

DETERMINATION OF NONVOLATILE CARBOXYLIC ACIDS IN NATURAL WATERS

I. A. Goncharova, A. N. Khomenko and A. D. Semenov
Hydrochemical Institute, Novocherkassk

Organic acids enter water as a result of the vital activities¹¹⁶ of aquatic animals and plants [1-4], as well as with soil solutions [5, 6].

Oxalic, tartaric, succinic, citric, malic and lactic acids have been found among the nonvolatile carboxylic acids in the secretations of algae [1, 2]. Oxalic, succinic, fumaric, gallic and certain other acids also have been found in soil solutions [5, 6].

As the first investigations have shown, the group of carboxylic acids in natural waters is very large and diverse in composition. It includes acids which differ in structure, molecular weight, physical and chemical properties and physiological activity. If it also is considered that the composition of the acids evidently is highly changeable and that the content of some of them is very small, it becomes clear that detection and identification of acids in the water is quite a difficult task. It has been successfully solved in part, by using partition chromatography to this end [7, 8].

Butyric, acetic, formic, lactic, aconitic and citric acids and others which could not be identified, have been found identified in river water [8]. The acids listed do not exhaust all of the complex set of acids dissolved in water and, moreover, it is not certain that some of them, as the authors of one of the works mentions, appear in water "as a result of alkaline hydrolysis of complex substances in a concentrated sample" [7]. In this manner, only preliminary data on the qualitative composition of this large group were obtained.

*Numbers in the margin indicate pagination in the foreign text. 1

At the present time, determination of organic acids by partition chromatography on silica gel is quite widespread [9]. In particular, for determination of butyric, propionic and acetic acids in natural water, a modification of this method was proposed /117* earlier, the use of which permitted information to be obtained on the composition of volatile organic acids in a number of samples of surface and subsurface water [10]. The results of use of this modification for determination of nonvolatile carboxylic acids are presented in this report.

To establish the optimum conditions for separation of non-volatile carboxylic acids, tests were conducted with solutions of the pure substances. A mixture consisting of 10 acids was prepared: formic, lactic, citric, fumaric, butyric, adipic, pyruvic, succinic, oxalic and gallic. The first three acids, as has been mentioned above, have been found in water [8], and the presence of the remainder has not been established. Acids adsorbed on a silica gel column, were eluted sequentially with 5, 10, 20 and 30% solutions of butanol in chloroform [10].

While each of the volatile acids (butyric, propionic, acetic) has its characteristic elution peak, which makes it possible to establish the presence of a given acid without additional identification, in the case of the nonvolatile acids, only group identification is possible, since one solvent elutes several acids. It was determined that the acids included in the mixture begin to be eluted only with 5% and higher butanol content in chloroform, and that they make up four groups (Table 1). Such a group chromatographic determination of the acids was carried out in samples of various natural waters. The results of the identification are given in Table 2.

TABLE 1
Composition of individual groups of organic acids, separated on a silica gel column

| Group | I | II | III | IV |
|----------------------------------|---|--------------------|------------------|--------|
| Butanol content in chloroform, % | 5 | 10 | 20 | 30 |
| Acid | Formic Fumaric Butyric Adipic Pyruvic | Lactic Succinic | Oxalic Gallic | Citric |

TABLE 2
Results of determination of acids in water

| Water Source | Date Sample Taken | Acid Content, $\mu\text{g-eq/l}$ | | | | | |
|--------------------------|-------------------|----------------------------------|-------------|------|------|------|--------|
| | | Volatile | Nonvolatile | | | | Total |
| | | | I | II | III | IV | |
| Don River | Nov '64 | 2.80 | 6.83 | 2.13 | 1.13 | 7.11 | 20.00 |
| Don River | Jan '65 | 0.38 | 2.80 | 0.25 | 0.38 | 0.28 | 4.09 |
| Kama River | Sep '63 | 11.05 | 4.23 | 2.21 | 2.40 | 3.60 | 23.49 |
| Volgograd reservoir | Oct '64 | 2.42 | 11.63 | 1.16 | none | 3.04 | 18.25 |
| Kuban drowned estuary | Nov '64 | 3.82 | 17.47 | 3.10 | 1.79 | 5.09 | 31.27 |
| Well in Maykop gas field | Jul '64 | 761.7 | 1.70 | 2.20 | 1.00 | 1.50 | 768.10 |
| Well No. 28 | Aug '64 | 963.0 | 1.90 | 2.10 | none | 0.95 | 968.0 |

For the purpose of determining the yield of acids, tests were 118 carried out, with addition of the 10 acids listed above to a sample of natural water (Volgograd reservoir). The tests were conducted in the following manner. A mixture containing 100 $\mu\text{g-eq}$ of acid (10 $\mu\text{g-eq}$ each) was added to 0.5 l of water, caustic soda was added to pH 9 and it was evaporated almost to dryness. The residue was dried in a desiccator, at a temperature of 60°C, and dissolved in two ml of HCl solution (1:1), after which the organic acids were extracted twice with 50 ml of diethyl ether by shaking in a separatory funnel for a period of 10 min. The ether extract (100 ml)

was neutralized with 0.01 N alkali solution, the ether was removed and the water residue of organic acid salts was evaporated to 1 ml, acidified with 0.5 N sulfuric acid and placed in a column for chromatography. The yield of the group of nonvolatile organic acids indicated in Table 1, with the background content subtracted, is presented in Table 3.

TABLE 3
Yield of individual groups of nonvolatile acids

| Added μg-eq | I | | II | | | III | | | IV | | | Total μg-eq | Average Yield, % |
|----------------|-------|----|----------------|-------|----|----------------|-------|----|----------------|-------|----|----------------|---------------------|
| | Found | | Added μg-eq | Found | | Added μg-eq | Found | | Added μg-eq | Found | | | |
| | μg-eq | % | | |
| 50 | 43.1 | 86 | 20 | 15.4 | 77 | 20 | 7.2 | 36 | 10 | 6.1 | 61 | 71.8 | 72 |
| 50 | 42.9 | 86 | 20 | 12.9 | 65 | 20 | 7.6 | 38 | 10 | 6.1 | 61 | 69.5 | 70 |

After separation in the column and quantitative determination of the acids in the eluate, paper chromatography was used to identify them. A satisfactory separation of the acids on standard chromatograms was obtained by use of the following solvents: Phenol-1% formic acid solution in 7:3 ratio for Group I and II acids, and butanol-formic acid-water in 18:2:9 ratio for Group III and IV acids [5]. The R_f values obtained for standard elution samples and for the acids separated from natural water, are given in Table 4. Formic acid, as a volatile in these solvent systems, cannot be identified.

Grade "m" chromatographic paper of the Leningrad mill was used for the chromatography (for slow absorption). Duration of the chromatography was 8 hours. Upon completion of the test, the solvent was removed by drying (the chromatograms usually were left overnight at room temperature). The locations of the spots of organic acids on the chromatogram were determined by spraying with 0.04% alcohol solution of bromocresol green.

TABLE 4

R_f values obtained for standard chromatograms and for acids separated from water

| Group | Acid | R_f values | | | | | |
|-------|----------|----------------------|---------------------|-----------|------------|---------------------|-----------------------|
| | | For Individual Acids | In Mixture of Acids | Don River | Kama River | Volgograd Reservoir | Kuban Drowned Estuary |
| I | Pyruvic | 0.49 | 0.26 | 0.29 | 0.16 | 0.22 0.23 | 0.24 0.28 |
| | Fumaric | 0.54 | 0.47 | -- | -- | 0.41 | 0.49 |
| | Butyric | 0.79 | | -- | -- | 0.64 | -- |
| | Adipic | 0.86 | | 0.81 | -- | -- | 0.64 |
| II | Succinic | 0.49 | 0.48 | -- | 0.12 | 0.18 | 0.24 |
| | Lactic | 0.54 | 0.53 | -- | -- | -- | 0.51 |
| III | Oxalic | 0.14 | 0.11 | -- | 0.13 | -- | 0.15 |
| | Gallic | 0.64 | 0.66 | -- | -- | -- | -- |
| IV | Citric | 0.40 | -- | 0.17 | 0.27 | | 0.26 |
| | | | | 0.61 | | | |
| | | | | 0.70 | | | |

Note: A dash indicates the absence of data

It is evident from the data of Table 4 that the R_f values in the first group of acids, determined separately for each acid, are higher than for mixtures of them. Moreover, fumaric and glutaric acids demonstrate identical mobility in the mixture; therefore, there may be inaccuracies in identification of unknown acids in mixtures of them.

As a result of separation of the nonvolatile acids on the silica gel column (see Table 2) and subsequent paper chromatography, four groups of acids were found in samples of natural water/119 (see Table 4), and some of them were identified.

It can be noted from the data of Table 4 that the R_f values obtained in the first group of acids correspond to the values of pyruvic (Don River, Volgograd reservoir, Kuban drowned estuary)

and fumaric (glutaric) acids (Volgograd reservoir and Kuban drowned estuary), obtained on the standard chromagram.

The R_f values found in the second group of acids are close to those for lactic acid (Kuban drowned estuary) and, in the third group, for oxalic acid (Kama River, Kuban drowned estuary).

Besides, other spots were noted, the R_f values of which are absent in the standards used.

Other acids, present in very low concentrations, do not give sufficiently distinct spots for identification of them. The minimum content of acid, necessary for identification of them under the conditions adopted, should be at least 0.5 $\mu\text{g-eq}$ of each acid.

Conclusions

1. The proposed modification of partition chromatography on silica gel allows separation of a mixture of formic, pyruvic, fumaric, glutaric, adipic, lactic, succinic, gallic and citric acids into four groups. The acids can be identified by paper chromatography in the following solutions: Phenol-1% formic acid solution 7:3 (for Groups I and II acids) and butanol-1% formic acid-water 18:2:9 (for Groups III and IV acids), with the content of each of /120 them at least 0.5 $\mu\text{g-eq}$.

2. Four groups of nonvolatile organic acids were separated from samples of natural water, among which acids with R_f values close to the R_f values of oxalic, pyruvic, fumaric (glutaric) and lactic acids were found. The presence of other unidentified non-volatile acids also was established in the samples studied.

REFERENCES

1. Goryunova, S. V., Khimicheskiy sostav i prizhiznennoye vydeleniye vodorosli Oscillatoria [Chemical Composition and Living Secretions of the Alga Oscillatoria], USSR Acad. of Sci. Press, Moscow-Leningrad, 1950.
2. Goryunova, S. V., "Living secretions of vegetable acids in the water environment of the blue-green alga Oscillatoria," Dokl Akad Nauk SSSR 60 (8), 1409-1411 (1948).
3. Kuznetsov, S. I., Rol' mikroorganizmov v krugovorote veshchestv y ozerakh [The Role of Microorganisms in the Matter Cycle in Lakes], USSR Acad. of Sci. Press, Moscow, 1952.
4. Omelyanskiy, V. L., "Methane fermentation of cellular tissue," Arkh Biol Nauk 9 (3) (1902).
5. Kononova, M. M., Problemy pochvennogo gumusa i sovremennyye zadachi ego izucheniya [Problems of Soil Humus and Contemporary Tasks in Study of It], USSR Acad. of Sci. Press, Moscow, 1951.
6. D'yakonova, K. V., "The nature of humus matter in soil solutions, their dynamics and methods of study," Pochvovedeniye (4), 57-66 (1964).
7. Mueller, H. F., F. E. Larson, W. I. Lennarz, "Chromatographic identification and determination of organic acids in water," Anal. Chem. 30 (1), 41-45 (1958).
8. Mueller, H. F., F. E. Larson and M. Feretti, "Chromatographic determination and identification of organic acids," ibid 32 (6), 687-690 (1960).
9. Goncharova, I. A. and A. G. Stradomskaya, "Method of separate determination of organic acids in natural waters," Gidrokhim. Mater. 39 (1965).
10. Stradomskaya A. G. and I. A. Goncharova, "Separate determination of butyric, propionic and acetic acids in natural waters by partition chromatography on silica gel," ibid.
11. Magnitskiy, K. P., Yu. A. Shugarov and V. K. Malkov, Novyye metody analiza rasteniy i pochvy [New Methods of Analysis of Plants and Soils], Sel'khozgiz Press, Moscow, 1959, pp. 225-227.