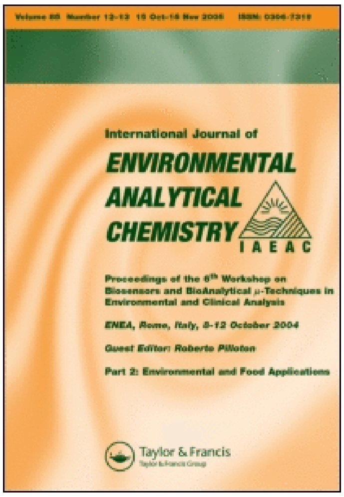

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Determination of Nitrilotriacetic Acid in Waters and Waste Waters by Gas-Liquid Chromatography, Differential Pulse Polarography and a Colorimetric Method

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A comparison of gas-liquid chromatography, differential-pulse polarography and a colorimetric method for the determination of nitrilotriacetic acid in settled sewage, sewage effluent, potable water, soil extracted water and deionised water has been undertaken. Differential-pulse polarography has also been applied to the determination of nitrilotriacetic acid in saline samples. By statistical analysis of replicate determinations, accuracy and precision have been evaluated, and calibration linearity assessed. Interferences were observed for sewage samples when analysed by all three methods. Precision was generally higher for differential-pulse polarography down to $100\,\mu g\,l^{-1}$, although only gas-liquid chromatography is applicable to concentrations below $25\,\mu g\,l^{-1}$ in non-saline samples. The colorimetric method was not applicable to concentrations below $500\,\mu g\,l^{-1}$ of nitrilotriacetic acid.

KEY WORDS: Nitrilotriacetic acid, gas-liquid chromatography, differential-pulse polarography, colorimetry, water analysis.

INTRODUCTION

The sodium salt of nitrilotriacetic acid (NTA) is being considered as a replacement detergent builder in order to reduce phosphate discharges in sewage effluents. Phosphate is often considered to contribute to the eutrophication of slow moving rivers and lakes. The incorporation of NTA in detergent formulations was discontinued in the United States as a result of the concern expressed by the Surgeon General[1] regarding possible health effects. NTA is currently being used in Canada where it constitutes 15% of the total weight of detergents sold, and to a more limited extent in the Netherlands and Switzerland.[2] NTA has been shown to chelate toxic 'heavy' metals,[3] and has been cited as a carcinogen.[4,5]

The determination of NTA in waters and waste waters has previously been reviewed[6] and it was concluded that the most widely adopted analytical techniques were gas-liquid chromatography, polarography and colorimetry. Monitoring programmes have been undertaken, largely in Canada, to evaluate the extent of NTA removal during sewage treatment and to determine residual NTA in potable and ground water.[7-9] An interlaboratory comparison of the gas-liquid chromatographic method of Aue *et al.*[10] and a differential cathode ray polarographic method developed by Afghan and Goulden,[11] has been undertaken by the Proctor and Gamble Co. and the Canada Centre for Inland Waters, respectively. The results of this comparison have been summarised by Matheson,[8] who states that the two methods produced statistically different results, the Proctor and Gamble values being significantly higher on average than the corresponding Canada Centre for Inland Waters values. The reasons for this discrepancy were not examined.

The gas-liquid chromatographic method used in the interlaboratory comparison and in this study was that of Warren and Malec,[12] as modified by Aue *et al.*.[10] NTA is isolated by anion-exchange concentration and the dried extract derivatized to form the tri-*n*-butyl ester, which is determined by gas-liquid chromatography using flame ionization. Differential cathode ray polarography has now been superseded by the more versatile and sensitive differential-pulse polarography[13] which has been adopted for this study. Of the other techniques available a colorimetric method developed by Longman *et al.*,[14] has found widest application although it is less sensitive. All three techniques are subject to interference[6] by both organics and inorganics in the water sample. Relatively little information is available on the extent of this interference or the accuracy and precision obtainable in various matrices.

This study was carried out to assess the applicability of gas-liquid chromatography, differential-pulse polarography and a colorimetric method to the determination of NTA in settled sewage, sewage effluent, potable water, soil extract and seawater where applicable.

MATERIALS AND METHODS

Primary settled sewage and final effluent were collected from Hogsmill Valley Water Pollution Control Works (Thames Water Authority, U.K.).

Grade 1 agricultural soil was obtained from Wye College Farm (University of London). Potable water was taken from the supply to the Public Health Laboratory and seawater taken from the Baltic sea was supplied by the Aquarium, Regents Park Zoological Gardens.

To obtain soil extract soil was shaken for 4 h with deionised distilled water and left to settle. All samples were filtered through GF/C glass microfibre filters (Whatman, U.K.) and subsequently through 0.45 μm microporous filters (Amicon, U.K.) and stored at 8°C in borosilicate aspirators.

Gas-liquid chromatographic analysis

An Erba Science Fractovap 4200 equipped with a flame ionization detector was used. The column was 2 m × 3 mm I.D. glass, packed with 3% OV-210 on 80–100 mesh Chromosorb W HP,[15] operated isothermally at 200°C. Helium was used as the carrier gas at a flow rate of $40\,\mathrm{ml\,min^{-1}}$. Hydrogen and air flows were optimised for the detector. The injector and detector temperatures were 240 and 280°C, respectively.

Solutions and reagents

All reagents used were AnalaR grade except where indicated. The anion exchange resin was Bio-Rad AG1-X2; 50–100 mesh, formate form.

Derivatizing reagent preparation: Hydrogen chloride generated by adding concentrated sulphuric acid to solid sodium chloride, was dried by passage through two sulphuric acid towers in oven-dry apparatus before dissolving in Aristar butan-1-ol (B.D.H., U.K.). The hydrogen chloride was adjusted to 3 M by weight increase.

Internal standards: dibutyl phthalate (99%, Aldrich Chemical Co., U.K.) and nitrilotripropionic acid (Sigma Chemical Co., U.K.).

Gas-liquid chromatography solvent: Pesticide grade dichloro-methane (Fisons, U.K.).

Procedure

The procedure used prior to analysis was that developed by Aue *et al.*.[10] Anion-exchange concentration followed by derivatization to the tri-n-butyl ester allowed NTA to be determined by flame-ionization gas-liquid chromatography. The column packing was that of Williams *et al.*.[15] Dichloromethane was chosen as the solvent in preference to acetone to reduce solvent tailing and column degradation. Dichloromethane (200 μl) was added to each dry derivative by syringe prior to duplicate 2 μl injections.

A modified preparative procedure outlined by Games *et al.*[16] was applied to certain samples, and consisted of narrowing the elution molarities of the formic acid. In order to evaluate the effect of this procedure on recovery a series of experiments was undertaken to analyse deionised water and settled sewage spiked with $50\,\mu g\,l^{-1}$ of NTA. Five replicates of each matrix were prepared by the method of Aue *et al.*[10] and a further five replicates of each by the modified method of Games *et al.*.[16]

Concentrations were determined by comparison with calibration results using peak height measurements. Dibutyl phthalate[17] and nitrilotripropionic acid[16] were evaluated as internal standards, but neither improved quantification at NTA concentrations above $100\,\mu g\,l^{-1}$ and at lower concentrations they proved to be difficult to determine due to solvent tailing interference.

The identity of the tri-*n*-butyl ester of NTA was confirmed by GC-mass spectrometry using a JEOL DS300 mass spectrometer coupled to an Erba Science Fractovap 4200 with a 25 m fused silica capillary column coated with OV-101.

Polarographic analysis

A Princeton Applied Research Corporation (PAR) Model 384 Polarographic Analyser was used together with a PAR Model 303 Static Mercury Drop Electrode and a Houston Instruments Model DMP-2G Digital Plotter. The Model 303 electrode system incorporates a Ag/AgCl reference electrode separated from the analyte by a vycor frit, and a PTFE-coated platinum counter electrode.

Solutions and reagents

All reagents used were AnalaR grade except for Aristar hydrochloric acid (BDH, U.K.).

Acetate electrolyte: 0.5 M sodium acetate in 9.5 M acetic acid.

Bismuth/acetate electrolyte: dissolve 50 mg ± 2 mg of $Bi(NO_3)_3 . 5H_2O$ in 100 ml of acetate electrolyte.

Chloride electrolyte: 1 M Aristar hydrochloric acid.

Bismuth/chloride electrolyte: dissolve 50 mg ± 2 mg of $Bi(NO_3)_3 . 5H_2O$ in 100 ml of chloride electrolyte.

Hydroxylammonium sulphate and hydroxylammonium chloride were prepared weekly as 2% wt/vol. solutions.

Procedure

The method is based on the formation of a Bi-NTA complex which is determined by differential-pulse polarography as described by Hoover.[13]

Two supporting electrolytes were applied, an acetate electrolyte for non-saline waters and a chloride electrolyte for saline waters.

Method 1

Applicable to settled sewage, sewage effluent, tapwater, soil water and other natural and waste waters having a chloride content less than 0.1%.

Pipette 5.0 ml of sample into each of two 10 ml volumetric flasks. Add 0.1 ml of hydroxylammonium sulphate solution by Eppendorf micropipette (Anderman & Co., U.K.) and heat to nearly boiling to remove interference from Fe^{3+}. Cool to room temperature and add 0.1 ml of acetate electrolyte to the reference and 0.1 ml of 1×10^{-3} M, bismuth/acetate electrolyte to the sample using a Micropettor (Alpha Laboratories Ltd., U.K.) positive displacement micropipette. Dilute each to the mark with deionised water, transfer to polypropylene electrochemical cells and analyse blank and sample consecutively purging with high purity argon saturated with the electrolyte. Instrumental parameters are listed in Table I.

Method 2

Applicable to seawater and other waters having a chloride content greater than 0.1%.

Pipette 5.0 ml of the sample into each of two 10 ml volumetric flasks. Add 0.1 ml hydroxylammonium chloride solution to each. Add 0.1 ml of chloride electrolyte to the blank and 0.1 ml of 1×10^{-3} M bismuth/chloride electrolyte to the sample, using an Eppendorf

TABLE I

Instrumental parameters for the Model 384 Polarographic Analyser

Parameter	Acetate electrolyte	Chloride electrolyte
Scan rate	2 m V sec^{-1}	2 m V sec^{-1}
Initial potantial[a]	−0.100 V	0.000 V
Final potential[a]	−0.400 V	−0.300 V
Pulse height	50 m V	50 m V
Operation mode	Differential pulse	
Drop time	1s	
Overrides:		
blank subtraction	ON	
tangent fit	ON	
peak location	ON	
real time data smoothing	OFF	

[a] Initial and final potentials of scans varied slightly due to ageing of the reference electrode vycor frit.

micropipette. Dilute each to the mark with deionised water, transfer to PTFE electrochemical cells and analyse blank and sample consecutively as before, using the instrumental parameters listed in Table I. Standards were prepared in 0.5 M potassium chloride.

NTA concentrations exceeding $100\,\mu g\,l^{-1}$ were scanned twice per sample, the average being computed and displayed automatically. At $100\,\mu g\,l^{-1}$ and below three replicate scans were made per sample. Between each scan the solution was purged for 30 s. Medium drop size was normally set on the Model 303 electrode system, which corresponded to a drop weight of 2.5 mg[18] and an area of $0.0156\,cm^2$ assuming a spherical drop. Where specified a large drop size was set which corresponded to a drop weight of 5.4 mg[18] and an area of $0.0261\,cm^2$, again calculated assuming a spherical drop.

Colorimetric analysis

A Pye Unicam SP8-100 U.V./visible spectrophotometer was used, set at 620 nm with a 1 nm bandwidth. Matched 20 mm glass cells were supplied by Frost Instruments, U.K.

Solutions and reagents

As detailed by Longman *et al.*[14] all reagents were AnalaR grade except for zincon which was laboratory reagent grade (B.D.H., U.K.).

Procedure

NTA was determined using the zinc–zincon procedure developed by Longman *et al.*,[14] but with minor modifications. The ion exchange columns were fitted with 50 ml dropping funnels (Corning, U.K.) allowing the samples to be introduced dropwise so as not to disturb the Chelex 100 (Bio-Rad Laboratories, U.K.) resin bed. The elutriation rate was never greater than the $1\,ml\,min^{-1}$ recommended and was usually in the range $0.5–0.75\,ml\,min^{-1}$. The first 50 ml of eluate were rejected.

Glassware

Borosilicate glassware was used throughout. Glassware was cleaned by soaking in 5% v/v Decon 90 detergent for 12 h, rinsing with distilled water, leached in 10% v/v AnalaR nitric acid for 12 h and finally rinsing at least three times in glass distilled water. Polarographic cells were similarly cleaned prior to use and dried at 50°C.

Culture tubes used in the gas-liquid chromatographic analysis were boiled in concentrated hydrochloric acid for 2 h and thoroughly rinsed with glass distilled water prior to use.

Addition of NTA

NTA was spiked from freshly prepared standards into the samples immediately prior to analysis using a 100 μl Eppendorf micropipette. The relative standard deviation of this addition was determined by weight on five replicates and was found to be 0.2%. All NTA concentrations are expressed as the anion (NTA^{3-}).

Statistical analysis of results

The values reported were those obtained by comparison with spiked deionised water standards (0.5 M potassium chloride in the case of seawater analysis by differential pulse polarography) producing a linear calibration graph, corrected by blank determinations for each matrix. For each set of replicates the mean concentration and relative standard deviation (per cent) were calculated together with the between groups analysis of variance (F-test).[19] Tukey's test[19] was applied to identify means that were statistically different at the 0.05 significance level.

RESULTS

Gas-liquid chromatography

Concentrations of 10, 100 and 1000 $\mu g\,l^{-1}$ of NTA in ten replicate subsamples of deionised water and five replicate samples of potable water and water extracted from soil together with sewage effluent and settled sewage were determined by the procedure of Aue *et al.*,[10] the results of which are indicated in Table II. In order to undertake a valid statistical comparison the settled sewage samples, which exhibited marked positive interference despite blank values of less than 1.0 $\mu g\,l^{-1}$ of NTA, were not included in the analysis of variance presented in Table II. A separate treatment of the results including settled sewage indicated that these results were significantly higher than for other matrices at each concentration at the 0.01 significance level.

Examination of Table II reveals that the F-test results were highly significant at 10 and 100 $\mu g\,l^{-1}$ while at 1000 $\mu g\,l^{-1}$ of NTA there was no significant difference between matrices, excluding settled sewage. Recovery was significantly higher from the soil extract, being 133.8% relative to deionised water at 100 $\mu g\,l^{-1}$ of NTA, and for potable water and sewage effluent with recoveries of 127.9% and 148.1% respectively at 10 $\mu g\,l^{-1}$ of NTA. Recoveries from other matrices appeared higher but were not significantly higher due to the relatively greater scatter. The observed relative standard deviation ranged from 1.9% for sewage effluent at

TABLE II

Variation in recoveries of spiked NTA by gas-liquid chromatography from deionised, potable and soil extracted water, sewage effluent and settled sewage

Concentration of spike $\mu g\,l^{-1}$	Matrix	F-Test result[a]	Mean[b] concentration $\mu g\,l^{-1}$	RSD %	Range $\mu g\,l^{-1}$
10	Deionised	0.01	9.73 a[c]	11.6	6.5–11.1
	Potable		12.79 b	8.9	11.0–13.8
	Soil extract		10.09 a	7.9	9.2–11.1
	Sewage effluent		14.81 c	5.7	13.5–15.6
	Settled sewage		91.8 [a]	9.3	76.8–100.9
100	Deionised	0.01	100.3 a	9.3	82.0–114.8
	Potable		109.3 a	6.5	97.5–117.4
	Soil extract		133.8 b	3.2	127.9–139.9
	Sewage effluent		106.0 a	6.2	93.6–111.9
	Settled sewage		302.7 [a]	6.4	274–324
1000	Deionised	N.S.	1000 a	7.0	838–1134
	Potable		1004 a	9.7	916–1167
	Soil extract		1027 a	5.3	966–1076
	Sewage effluent		1057 a	1.9	1028–1079
	Settled sewage		1285 [a]	8.3	1174–1404

[a] F-Test does not include settled sewage (see text).

[b] Of five replicate determinations except for deionised water.

[c] Means not followed by a common letter are significantly different at the 0.05 significance level.

$1000\,\mu g\,l^{-1}$ of NTA, up to 11.6% for deionized water at $10\,\mu g\,l^{-1}$ NTA. In general, standard deviation was highest for deionised water followed by potable water and settled sewage, while soil extract and sewage effluent had the lowest scatter.

The results of the comparison of preparative procedures are included in Table III. It may be seen that recovery was significantly higher for settled sewage using both methods, but there was no significant difference between methods for each matrix.

Three spiked samples per matrix were analysed to determine calibration linearity up to $1000\,\mu g\,l^{-1}$, the results are included in Table IV. Since the calibrations were undertaken under slightly differing instrumental conditions, they are not directly comparable, although all display good linearity. A study of NTA recovery at concentrations below $10\,\mu g\,l^{-1}$ was not carried out, although a limited number of analyses suggest that concentrations in this range could not be stated accurately using this procedure and would best be reported as being 'below $10\,\mu g\,l^{-1}$'.

TABLE III

Comparison of recoveries from deionised water and settled sewage applying the procedures of refs. 10 and 17

Procedure	Matrix	F-Test result	Mean[a] recovery %	RSD %
Aue *et al.*[10]	Deionised		100.0 a[b]	14.9
	Settled sewage		137.5 b	13.8
		0.01		
Games *et al.*[17]	Deionised		106.9 a	15.4
	Settled sewage		156.9 b	6.4

[a] Of five replicates, relative to deionised water analysed by the method of Aue *et al.*.[10]

[b] Means not followed by a common letter are significantly different at the 0.05 significance level.

TABLE IV

Calibration results for the determination of NTA by gas-liquid chromatography between 10 and 1000 $\mu g\, l^{-1}$.

Matrix	a[a]	b[b]	Correlation coefficient
Deionised	−214	56.18	0.9999
Potable	+93.4	54.63	1.0000
Soil extract	−530	59.04	0.9999
Sewage effluent	−348	55.84	0.9999
Settled sewage	−551	58.43	0.9999

[a] Peak height (mm) × attenuation.

[b] Peak height (mm) × attenuation per $\mu g\, l^{-1}$.

DIFFERENTIAL-PULSE POLAROGRAPHY

Non-saline water analysis

Subsamples of deionised, potable and soil extracted water, sewage effluent and settled sewage were analysed by differential-pulse polarography in acetate electrolyte. Five replicate samples using automatic blank subtraction were determined for each matrix at 100, 500, 1000 and 2500 $\mu g\, l^{-1}$ of NTA, and a statistical analysis undertaken the results of which are included in Table V. Unless otherwise specified analyses were undertaken using the medium drop size.

Examination of the results indicates that the F-test was highly significant at all concentrations. At concentrations greater than 100 $\mu g\, l^{-1}$

TABLE V

Variation in recoveries of spiked NTA by differential pulse polarography from deionised, potable and soil extracted water, sewage effluent and settled sewage

Concentration of spike $\mu g\ l^{-1}$	Matrix	F-Test result	Mean[a] concentration ($\mu g\ l^{-1}$)	RSD (%)	Range ($\mu g\ l^{-1}$)
100	Deionised	0.01	100 a[b]	6.3	91–106
	Potable		118 b	4.6	110–125
	Soil extract		98 a	4.4	93–103
	Sewage effluent		136 c	3.6	131–137
	Settled sewage		91 a	5.0	86–97
500	Deionised	0.01	500 a	1.1	493–507
	Potable		475 b	1.2	464–483
	Soil extract		501 a	1.7	491–513
	Sewage effluent		467 b	1.0	472–483
	Settled sewage		388 c	1.9	378–397
1000	Deionised	0.01	1000 a	2.4	987–1039
	Potable		887 b	1.7	856–896
	Soil extract		906 b	2.1	881–930
	Sewage effluent		800 c	1.0	787–809
	Settled sewage		725 d	0.7	720–732
2500	Deionised	0.01	2500 a	2.4	2417–2579
	Potable		2213 b	1.5	2179–2257
	Soil extract		2237 b	0.4	2228–2247
	Sewage effluent		1938 c	1.6	1893–1965
	Settled sewage		1804 d	1.0	1778–1819

[a] Of five replicate determinations.

[b] Means not followed by a common letter are significantly different at the 0.05 significance level.

recovery was generally lower than from deionised water, the divergence becoming more marked as NTA concentration increased. Settled sewage exhibited the lowest recovery at all concentrations studied, although at $100\,\mu g\,l^{-1}$ recovery was not significantly different from deionised water at the 0.05 significance level. Potable water and sewage effluent exhibited high recoveries at $100\,\mu g\,l^{-1}$ of 118% and 136% respectively. All the results display little scatter, the highest relative standard deviation being 6.3% for deionised water at $100\,\mu g\,l^{-1}$ of NTA. Relative standard deviations above $100\,\mu g\,l^{-1}$ did not exceed 2.4%.

A series of samples were analysed to determine calibration linearity in the ranges 100 to $300\,\mu g\,l^{-1}$ and 500 to $2500\,\mu g\,l^{-1}$, the results are presented in Table VI. Linearity was generally good, but differences in the slope of the calibrations in the two concentration ranges were observed.

TABLE VI

Calibration results for the determination of NTA by differential pulse polarography

Matrix	No. of points	Concentration range (mg l^{-1})	a(nA)	b(nA/mg l^{-1})	Corellation coefficient
Deionised	3	0.1–0.3	−2.13	62.77	0.9997
	5	0.5–2.5	−10.68	79.93	0.9999
Potable	3	0.1–0.3	−1.04	58.75	0.9962
	5	0.5–2.5	−5.77	67.24	0.9996
Soil extract	3	0.1–0.3	−1.20	58.90	0.9993
	5	0.5–2.5	−1.07	67.74	0.9967
Sewage effluent	3	0.1–0.3	−0.18	52.35	0.9998
	5	0.5–2.5	−1.93	57.72	0.9999
Settled sewage	3	0.1–0.3	−1.62	49.95	0.9997
	5	0.5–2.5	−3.86	54.33	0.9998
0.5 M Potassium	3	0.1–0.3	0.01	73.90	0.9920
chloride	5	0.5–2.5	3.23	43.30	0.9990
Seawater	3	0.1–0.3	−2.62	53.55	0.9516
	5	0.5–2.5	−3.55	40.49	0.9996

The intercept was negative in each case and generally decreased for the lower calibration suggesting a curvature at low concentrations. NTA concentrations of 50 $\mu g\,l^{-1}$ could be detected in all samples on medium drop size although greater sensitivity was achieved using a large drop size, with an inherent noise increase. Five replicate determinations were carried out on settled sewage spiked with 50 $\mu g\,l^{-1}$ of NTA using a medium drop size and subsequently a further five replicates using a large drop size, under otherwise identical conditions. The results are presented in Table VII. Using large drop size concentrations between 20 and 30 $\mu g\,l^{-1}$ NTA could be detected.

TABLE VII

Effect of drop size on peak height for settled sewage spiked with 50 $\mu g\,l^{-1}$ of NTA

Drop size	Mean[a] current (nA)	RSD (%)	Range (nA)
Medium	1.686	10.9	1.424–1.870
Large	4.226	9.2	3.800–4.650

[a] Of five replicates.

It was evident that the composition of the standards is of vital importance and ideally similar matrices, not containing NTA, should be used to construct calibrations, otherwise recovery losses would have to be taken into account.

Saline water analysis

An initial study of the electrolyte composition of the standards confirmed that this was of vital importance in determining the peak height of the bismuth-NTA complex. Comparable recovery to seawater was obtained from a 0.5 M potassium chloride solution which had a chloride content close to that of the seawater itself.

Five replicate samples of seawater and 0.5 M potassium chloride solution were analysed at NTA concentrations of 100, 500, 1000 and 2500 $\mu g\,l^{-1}$ of NTA, using automatic blank subtraction. The results, including a statistical analysis of the data are presented in Table VIII. Recovery and calibration analyses were undertaken using the medium drop size.

Examination of Table VIII indicates that the T-tests were highly significant at 100 and 2500 $\mu g\,l^{-1}$, while there was no significant difference at 500 and 1000 $\mu g\,l^{-1}$ of NTA. Recovery of NTA from seawater was 65, 101, 88.3 and 85.4% at spiked concentrations of 100, 500, 1000 and

TABLE VIII

Variation in recoveries of spiked NTA by differential pulse polarography from 0.5 M potassium chloride and seawater

Concentration of spike $\mu g\,l^{-1}$	Matrix	T-Test result[b]	Mean concentration ($\mu g\,l^{-1}$)	RSD (%)	Range
100	0.5 M KCl	0.01	100	15.7	84–123
	Seawater		65	23.8	50–88
500	0.5 M KCl	N.S.	500	6.6	481–559
	Seawater		505	7.2	447–542
1000	0.5 M KCl	N.S.	1000	7.9	899–1111
	Seawater		883	12.8	741–1041
2500	0.5 M KCl	0.01	2500	2.6	2422–2583
	Seawater		2136	0.9	2109–2155

[a] Of five replicate determinations.

[b] N.S. not significant at 0.05 significance level.

$2500\,\mu g\,l^{-1}$. The scatter of results was generally higher for seawater than 0.5 M potassium chloride, except at $2500\,\mu g\,l^{-1}$. A comparatively high relative standard deviation of 23.8% was recorded at $100\,\mu g\,l^{-1}$ of NTA in seawater.

A series of spiked samples was analysed to determine calibration linearity in two ranges as for the acetate electrolyte, the results are presented in Table VI. Linearity was good, but seawater and 0.5 M potassium chloride diverged markedly at low concentrations. The detection limit was around $50\,\mu g\,l^{-1}$ of NTA, using a large drop size and three replicate scans per sample.

It appears that standard composition is again of great importance for the construction of calibration curves and that chloride content was not the only important parameter.

Colorimetric analysis

Five replicate subsamples of deionised, potable and water extracted from soil, sewage effluent and settled sewage together with two blanks for each matrix were analysed by the colorimetric procedure, containing NTA at concentrations of 500, 1000 and $2500\,\mu g\,l^{-1}$. The results together with a statistical analysis are indicated in Table IX.

Examination of Table IX reveals that settled sewage results exhibited a marked positive interference despite an insignificant blank determination. The F-test comparing all matrices indicated that the settled sewage results were highly significant at each concentration. However, since the settled sewage results were regarded as outliers the reported F-test values do not include settled sewage results. The other matrices produced a highly significant F-test value, positive interference being observed for sewage effluent at each concentration studied at around $200\,\mu g\,l^{-1}$. Since this is not within the limit of detection, blank subtraction may not have corrected for other chelants in the sewage effluent. Recovery for soil extract was highly quantitative with 100.2, 100.9 and 103.8% recoveries at 500, 1000 and $2500\,\mu g\,l^{-1}$, respectively, although the low scatter of the $2500\,\mu g\,l^{-1}$ results produced a significant difference at the 0.05 significance level. Generally, recovery was higher than from deionised water and scatter was significant, especially for sewage type samples at and below $1000\,\mu g\,l^{-1}$.

A calibration curve was constructed using a series of deionised water standards containing NTA in the range 1000–$20000\,\mu g\,l^{-1}$. It was determined, by visual examination of the calibration plot, that the calibration was linear up to $5000\,\mu g\,l^{-1}$, although higher concentrations could be analysed with reduced precision up to $15000\,\mu g\,l^{-1}$. A five point

TABLE IX

Variation in recoveries of spiked NTA by colorimetric analysis from deionised, potable and soil extracted water, sewage effluent and settled sewage

Concentration of spike $\mu g\ l^{-1}$	Matrix	F-Test result	Mean[a] concentration $\mu g\ l^{-1}$	RSD %	Range $\mu g\ l^{-1}$
	Deionised		502 a[c]	12.1	381–584
	Potable		688 b	15.6	611–703
500	Soil extract	0.01	501 a	7.5	438–537
	Sewage effluent		684 b	25.6	422–900
	Settled sewage		2254 [b]	29.5	1584–3179
	Deionised		1000 a	5.2	891–1114
	Potable		935 a	5.0	878–982
1000	Soil extract	0.01	1009 a	4.1	961–1073
	Sewage effluent		1229 b	10.1	1114–1435
	Settled sewage		2407 [b]	22.6	1737–3209
	Deionised		2500 a	2.9	2371–2645
	Potable		2558 ab	1.5	2498–2601
2500	Soil extract	0.01	2596 b	0.6	2577–2618
	Sewage effluent		2730 c	2.0	2675–2813
	Settled sewage		4030 [b]	1.8	3962–4130

[a] Of twenty replicates for deionised water, five for other matrices.
[b] F-test does not include settled sewage (see text).
[c] Means not followed by a common letter are significantly different at the 0.05 significance level.

calibration between 1000 and 5000 $\mu g\ l^{-1}$ produced the following regression equation in terms of absorbance.:

$$Y(\text{Absorbance}) = 0.0093 + 0.0737 \times (\text{mg NTA l}^{-1})$$

with a correlation coefficient of 1.0000. Concentrations less than 500 $\mu g\ l^{-1}$ could not be analysed with any consistency.

DISCUSSION

Interference is a problem frequently encountered in the analysis of NTA in raw and waste waters. The gas-liquid chromatographic method of Aue *et al.*,[10] is prone to both inorganic and organic interference, derivatization being impeded by the presence of alkali and alkaline earth metals together with certain transition metals and 'heavy' metals; while naturally occurring carboxylic acids such as citric acid can cause positive interference due to poor resolution between the esters of the acids.[16] Positive interferences in sewage type samples has previously been reported by Aue *et al.*[10] and

Games *et al.*,[16] the latter applying radiolabelled ^{14}C-NTA to overcome variable recovery problems from sewage influent and effluent samples.

The gas-liquid chromatography results reported in this study display severe matrix interference for settled sewage samples which could not be accounted for by blank recoveries which were near the detection limit of the method. The interference was not decreased by the formic acid elution molarity modifications outlined by Games *et al.*.[16] The separate use of two internal standards, dibutyl phthalate[17] and nitrilotripropionic acid,[16] did not improve recovery at high concentrations and neither could be easily chromatographed at low concentrations using a flame ionization detector and packed column. Since dibutyl phthalate is added prior to injection or nitrilotripropionic acid prior to derivatization neither corrects for losses over the complete determination procedure. The use of an NPD detector[15] may offer an increase in resolution due to reduced solvent tailing although Games *et al.*[16] report wide day-to-day and daily fluctuations from this type of detector.

The analysis of potable water by gas-liquid chromatography exhibited a positive interference which became statistically significant at low concentrations, while soil extract displayed a positive interference which appeared to be reduced at low concentrations, a phenomenon which may be explained by the variable recovery previously observed. Excluding the settled sewage results only soil extract exhibited a statistically significant difference from deionised water containing NTA at $100 \mu g l^{-1}$, while at $1000 \mu g l^{-1}$ none of the other matrices exhibited a significant difference.

In practice, the method proved to be a long and painstaking procedure, care being required to avoid overheating the extracts during the two drying stages, a significant loss of ester having been previously reported by Aue *et al.*[10] and Games *et al.*.[16]

The differential-pulse polarographic procedure is also prone to interference due to high stability metal complexes preventing the formation of bismuth-NTA, and other chelants in the sample complexing the bismuth. Significant suppression was evident for sewage type samples and potable water above $100 \mu g l^{-1}$ of NTA while soil extract also exhibited suppression above $500 \mu g l^{-1}$ of NTA. Hoover[13] stated that sewage effluent produced a lower response to NTA than 'clean' water, although precision improved. Scatter was generally small using differential-pulse polarography. The production of standards using a similar uncontaminated matrix to the one being analysed would produce the most quantitative results. Due to the negative intercept and calibration slope variation observed at low NTA concentrations standard additions would not be expected to improve accuracy. The detection limit of $10 \mu g l^{-1}$ of NTA claimed by Hoover[13] was found to be unattainable in any matrix using this technique, 25 $\mu g\,l^{-1}$ being a more realistic detection limit in non-saline waters.

NTA analysed in saline samples according to the method of Hoover[13] could be detected at 50 $\mu g\,l^{-1}$ which conforms to the stated detection limit. Interference due to the high inorganic content of seawater prevented the application of gas-liquid chromatography or colorimetry to saline waters. The variation in peak height with chloride content suggests that a lower limit of detection may be attainable in estuarine waters.

Colorimetric analysis exhibited severe positive interference in settled sewage samples and some interference in sewage effluent samples. The blank corrections were in all cases lower than the detection limit set at 500 $\mu g\,l^{-1}$ of NTA. Precision was high at 2500 $\mu g\,l^{-1}$ and was generally good at 1000 $\mu g\,l^{-1}$, except for settled sewage samples. However, it decreased markedly at low concentrations, recovery becoming highly variable at 500 $\mu g\,l^{-1}$ of NTA for sewage samples and for all matrices below this concentration. The colorimetric method is not specific to NTA since it determines chelating capacity but has found use in controlled experiments where blank determinations are possible.

CONCLUSIONS

Interference is exhibited in sewage type samples analysed for NTA by gas-liquid chromatography, differential-pulse polarography and the colorimetric method. Precision is generally greater for the differential-pulse polarographic method down to 100 $\mu g\,l^{-1}$ of NTA. For the determination of NTA at concentrations lower than 25 $\mu g\,l^{-1}$ gas-liquid chromatography is necessary. Above 25 $\mu g\,l^{-1}$ the lengthy analysis time required favours polarography. The colorimetric method is not applicable to NTA concentrations less than 500 $\mu g\,l^{-1}$.

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