess hydrochloric acid remains behind in the aqueous phase of the concentrate. The extraction is run overnight for convenience at a rate of 5 ml. per minute. A minimum of 4 hours has been reported by Neish as satisfactory.

After extraction is complete, the extract is titrated with sodium hydroxide to a phenol red end point. The neutralized solution is then warmed on a steam bath to volatilize the ether layer, and the aqueous phase containing the acid salts is finally evaporated to near dryness, acidified with sulfuric acid, adsorbed on silicic acid, and chromatographically separated.

CHROMATOGRAPHIC TECHNIQUE

Five grams of silicic acid (Mallin-

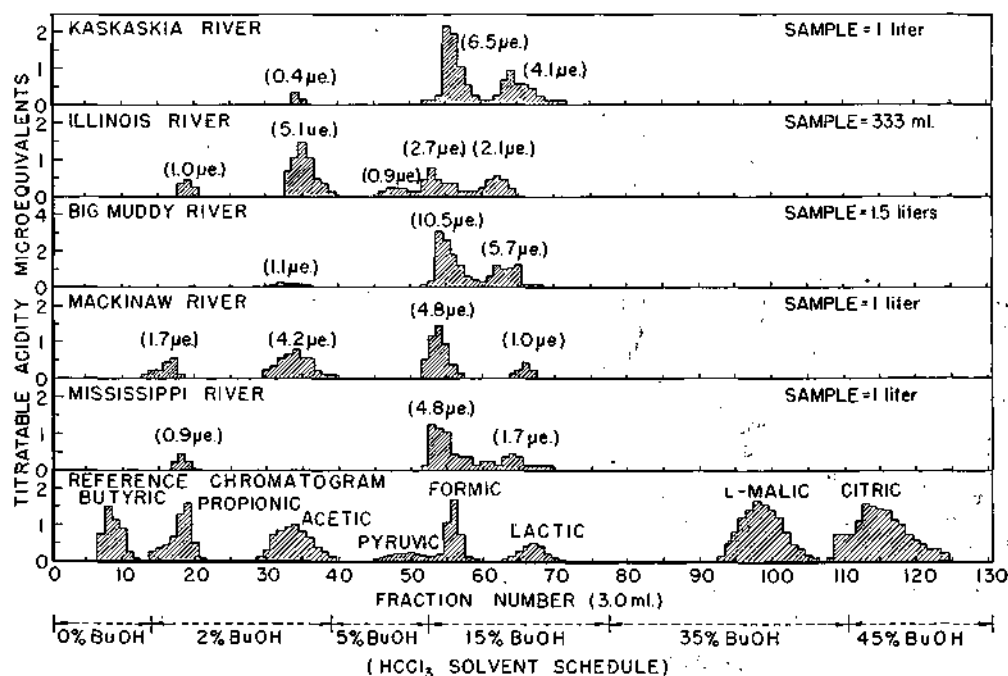
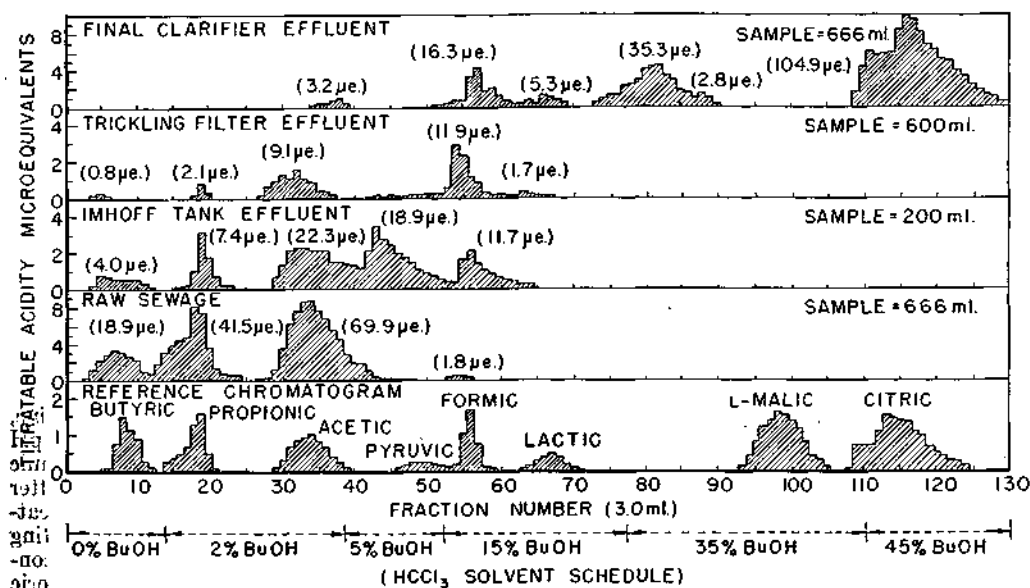


Figure 1. Separation of organic acids from river waters

Figure 2. Separation of organic acids from sewage effluent



ckrodt 100 mesh, specially prepared for chromatography), washed free of fine particles, are thoroughly mixed with 3.5 ml. of 0.5N sulfuric acid as the aqueous phase. Chloroform, previously equilibrated with 0.5N sulfuric acid, is added to the silicic acid and the resulting slurry is transferred to the chromatographic tube (10 mm. in inside diameter). A glass wool plug supports the column. A gas pressure of 10 to 12 cm. of mercury hastens removal of excess chloroform. An approximately 3-cm. depth of chloroform is retained above the column to ensure thorough wetting of the sample.

The 1.0-ml. sample, previously prepared, is acidified to a pH of 2 and thoroughly mixed with 2 grams of dry silicic acid. The dry preparation is quantitatively transferred to the top of the column and a glass wool plug is forced down firmly on the sample surface. This packing action thoroughly wets the sample preparation, giving a uniform column. The selected solvent schedule for the development of the chromatogram is shown beneath the reference chromatograms in Figures 1 and 2. Each subsequent solvent is added as the previous solvent just enters the glass wool capping. Gas pressure of 10 to 12 cm. of mercury, depending on freedom from fine particles, provides a flow rate of approximately 3.0 ml. per minute. Effluent fractions of 3.0 ml. are delivered by an intermittent siphon. To each fraction are added 6.0 ml. of water and the concentration of acid is measured by titration with 0.02N sodium hydroxide. A Cannon automatic titrator, with a Beckman Model G pH meter, is used for precise end point detection.

Blank values are determined on each solvent system and subtracted from the respective effluent titration values. The holdup volume of the column (approximately 14.0 ml.) is compensated for by subtracting each new blank value from the fifth 3.0-ml. fraction subsequent to the addition of each solvent system.

Each acid has a characteristic peak

effluent volume, which is the fraction having the highest titration value. The positions of various acids on the reference chromatogram and their recoveries by the technique described are indicated in Table I. The location of each acid was determined by repeated appearance in the position indicated, when chromatographed both singly and in mixtures. Formic, fumaric, and glutaric acids, eluted as one acid, require secondary treatment for complete separation (1). When both lactic and succinic acids are present, they may be

effectively separated by the method of Neish (7).

The method described here permits the detection of as little as 1.0 μ eq.; however, the column is best suited for separations of 2 to 75 μ eq. of each organic acid.

TECHNICAL APPLICATION

River Samples. The amounts of several organic acids found in five river samples are graphically summarized in Figure 1. A comparison of these data with the reference chromatogram shows several peaks corresponding to the peak effluent volumes for several known acids. Samples taken from the Illinois, Mackinaw, and Mississippi rivers showed traces of acids in the fractions corresponding to propionic acid. Acetic acid appeared in samples from the Illinois and Mackinaw rivers, but only traces were noted in the Kaskaskia and Big Muddy river samples. Peaks corresponding to formic and lactic acids on the reference chromatogram were observed on each of the river waters chromatographed. The degree of separation provides only a tentative means of their identification. Acids eluted beyond the 15% butanol in chloroform solvent were not further separated and identified in this preliminary study. Later studies have shown that the anionic detergents and other acids are adsorbed and subsequently eluted in the same fractional volume as formic acid.

Sewage Samples. Sewage samples obtained from the local treatment plant were treated in the same manner as the river waters. The nature of the organic acids observed in the effluents of several stages of the treatment process is illustrated in Figure 2. Peaks corresponding to butyric, propionic, acetic, and formic acids were

Table I. Positions and Recovery of Organic Acids on Chromatogram (Peak effluent)

Acid	Fraction ^a	% Recovery
Butyric	7	90.6
	8	104.0
	7	101.0
Propionic	18	96.4
	19	100.4
	18	104.0
Acetic	32	95.0
	32	100.0
	34	94.0
Formic	56	101.7
	55	93.0
Fumaric	55	97.3
Lauryl hydrogen sulfate	54	97.0
	54	113.0
	54	113.0
	56	104.0
Lactic	65	100.0
	67	97.2
	67	94.5
Gallic	78	94.0
	77	104.0
Succinic	64	100.8
	66	97.0
L-Malic	98	91.7
Citric	113	105.0

^a Fraction = 3 ml.

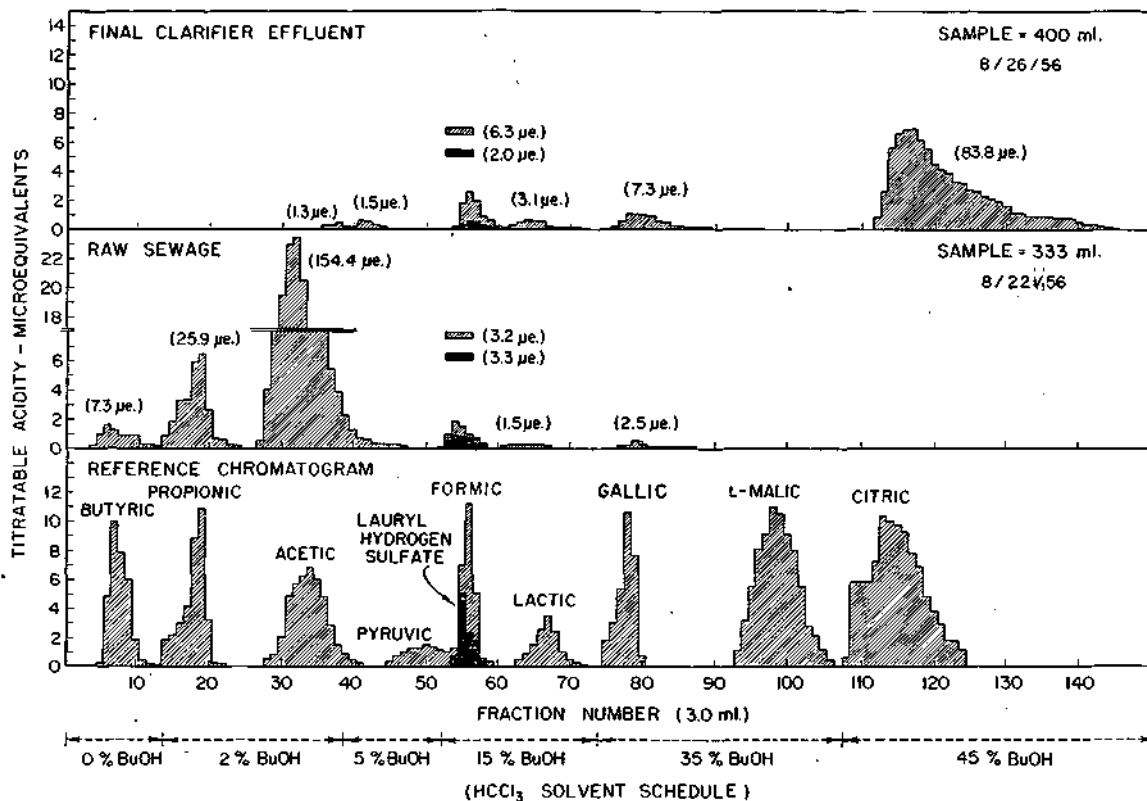


Figure 3. Separation of organic acids from sewage effluent

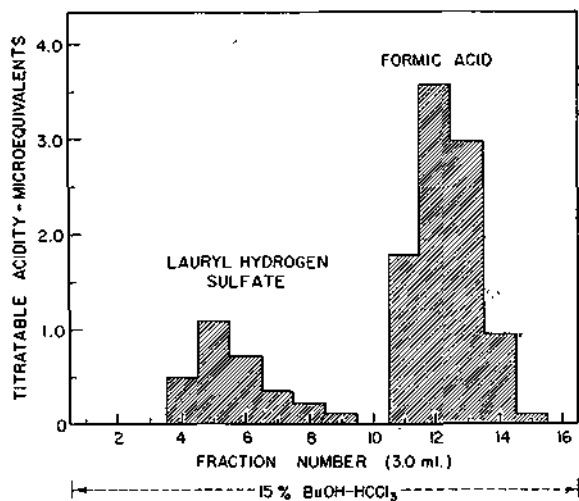


Figure 4. Secondary separation of lauryl hydrogen sulfate and formic acid

observed on samples of raw sewage, Imhoff tank, and trickling filter effluents. Butyric and propionic acids were absent in the final clarifier effluent; however, traces of an acid in the fractional range of acetic acid were observed. The Imhoff tank effluent presented an unidentified acid immediately following the elution of acetic acid. In the trickling filter effluent, smaller erratic peaks were noted in the fractional area of this unidentified peak. The results obtained on the final clarifier effluent are not readily interpretable. Peaks corresponding to the effluent volumes of formic and lactic acids, though present, were not clearly defined. This observation could be at-

tributed to the acids that are not resolvable with formic and lactic acids—i.e., formic-fumaric-glutaric acids and lactic-succinic acids. Two other peaks following the elution of fractions corresponding to lactic acid were noted and were not identified with the reference chromatogram. The presence of these acids in the final clarifier effluent was further substantiated by a repeat analysis on another effluent sample (Figure 3).

Detergents. The feasibility of extending the methods to synthetic detergents was also studied. These constitute alkyl sulfates and alkyl aryl sulfonates, and are ordinarily present as the sodium salts.

Preliminary investigation has shown that both detergent types are adsorbed by silicic acid and are subsequently eluted with butanol in chloroform in the fractional area characteristic of formic acid.

Detergent in the eluate may be determined either by titration with sodium hydroxide, or colorimetrically, by means of the blue complex formation between detergent and methylene blue (5). It was of interest to determine the amounts of anionic detergent in sewage effluents. Samples were selected from the inflow of raw sewage and from the effluent of the final clarification tank. After titration of the fractions, common to both the elution of formic acid and anionic detergents, the amount of detergent present in the fractions was determined colorimetrically (Figure 3). Assuming no loss in the procedure, the values were converted to microequivalents of acid and plotted for the respective fractions. The difference in acidity between the titrimetric and colorimetric procedures is attributed to formic acid or an unidentified acid. Confirmatory identification of formic acid has not been made.

It has since been demonstrated that formic acid and anionic detergents may be completely separated on a second column, when the initial solvent system used is 15% butanol in chloroform. The separation is quantitative for each acid (Figure 4).

Modification of the colorimetric method by first chromatographically

separating the detergent suggests increased accuracy by elimination of interferences in the usual procedure. Substances that normally interfere with the colorimetric method—thiocyanate, nitrite, peptone, and urine (5)—would not be expected to pass through the column with the detergent.

As the peak effluent volume of this anionic detergent coincides with that of formic acid, peaks previously observed in the fractional area of formic acid of both the river waters and sewage effluents may be partly or totally due to alkyl acid sulfates.

Anaerobic Digester Liquors. Analyses on the supernatant liquor from both the primary and secondary anaerobic digesters of the local treatment plant were of interest. Peaks corresponding to the commonly occurring fatty acids—butyric, propionic, and acetic—were observed in all samples chromatographed. The presence of these acids would be expected and is attributed to the metabolic activity of the organism types present. The repeated appearance in several analyses of a peak in the fractional area characteristic of synthetic detergents and formic acid, however, is not readily explainable. After titration of the fractions, common to the elution of these acids, the presence of anionic detergents was assayed colorimetrically. The results of one such assay showed 0.32 μ eq. of detergent present, or 4.3% of the acidity (7.4 μ eq.) as determined by titration. A similar analysis, which compared favorably in the quantities of acids present, showed 11.3% of the acidity in this fractional area accounted for

as synthetic detergent. Formic acid was not determined in the fractions.

Fractionation of the acids present in the distillate from the supernatant liquor resulted in peaks corresponding to butyric, propionic, and acetic acids; however, the peak common to synthetic detergents and formic acid, present in the analysis of the supernatant liquor, was not observed. Hence, the absence of formic acid in the chromatograph suggests the presence of another acid or acids that are coincident with the elution of synthetic detergents and formic acid. Fumaric and *a*-ketoglutaric acids are unresolvable with formic acid by the solvent system employed; however, their presence in such high concentration is improbable.

DISCUSSION AND SUMMARY

Preliminary data on river waters though not intended for comparative purposes, show a somewhat uniform correlation in the acid types appearing. These commonly occurring acids are likely to be of biological origin, but may come from other sources. It is possible that acids appear as a result of alkaline hydrolysis of complex materials during concentration of the sample. Also, anionic detergents and other acids are eluted in the same fractional area as formic acid.

On the other hand, the chromatographic analyses of the sewage effluents at various stages of the treatment process are not as readily interpreted as the analyses of the river waters. Several known and identifiable acid peaks are evident in each stage, but complex and erratic peaks of unknown origin are also present. This is ??

expected in view of the heterogeneity of waste materials and metabolic processes involved in their degradation.

The methodology and data presented only begin to illustrate the potentialities of the chromatographic analysis of natural waters. The procedure is not complicated and is quantitative for the acids included in the reference chromatogram. At present the method is capable of separating many organic acids occurring in natural waters. Further work is desirable to extend the number of organic acids identifiable by column and solvent modifications, and to provide supplemental tests for confirmatory identification of the acids by colorimetric means, as in the case of synthetic detergents, by selective enzymes, or by ionization product.

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