Determination of β-alaninediacetic acid in waste waters and aquatic environment using GC-NPD

Mika Sillanpää,*[a] Vida Vickackaite,[b] Jaakko Rämö[a] and Lauri Niinistö[a]

[a] *Laboratory of Inorganic and Analytical Chemistry, Helsinki University of Technology, PO Box 6100, FIN-02015 Espoo, Finland*
[b] *Department of Analytical Chemistry, Vilnius University, Naugarduko 24, LT-2006 Vilnius, Lithuania*

Received 1st June 1998, Accepted 17th July 1998

New chelating agents need to be applied to industrial purposes, because the conventional compounds, such as ethylenediaminetetraacetic acid (EDTA), have been demonstrated to cause adverse environmental effects. A promising compound in this respect is β-alaninediacetic acid (β-ADA). A reliable analytical procedure for the determination of β-ADA in waste waters and natural waters is presented in this study. The method is also applicable to sediments after extraction of β-ADA with phosphate. An aqueous sample was evaporated to dryness and treated with an esterification reagent containing ethanol, propanol or butanol. The resulting esters were determined by capillary gas chromatography using a nitrogen–phosphorus specific detector (GC-NPD). The best results were obtained by esterification with propanol at 86 °C for 2 h. The response was linear up to 10 mg dm^{-3}. The limits of detection in distilled, waste, lake and sea waters were 2.0, 2.7, 2.5, 2.9 μg dm^{-3}, respectively, and 0.21 μg g^{-1} in sediments. The relative standard deviation (RSD) values of retention times did not exceed 0.46%. The run-to-run and day-to-day repeatabilities (calculated as RSD) in distilled water were below 7%, except near the limit of detection. In the natural waters studied the recoveries were 49–111%, in sediments only 19–21%. High concentrations (above 10 mg dm^{-3}) of Fe^{3+} interfere with the determination of β-ADA.

Introduction

β-Alaninediacetic acid (β-ADA) is a strong chelating agent being thus a potential substitute in industrial applications for the more conventional compounds, such as ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). These complexing agents are used in increasing amounts in the pulp and paper industry to remove or inactivate metal ions which catalyze the decomposition of added bleach (ozone, peroxide, dithionite). EDTA especially is relatively slowly degradable in waste water treatment plants (degrades up to 30%) and therefore significant amounts of these compounds can be released to the aquatic environment.[1–6] Their environmental fate has recently been reviewed.[7] As there are reasons for environmental concern for EDTA and DTPA, the applicability of alternative compounds should be investigated.

β-ADA is a newer chelating agent and has been applied in photographic processes only for a few years. Its biodegradability and aquatic toxicity have recently been investigated.[8] It was shown that β-ADA degrades significantly more readily (up to 99%) in waste water treatment plants than EDTA and DTPA. In addition, β-ADA has a very low aquatic toxicity.[8]

We have studied β-ADA in connection with peroxide bleaching process simulation.[9] Preliminary results show that β-ADA inactivates metal ions almost as effectively as EDTA and DTPA, and is therefore compatible with EDTA and DTPA in the pulp and paper industries, but superior to them in terms of degradability and aquatic toxicity. In order to study the behaviour of β-ADA in pulp and paper processes, waste water treatment plants and especially in the aquatic environment in more detail, a sensitive method for its determination is needed. Very recently a method to determine β-ADA by liquid chromatography (LC) has been presented and this appears to be the only available method reported in the literature.[8] With an enrichment of the sample in the ratio 10 : 1 a detection limit of 100 μg dm^{-3} for β-ADA was obtained. In natural waters, however, the expected level of concentration is much lower. For instance, concentrations of the less degradable EDTA and DTPA in the waters influenced by pulp and paper mill effluents are 2–50 and 10–20 μg dm^{-3}, respectively.[10,11] As the limits of detection for the corresponding compounds, namely EDTA and DTPA, were found in our earlier studies to be much higher for the LC method[12] than for gas chromatographic (GC) determination,[13,14] we expected that the sensitivity could be improved for β-ADA analysis by developing an analytical procedure based on GC.

Experimental

Apparatus

The apparatus consisted of the following: Hewlett-Packard HP 5860 Series II Plus (Avondale, PA, USA) gas chromatograph equipped with a nitrogen–phosphorus detector (NPD) and a model HP 7673 automatic liquid sampler with a 10 μl syringe. The analytical column used was an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm). The data were analyzed by an HP 3365 A ChemStation.

The injector temperature was 250 °C, the detector temperature was 300 °C. The column oven temperature in the case of determination of single β-ADA was as follows: initial temperature 100 °C (held for 1 min), increased at 60 °C min^{-1} to 200 °C (held for 8 min). In the case of simultaneous determination of β-ADA, EDTA and DTPA, the oven temperature was: initial temperature 100 °C (held for 1 min); increased at 60 °C min^{-1} to 200 °C (held for 5 min), increased at 50 °C min^{-1} to 245 °C (held for 9.3 min). Injection of a 1 μl sample was performed in the splitless mode with a 1 min purge off using nitrogen as a carrier gas at a constant flow rate of 3.5 ml min^{-1}.

Reagents and solutions

H_2SO_4, $KHCO_3$, anhydrous Na_2SO_4, $NaH_2PO_4 \cdot H_2O$ and C_4H_9OH were obtained from Merck (Darmstadt, Germany), C_2H_5OH from Primalco (Rajamäki, Finland), C_3H_7OH from BDH (Poole, Dorset, UK), heptadecanoic acid nitrile (HDAN) and $CaNa_3$–DTPA from Fluka (Buchs, Switzerland), toluene from Lab-Scan (Dublin, Ireland), Na_4-EDTA from Sigma (St. Louis, MO, USA), β-ADA from BASF (Ludwigshafen, Germany). All chemicals were of analytical-reagent grade and were used without further purification.

A standard stock solution containing 100 mg dm^{-3} of β-ADA was prepared by dissolving β-ADA in distilled water and was stored in the dark at room temperature for not more than 2 months. An internal stock standard solution of 1000 mg dm^{-3} was prepared by dissolving 100 mg of HDAN (used as an internal standard) in 100 ml of C_2H_5OH and was stored in a refrigerator for 1 month at the most. HDAN was chosen as an internal standard on the basis of having similar chemistry to the analyte.

Esterification reagents were prepared in a 50 ml calibration flask by mixing 2.5 ml of H_2SO_4 and about 30 ml of C_2H_5OH, C_3H_7OH or C_4H_9OH, adding 2.5 ml of 10 mg dm^{-3} internal standard solution and diluting to volume with C_2H_5OH, C_3H_7OH or C_4H_9OH, respectively.

Samples

Water samples were prepared in four different matrices: distilled water, lake water, sea water and waste water. The lake water was from Lake Saimaa (SE Finland). The sea water samples were from the Gulf of Finland at Tvärminne . Waste water samples were collected from the effluents of pulp and paper mills located on the shore of Lake Saimaa. Surface sediment samples were collected at Lake Saimaa at depths of between 5 and 10 m.

As β-ADA is not yet used in industrial applications to a significant extent, we expected it to be absent in the sample matrices studied. Experiments with the blanks showed no detectable amounts of β-ADA in the samples.

Sample preparation

A 20 ml water sample was spiked with β-ADA to give the desirable concentration (5–5000 μg dm^{-3}). The spiked sample was evaporated to dryness in an oven at 110 ± 5 °C, cooled to room temperature, after which 1 ml of esterification reagent was added and esterification was executed for 2 h in a water bath in a vial sealed with a screw-cap. After esterification the sample was extracted into 1.5 ml of toluene and neutralized with 10 ml of 1 mol dm^{-3} $KHCO_3$. These esterification conditions were found to be optimum for the analysis (see Esterification section). The extracted organic phase was dehydrated by anhydrous Na_2SO_4 and analysed by GC-NPD.

Sediments were dried in an oven at 90 °C and 1 g of the sample was spiked with 1 ml of a solution containing a desirable concentration of β-ADA. These sediments were left overnight to adsorb, then dried again at 90 °C and extracted with 20 ml of 2 mmol dm^{-3} $NaH_2PO_4 \cdot H_2O$ solution for 10 min. The suspension was filtered through a dense filter (Macherey–Nagel, 640 d) and the filtrate was analysed as described above.

Treatment of results

To compare the different esterification methods and the influence of the esterification conditions, the area ratio of the individual chromatographic peak of β-ADA and that of the internal standard was measured. This relative peak area was used in all further calculations. Recoveries from the natural water, waste water and sediment matrices were calculated by comparing the relative peak areas of spiked sea, lake and waste water and sediment samples with the respective relative peak areas of spiked distilled water samples. The detection limits were determined with the criterion of signal-to-noise 3.

Results and discussion

Preliminary tests

Preliminary tests were performed using ethanol, propanol and butanol for β-ADA esterification. After an hour's esterification, ethyl, propyl and butyl esters of β-ADA were extracted into toluene, neutralized by $KHCO_3$, dehydrated by anhydrous Na_2SO_4 and run by GC. Fig. 1 reveals that the retention times of butyl esters were longer than those of propyl esters and especially those of ethyl esters. Also, butyl ester gave significantly lower recovery. Therefore, ethylation and propylation were used in further experiments.

The effect of the sample pH on the recoveries was investigated in the pH range 2–11. Distilled water was used as a sample matrix with a sample size of 20 ml, pH was adjusted with H_2SO_4, $KHCO_3$ or NaOH, the sample was evaporated to dryness and esterified. It was found that pH had no observable effect on the results.

Esterification

To examine the influence of temperature, esterification reactions were carried out at 60, 80, 86, 100 and 120 °C with reaction times between 10 min and 5 h. Samples were heated in an oven or in a water bath (86 °C). Peak areas of β-ADA ethyl ester and β-ADA propyl ester at different temperatures are presented in Fig. 2. At 60 °C, reaction of β-ADA with both esterification reagents was slow, resulting in low recoveries. Instead, at higher temperatures recoveries increased. However, after 2.5–3 h at 86 °C and higher temperatures, peak areas began to decrease, most likely due to decomposition of β-ADA esters. In addition, at high temperatures the degradation of internal standard was observed. In Fig. 3, relative peak areas as a function of time are presented. Based on Fig. 3 and the above discussion, the experimental conditions of 86 °C and 2 h were chosen.

To investigate the optimal amount of esterification reagent, 20 ml of 50 mg dm^{-3} β-ADA water sample was esterified with 0.5–2.5 ml of esterification reagent containing either ethanol or propanol. In both cases, 1 ml was found to be sufficient.

Further, β-ADA esters were extracted with 1.5 ml of toluene and neutralized. A solution of 1 mol dm^{-3} $KHCO_3$ was used in the neutralization stage. As can be seen from Fig. 4, pH 7 is reached after addition of 6 ml $KHCO_3$ solution. Therefore, a minimum of 10 ml of the neutralization solution was used.

Both ethanol and propanol can be used for β-ADA esterification. If there are other complexing agents present, such as EDTA and DTPA, a simultaneous determination of all three chelating agents can be performed by ethylation (Fig. 5).

However, the propyl ester of β-ADA has higher molecular mass than the corresponding ethyl ester and, therefore, higher boiling point and retention. This can be sometimes useful when higher resolution from matrix peaks is required. Therefore, in the method validation section below, propylation was used.

Extraction from sediments

Sediment samples were prepared as described in the Experimental section. For the extraction, 20 ml of 2 mmol dm^{-3} $NaH_2PO_4 \cdot H_2O$ solution was used. Because phosphate has been

found to be able to compete with metal-EDTA complexes for adsorption sites on iron oxides,[15] phosphate solution was applied for EDTA and DTPA extraction from sediments.[11] Thus, the same step was relatively successfully applied to β-ADA analysis. The extraction times of 10, 15, 20 and 30 min were investigated. The results revealed that the slight differences obtained were within analytical precision. Thus, the extraction time of 10 min was chosen.

Influence of metal ions

The interference of several metal ions on β-ADA analysis was studied. The influence of Fe(III), Cd, Ni, Pb, Cu(II), Hg(II), Mn(II) and Cr(III) on the recovery of β-ADA was investigated by analysing 20 ml spiked samples containing 10 μg dm^{-3} and 5 mg dm^{-3} β-ADA and 1×10^{-3} mol dm^{-3} metal ion. For 5 mg dm^{-3} β-ADA concentration there was no interference. In the case of 10 μg dm^{-3} β-ADA, there was no interference with one exception: no β-ADA peak was observed in the chromatogram, when iron was added prior to analysis at the concentration mentioned above. The influence of iron was further investigated by analysing 20 ml 10 μg dm^{-3} β-ADA samples containing 0.05, 0.1, 1.0, 5.0, 10.0, and 30.0 mg dm^{-3} Fe(III). Iron starts to interfere at concentrations above 10 mg dm^{-3}. In Finnish natural waters the iron content is below 1 mg dm^{-3}.[13] In the lakes containing clay or during the overflow, the concentration can exceed 1 mg dm^{-3}. Hence, the interference of iron is expected to be significant only in the analysis of heavily contaminated waters, especially untreated waste waters.

Linearity of the method and quantitative aspects

The calibration graph was linear in the range of 0–10 mg dm^{-3} β-ADA. Correlation coefficients of the linear calibration graphs were between 0.998-0.999 (n = 5). The equation of the calibration curve is $y = (2.382 \pm 0.042)x + (0.243 \pm 0.018)$.

The detection limits obtained from the four different water sample matrices were 2.0, 2.5, 2.9, and 2.7 μg dm^{-3} for distilled, lake, sea and waste water, respectively. For sediments the detection limit was 0.21 μg g^{-1}.

The retention time for β-ADA propyl ester was about 6.1 min. In natural and waste waters, the chelating agent studied can interact with different metal ions, humic and fulvic acids, as well as with sediments and organisms. Thus, a stable retention time of the analyte is essential if the identification is based on the retention. The RSD values (%) of the retention time of β-ADA were 0.15, 0.12, 0.19, 0.46 and 0.45 in distilled water, lake

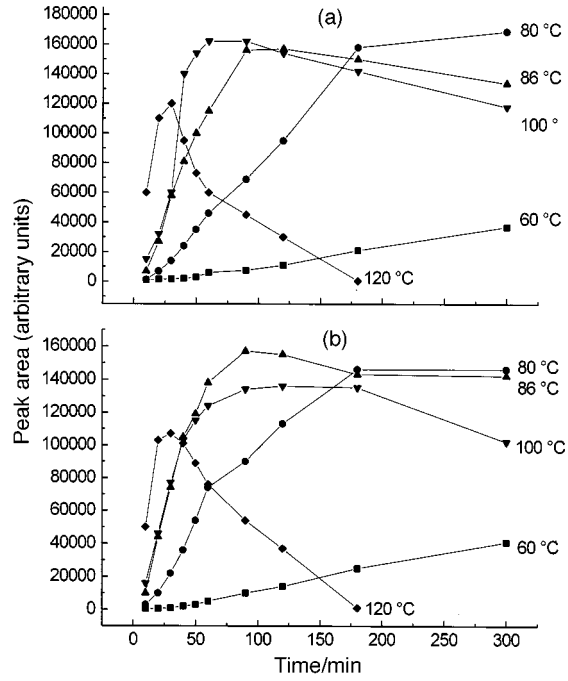

Fig. 2 Formation and decomposition of β-ADA ethyl (a) and propyl (b) esters (based on peak areas). β-ADA concentration is 0.5 mg dm^{-3}.

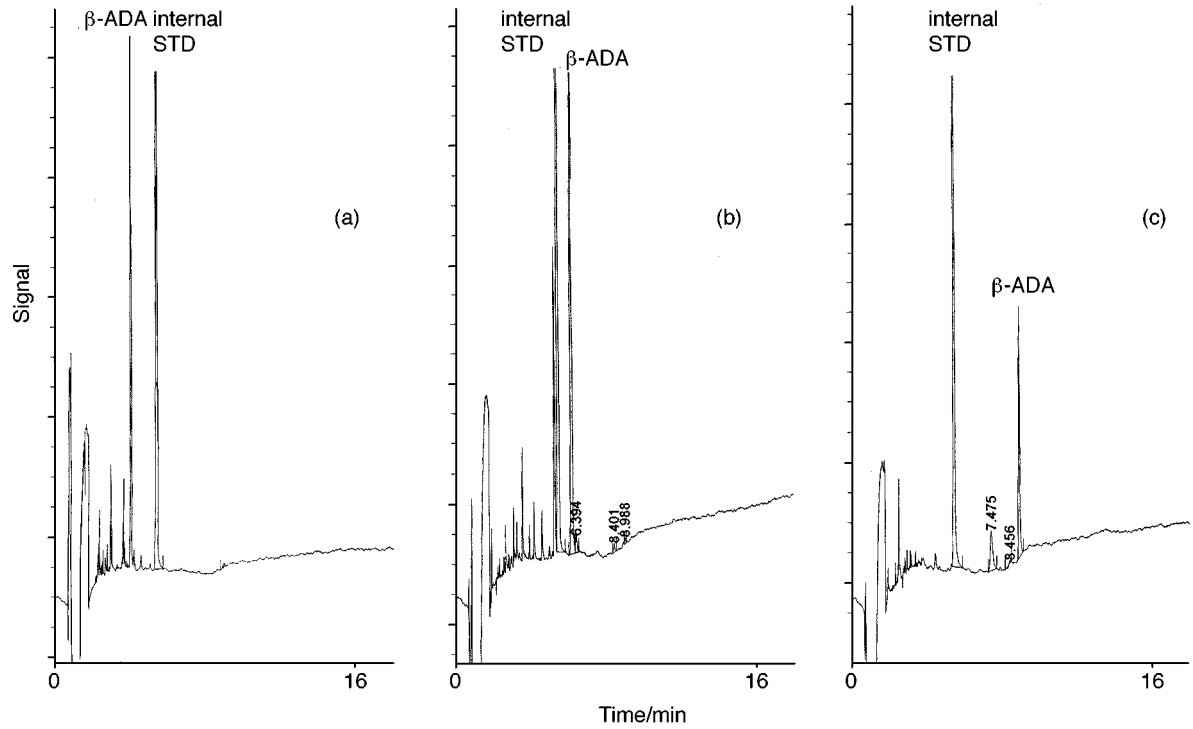

Fig. 1 Chromatograms of 0.5 mg dm^{-3} of β-ADA esterified with ethanol (a), propanol (b) and butanol (c). Separation conditions are presented in the Experimental section.

water, sea water, waste water and sediment, respectively (n = 15). There are no significant differences between distilled water, lake water and sea water matrices having the retention time RSDs below 0.2%. On the other hand, RSDs are higher in the case of waste water and sediment but do not exceed 0.5%.

The chromatographic repeatabilities (RSD) were calculated between eight concurrent measurements for the standard solutions containing 5–50000 μg dm^{-3} of β-ADA. The obtained run-to-run repeatabilities are presented in Table 1. In most cases (except close to the limit of detection) the relative repeatability values were lower than 4%. The day-to-day repeatabilities were calculated from the data obtained during a five day period. As it was expected, RSD values were higher (with 2 exceptions) than in the run-to-run measurements (Table 1), probably due to slow degradation of the formed ester.

In reproducibility tests the five concurrent samples underwent through the whole sample preparation procedure. In most cases the reproducibilities in distilled water were higher than the corresponding repeatabilities (Table 1). Therefore, the sample preparation procedure slightly affected the results. The only conclusive difference between the reproducibilities in different water matrices (Table 2) was that the reproducibility of sea water matrix was higher than those of lake and waste water, suggesting that marine salts might interfere with the analysis to some extent. Reproducibilities were higher for sediments ranging between 9 and 30%.

Recovery experiments for water matrices were performed at five concentration levels. The recoveries were lower for all matrices close to the limit of detection than at higher concentrations (Table 3). For sediment samples, recovery studies were performed at 0.2, 1.0 and 10 μg g^{-1}. The values were small for all concentrations and varied from 19 to 21%.

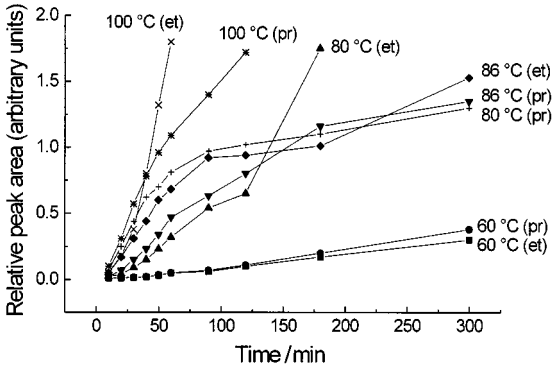

Fig. 3 Formation and decomposition of β-ADA ethyl (et) and propyl (pr) esters (based on relative peak areas). β-ADA concentration is 0.5 mg dm^{-3}.

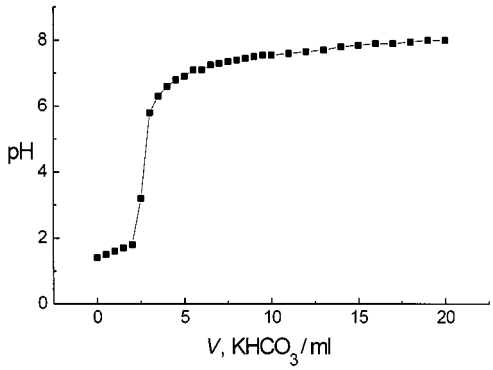

Fig. 4 Influence of 1 mol dm^{-3} $KHCO_3$ solution volume on the pH of β-ADA solution after addition of 1 ml of esterification reagent.

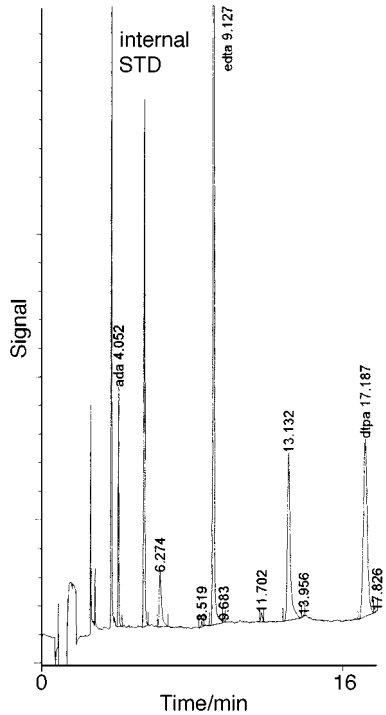

Fig. 5 Chromatograms of β-ADA, EDTA and DTPA ethyl esters. Separation conditions are presented in the Experimental section.

Table 1 Run-to-run and day-to-day repeatabilities and reproducibilities for β-ADA in distilled water

Concentration/ μg dm^{-3}	Repeatability run-to-run (n = 8; %)	Repeatability day-to-day (n = 5; %)	Reproducibility (n = 5; %)
5	36.0	25.7	23.9
10	8.6	6.5	4.5
50	3.6	6.6	5.8
100	1.6	1.6	6.8
500	2.6	3.4	10.7
1 000	1.6	3.6	5.0
5 000	0.76	3.0	2.2
10 000	1.7	2.9	3.6
50 000	3.4	4.0	5.4

Table 2 Reproducibilities for β-ADA in different water matrices (n = 5)

Concentration/ μg dm^{-3}	Reproducibility (%)		
	Lake water	Sea water	Waste water
5	14.2	21.7	11.0
10	9.6	15.7	12.3
50	4.8	5.7	4.4
500	4.1	11.5	7.5
5000	6.5	7.4	5.0

Table 3 Recoveries of β-ADA in different water matrices (n = 5)

Concentration/ μg dm^{-3}	Recovery (%)		
	Lake water	Sea water	Waste water
5	60	69	61
10	66	49	55
50	111	98	107
500	102	105	91
5000	97	90	97

Conclusion

The proposed procedure for GC-NPD determination of β-ADA after esterification with ethanol or propanol is simple and suitable for analysis of different water samples. High concentrations of Ni, Co, Cu(II), Hg(II), Mn(II), Pb, Cd and Cr(III) do not interfere with the β-ADA determination. Iron(III) interferes only at concentrations far beyond those normally found in natural waters. The influence of iron is expected to be significant only in the analysis of heavily contaminated water or waste water.

The Method validation section shows that the developed procedure is reliable for practical purposes. However, the recoveries of β-ADA from sediments were low probably because of a strong adsorption of certain β-ADA–metal complexes. Thus, the extraction step of β-ADA from sediments should be improved.

Acknowledgements

One of the authors (V.V.) received a postdoctoral scholarship from the Centre for International Mobility (CIMO) which is gratefully acknowledged. M.S. and J.R. would like to thank the Emil Aaltonen Foundation, the Foundation of Technology and the Kemira Foundation for financial support.

References

1 N. S. Thom and A. R. Agg, *Proc. R. Soc. London, Ser. B: Biol. Sci*., 1975, **189**, 347.
2 J. Gardiner, *Water Res*., 1976, **10**, 507.
3 H. Siegrist, A. Alder, W. Gujer and W. Giger, *Wasser–Abwasser, Abfall.*, 1988, **68**, 101.
4 A. Alder, H. Siegrist, W. Gujer and W. Giger, *Water Res.*, 1990, **24**, 733.
5 F. G. Kari and W. Giger, *Water Res.*, 1996, **30**, 122.
6 M. Sillanpää, *Chemosphere*, 1996, **33**, 293.
7 M. Sillanpää, *Rev. Environ. Contam. Toxicol.*, 1997, **152**, 85.
8 L. Nitscke, A. Wilk, C. Cammerer, G. Lind and G. Metzner, *Chemosphere*, 1997, **34**, 807.
9 J. Rämö, M. Sillanpää, M. Orama, V. Vickackaite and L. Niinistö, to be published.
10 M. Sillanpää and A. Oikari, *Toxicol. Environ. Chem*., 1996, **57**, 79.
11 M. Sillanpää, V. Vickackaite, L. Niinistö and M.-L. Sihvonen, *Chemosphere***,** 1997, **35**, 2797.
12 M. Sillanpää, R. Kokkonen and M.-L. Sihvonen, *Anal. Chim Acta*., 1995, **303**, 187.
13 J. Sorvari, M. Sillanpää and M.-L. Sihvonen, *Analyst***,** 1996, **121**, 1335.
14 M. Sillanpää, J. Sorvari and M.-L. Sihvonen, *Chromatographia*, 1996, **42**, 578.
15 B. Nowack, F. G. Kari, S. U. Hilger and L. Sigg, *Anal. Chem.*, 1996, **68**, 561,

Paper 8/04065F