

CHROM. 6190

THE DETERMINATION OF PART-PER-BILLION LEVELS OF CITRIC AND NITRILOTRIACETIC ACIDS IN TAP WATER AND SEWAGE EFFLUENTS*

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(Received June 5th, 1972)

SUMMARY

Citric and nitrilotriacetic acids can be determined at the 1-10,000 p.p.b.** levels in aqueous systems ranging from tap water to sewage effluents by use of anion-exchange clean-up, derivatization with butanol-HCl and gas chromatography. A variety of metals present at legal tolerance limits do not interfere. The two esterified acids separate well on a special gas chromatographic phase; however, citric acid can also be separated from nitrilotriacetic acid by ion exchange prior to derivatization, if so desired.

INTRODUCTION

Both the "natural" citric and the man-made nitrilotriacetic acid (NTA) are efficacious complexing agents that can be used as "builders", *i.e.*, detergent additives that soften the water. The fate and effects of NTA have been the subject of much controversy, focusing on its biodegradability, its toxicological effects, and its role in the transport of heavy metals in natural bodies of water (see, for instance, refs. 1-13). The use of NTA in detergents is currently permitted in Canada and prohibited in the United States; however, recent U.S. Government decisions make its re-introduction under restricted conditions almost certain¹⁴.

Analytical methods for NTA and citric acid abound^{1,2,15-55}. The zinc-Zincon², polarographic²¹⁻²⁹ and gas chromatographic (GC) techniques³⁰⁻³³ are most often used for NTA, while citric acid is usually determined by either liquid chromatography³⁶⁻⁴⁴ or GC⁴⁵⁻⁵⁵.

The reliable determination of complexing agents present at very low levels in motley waters involves the elimination of inorganic interferences as the main problem. Soft waters are fairly easy to handle but waters with a high degree of hardness or significant levels of heavy metal ions are difficult to analyze by all the methods listed.

* Parts of this study are a contribution from the Missouri Agricultural Experiment Station, Journal Series No. 6420. It was supported by a grant from the Rockefeller Foundation.

** Throughout this article the American (10⁹) billion is meant.

That metal ions may interfere with analytical methods based on chelation equilibria is fairly obvious. However, gas chromatographic methods suffer no less. The hardness of Columbia water, for instance, effectively prevents the esterification of NTA even at the relatively high 1 p.p.m. level, and ion-exchange clean-up consequently becomes necessary. Other chelating agents, such as naturally occurring carboxylic, hydroxy-carboxylic or amino acids, as well as NTA metabolites, can also interfere with both types of methods. Furthermore, acidification of turbid or contaminated water samples is often necessary to release the full amount of chelating agents for analysis.

Under these circumstances, the concomitant use of two disparate techniques, *e.g.*, polarography and GC, undoubtedly improves analytical reliability. This study is concerned with the GC method for NTA and citric acid, the improvement of its accuracy and sensitivity down to the lower p.p.b. ranges, using aqueous systems with high levels of inorganic and organic contaminants. The need for these improvements arose during an analytical project involving 300 samples of tap water, river water and sewage treatment plant influents and effluents.

For this project in particular, and for similar problems in general, the limits of detection were in need of improvement. Minute traces of NTA can occur in drinking water in regions where it is used as a detergent additive. Citric acid is a common metabolite whose presence in water is interesting with regard to biological activity. Both compounds, of course, strongly influence metal equilibria in aqueous systems. Consequently, precise concentration data are necessary to estimate the relative amounts of metals that are chelated, non-chelated, or, for that matter, may be solubilized from sediments under equilibrium conditions.

NTA is frequently monitored in the input and output of sewage treatment plants. Interest in these data is based on the bacterial degradation of NTA in the activated sludge and the role of residual NTA in the transport of heavy metals through the treatment into the plant effluent. Citric acid can be present in these effluents at fairly high levels and, unfortunately, its ester derivatives interfere with those of NTA on most GC phases.

Consequently, one of the first problems approached in this study was the separation of the NTA and citric acid esters by gas-liquid chromatography (GLC). A second problem involved the removal of materials that interfered with the derivatization. Initial experiments in our laboratory had shown that not all methods described in the literature gave satisfactory results with Columbia water, which is hardly surprising in view of its hardness (Table I); however, we felt that this very fact gave us a chance to study a particularly difficult matrix. In a third set of experiments, several metals with high chelate formation constants (Table II) were added to Columbia water and the analytical technique adjusted accordingly in order to exclude their interference. Finally, we searched for particular conditions under which most of the citric acid could be separated from NTA by ion exchange prior to GC. Although our GC separation of the two derivatives was adequate at comparable concentrations, a great excess of the earlier-eluting citric acid would have interfered with the NTA determination.

Much of the analytical technique used in this study is based on the work by CHAU AND FOX³² and WARREN AND MALEC³³. Quite helpful was the methodologically related knowledge on the determination of amino acids, directly available to us from GEHRKE's group (*e.g.*, ref. 56). The packing material used for GC originated from one

TABLE I

COLUMBIA TAP WATER ANALYSIS

<i>Component</i>	<i>Amount (p.p.m.)</i>	<i>Component</i>	<i>Amount (p.p.m.)</i>
Bicarbonates	357	Calcium	59
Carbonates	0	Magnesium	27
Silica	8	Nitrate	0.3
Iron	0.02	Sulfate	16
Aluminum	0.1	Chloride	33
Manganese	0	Fluoride	1.2
Sodium	46		
Potassium	5.6		
Total residue		424	

TABLE II

METAL IONS ADDED TO COLUMBIA TAP WATER

<i>Metal</i>	<i>Added as</i>	<i>Metal concentration (p.p.m.)</i>
Lead	Pb(NO ₃) ₂	0.10 ^a
Copper	Cu(NO ₃) ₂	0.02 ^a
Nickel	Ni(NO ₃) ₂	0.80 ^a
Zinc	ZnCl ₂	0.10 ^a
Chromium	CrCl ₃	1.00 ^a
Cadmium	CdCl ₂	0.01 ^a
Mercury	Hg(NO ₃) ₂	0.005 ^b
Iron	Fe(NO ₃) ₃	1.00 ^c

^a Legal limit in Missouri water, adopted by the State Pollution Control Board, 1971.

^b Tentative standard, U.S. Department of Health, Education and Welfare.

^c Arbitrary value, no legal limit set.

of our own studies of non-extractable polymers on diatomaceous supports⁵⁷.

EXPERIMENTAL

General procedure

The water samples can be stabilized with 1% formaldehyde if they are not processed immediately. The analysis starts with the addition of 2 ml of 4 M formic acid to 50 ml of sample. The pH is checked and lowered to 2.5 by the addition of more formic acid if necessary. The 125 ml erlenmeyer flask containing the sample is then put into a water-bath at 60° and purified nitrogen is bubbled through the solution from a PTFE capillary for 30 min. After coming to room temperature, the sample can be transferred to the reservoir of the ion-exchange column.

Bio-Rad AG1-X2, 50-100 mesh (Bio-Rad Laboratories, Richmond, Calif.) is used to pack a 2-in. resin bed into a 150 mm × 5 mm I.D. column (Fischer and Porter Co., Warminster, Pa.). (Solvent reservoirs were blown from some of these columns to speed up the handling of multiple samples.) Before runs, 10 ml of 16 M formic acid are passed through the resin followed by de-ionized water up to a pH of 5-6.

The sample is poured into the reservoir and allowed to flow through the resin at full speed (*ca.* 3 ml/min for tap water). It is followed by two 5 ml portions of 0.1 M

formic acid, and then the NTA and citric acid are eluted from the column with 16 *M* formic acid, of which 8 ml are collected in a 16 × 75 mm culture tube with a PTFE-lined screwcap. (These culture tubes are boiled for 2 h in concentrated HCl and rinsed with de-ionized water before re-use.)

The formic acid is evaporated from the culture tube in a tube-heating block under a stream of nitrogen. The block maintains the tubes at 85° for 2 h and is then allowed to cool while the evaporation continues overnight.

A 2 ml volume of dry 3 *N* HCl-butanol⁵⁶ is added to the dried samples and the culture tubes are capped tightly and placed for 25 min in an ultrasonic water-bath at 75°. The ultrasonic stirring is used only for the first 5 min of reaction time. After esterification, the tubes are cooled, opened and the excess of reagent removed by a slow stream of nitrogen in the tube-heating block. The evaporation starts at 85°, but the heat is turned off after a few minutes and the tubes are removed immediately when they appear to be dry.

Just prior to GC analysis, 100 μl of dry acetone are added to the esterified acids for a 2 μl injection into the gas chromatograph. The 5 ft. × 4 mm I.D. glass column contains a non-extractable packing derived from Carbowax 20M on Celite 545, 100–120 mesh (details of the preparation of this particular column will be published separately⁵⁷. Its performance is roughly comparable to that of a well coated, well conditioned 0.3% Carbowax 20M on well acid-washed Chromosorb W). The Microtek 220 injection port is maintained at 230° and on-column injection is used for isothermal chromatography at 183°. Calculations are based on peak heights.

Tap water calibration curve

Citric acid and nitrilotriacetic acid trisodium salt were added to Columbia tap water in amounts corresponding to 1, 3, 10, 30, 100, 300, 1000, 3000 and 10,000 p.p.b. each of the free acids and the samples were analyzed as described above.

Metal ions in water

A number of metals likely to interfere with the analytical procedure were added to tap water (Columbia municipal water supply) at levels corresponding to legal limits, as shown in Table II. There is no legal limit for iron in Missouri and its concentration was arbitrarily chosen. The resulting water was spiked with NTA and citric acid at the 1, 3, 10, 30 and 100 p.p.b. levels and analyzed as described under *General procedure*.

Sewage samples

Effluent from the Columbia sewage treatment plant was spiked with NTA and citric acid at the 1, 3, 10, 30 and 100 p.p.b. levels, and analyzed as described under *General procedure*.

Removal of citric acid by ion exchange

Columbia tap water was spiked with 1–100 p.p.b. of NTA, accompanied by a 100-fold excess of citric acid thus ranging from 100 to 10,000 p.p.b. The general procedure was followed; however, the resin was washed with two 5 ml portions of 2 *M* formic acid after the sample had passed through. This fraction (which contains up to 99% of the citric acid) can be discarded. NTA was then eluted with 16 *M* formic acid and derivatized.

RESULTS AND DISCUSSION

The general procedure described above has been developed in response to analytical problems that arose during a sampling project. The additional experiments on citric acid and metal interference were conducted to ensure a broader applicability of the method.

The ion-exchange and derivatization steps are modifications of the procedures described by CHAU AND FOX³², WARREN AND MALEC³³ and GEHRKE and co-workers (*e.g.*, ref. 56). The purpose of most of the procedural details is self-evident, but two points may warrant additional discussion.

The initial sample heating at 60° for 30 min in formic acid of pH 2.5 under nitrogen serves several purposes. First, it avoids the formation of bubbles in the later ion-exchange chromatography during elution with 16 *N* formic acid, thus circumventing an upset of the resin bed. Second, a certain acid concentration is necessary to prevent heavy metals from interfering with the analytical method. This was established during initial studies of metal interferences, which involved the application of a neutral sample to the ion-exchange column. In this event, almost no acid derivatives were found by GC analysis. Third, the repeated analyses of sewage samples from different locations with varying acid concentrations in the initial step showed that often, but not always, the highest amount of NTA was released by high acid concentrations. It has not been established whether this effect arose from conjugate hydrolysis, desorption from particulates, dissolution of suspended materials, and/or release of chelated metals. It should be stressed at this point that we do not filter turbid samples as is common practice in NTA analysis. Our own choice of acid concentration clearly represents a compromise engendered by the disparity of samples. It should be optimized whenever NTA or citric acid levels are studied in one particular type of water.

The second point of interest concerns the choice of a final solvent for injection into the gas chromatograph. Initially, methylene chloride was used in analogy to amino acid analysis. However, our flame ionization detectors slowly became noisy and lost sensitivity, presumably from carbon deposits. Changing to Freon 113³³ avoided the deposits, but the NTA derivative was increasingly decomposed in the gas chromatograph, despite the on-glass column injection, when greater numbers of samples were processed in succession. Therefore we made the somewhat unlikely choice of acetone. For some time, *n*-octacosane was used as internal standard in a manner similar to literature methods, but was abandoned as its use did not result in improved precision.

Fig. 1 shows the separation of NTA and citric acid butyl ester derivatives from Columbia tap water spiked with 100 p.p.b. each of the free acids. Fig. 2 is a calibration curve of the two acids in Columbia tap water. The lower part of this calibration curve is enlarged in Fig. 3, and the results of experiments 3, 4 and 5 are superimposed. Columbia sewage effluents contained a peak corresponding to 10 p.p.b. of NTA derivative and the respective results therefore trail off at this level. All other results fall reasonably close to the two lines.

The results indicate that the method works well with Columbia tap and sewage effluent water and could be expected to do likewise with a variety of other waters. (Sewage influent, however, still presents problems in the lower concentration ranges.)

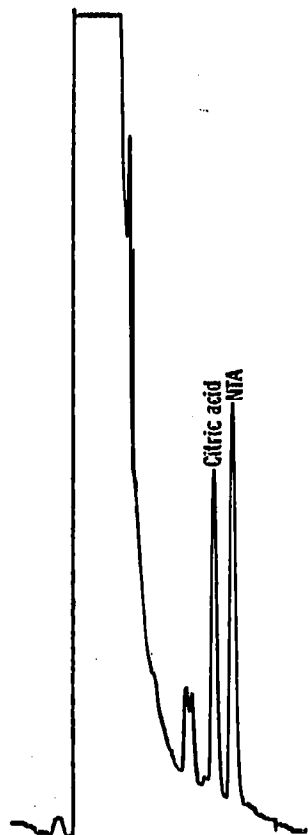
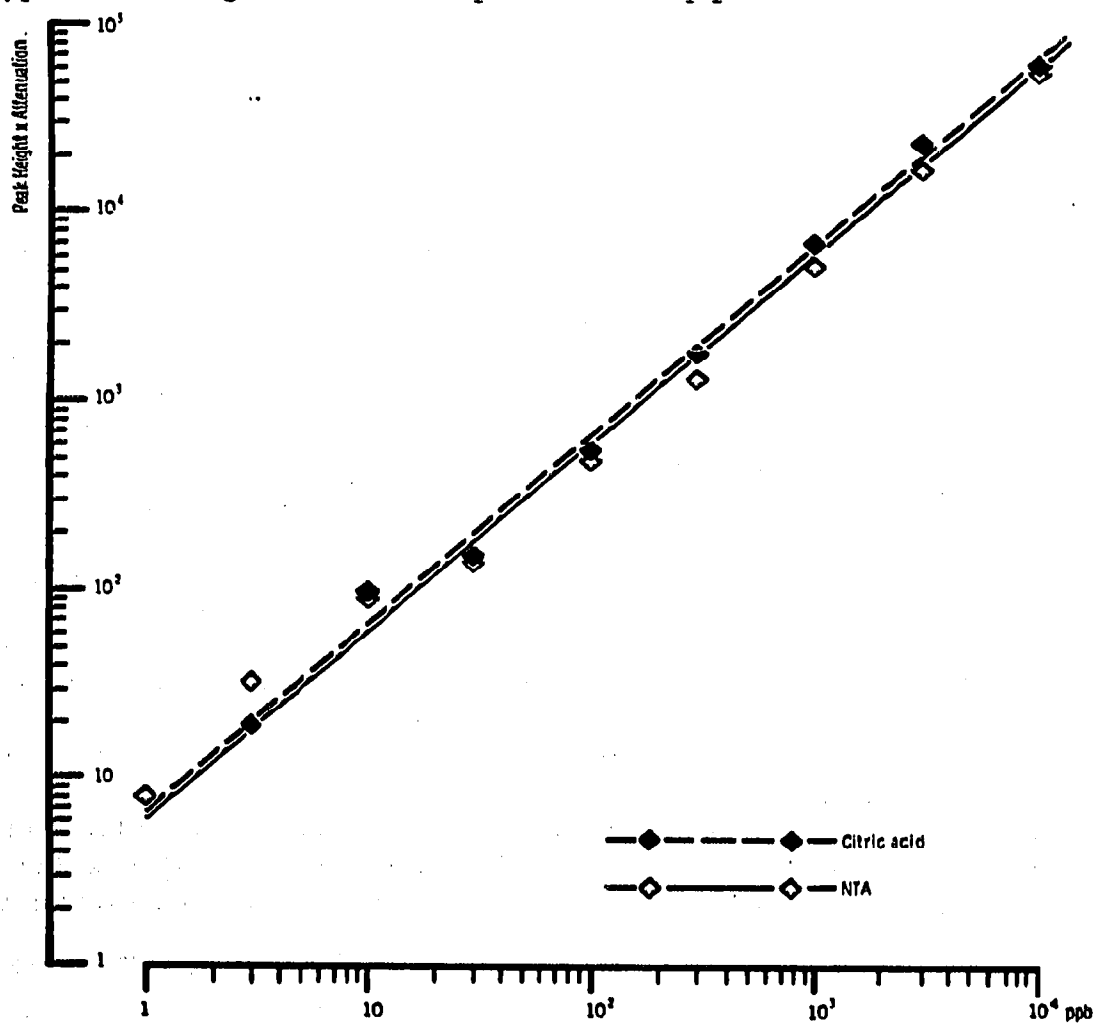


Fig. 1. Typical chromatogram from water spiked with 100 p.p.b. each of NTA and citric acid.



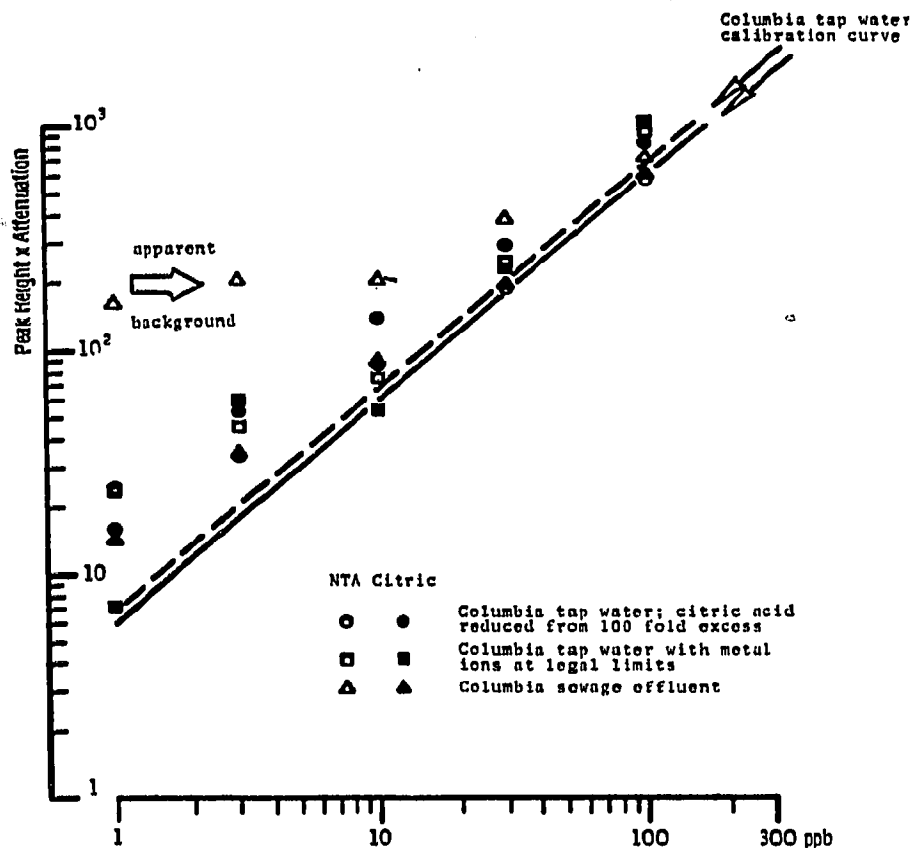


Fig. 3. Determination of NTA and citric acid under various conditions in the lower p.p.b. range.

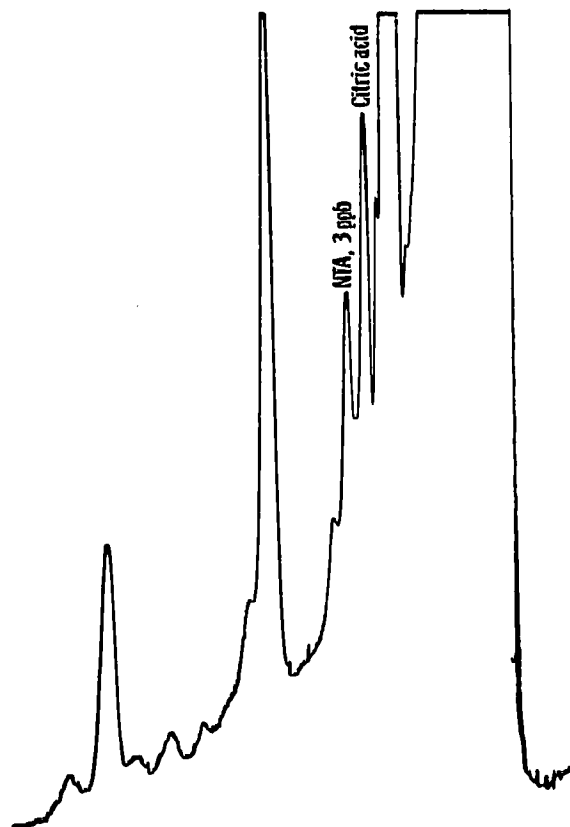


Fig. 4. Analysis of Columbia tap water containing 3 p.p.b. of NTA and 300 p.p.b. of citric acid, the latter reduced by ion exchange.

The metals selected did not interfere at the chosen conditions. The modified ion-exchange procedure worked well, reducing citric acid to approximately 1-2% of its original value. Fig. 4 shows a chromatogram of 3 p.p.b. of NTA determined in the presence of (originally) 300 p.p.b. of citric acid.

The method, as described, lowers the former limits of detection considerably, to about 1 p.p.b. of either acid. There is little interference from heavy metals and, generally, few extraneous peaks show up in the gas chromatogram. It should be noted, however, that the accuracy of the results, as is characteristic of most types of trace analyses in the p.p.b. range, could vary widely with waters of greatly different compositions. The analytical results obtained from such systems should therefore be interpreted with caution.

ACKNOWLEDGEMENTS

We thank Dr. C. W. GEHRKE and his group for considerable technical assistance and advice in the early stages of the project, Dr. R. L. DOWNEY for most helpful discussions and Mr. D. P. POZNIAK for skilful technical assistance.

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J. Chromatogr., 72 (1972) 259-267