



## ANALYSIS OF AMMONIA IN BRACKISH WATERS BY THE INDOPHENOL BLUE TECHNIQUE: COMPARISON OF TWO ALTERNATIVE METHODS

*The analysis of ammonia in waters of changing salinity by the indophenol blue technique may be carried out either by reading from a calibration curve obtained with standards prepared in distilled water, followed by the application of a correcting «salt effect factor» or by adjusting the magnesium contents of both standards and samples through the addition of convenient amounts of magnesium sulphate.*

*These two methods are compared statistically; the magnesium sulphate method is shown to be more accurate and only slightly less precise than the other; its increased accuracy is attributed to a better reproducibility of the pH of the final indophenol blue solution. The magnesium sulphate method is recommended for the manual analysis of ammonia in brackish waters.*

### 1 — INTRODUCTION

With no previous experience on the analysis of ammonia in seawater we were called upon to determine this water quality parameter in a coastal lagoon receiving a small freshwater input. A brief literature survey provided three manuals of methods of seawater analysis authored by STRICKLAND and PARSONS [1], GRASSHOFF [2] and CHAUSSEPIED [3], published respectively in 1972, 1976 and 1977.

The methods for the analysis of ammonia offered in those manuals are very similar. Indeed they are all based on the colourimetric technique proposed by SÓLORZANO in 1969 [4] which uses the synthesis of indophenol blue by the reaction between ammonia and phenol in alkaline solution, with hypochlorite as oxidant, nitroprusside as catalyst and citrate as a chelating agent for metals.

Since Grasshoff's method includes specific details about the analysis of brackish waters [5] this was the one elected.

When carrying out the indophenol reaction in solutions of different seawater content a «salt effect» is observed by which the colour intensity is suppressed at higher salinities. Recognizing that this is not a salt effect but rather a pH effect, resulting from the increased buffering capacity of seawater with respect to freshwater, the method offers two alternative correcting procedures. One, that will be referred hereafter as the citrate method (CM), assumes a correlation between salinity and pH and provides a table of «salt effect factors» for a number of different salinities. Absorbance readings shall be multiplied by the appropriate factors before converting them into concentrations. The other, that will be referred as the sulphate method (SM), obtains a virtually constant final pH by the addition of different quantities of magnesium sulphate estimated from the salinities of the sample so as to give an approximately constant final concentration of magnesium ion. Since precipitation of magnesium ion is desirable citrate is not added.

The lagoon we had to study is large, rather branched and shallow except in a deeper central part. A sampling boat could not be sailed close to the shore and would be used to collect samples along the central axis of the lagoon only, while sampling stations located in the side branches could be reached by car and canoe. Samples collected by boat would take

\* to whom all mail shall be addressed

6-8 hours to reach the laboratory while those collected by car would take less than half that time. The difficulty of preserving the samples for 6-8 hours recommended the addition of reagents *in loco*. Since there was not enough space on board for filtering the samples the sulphate method would be adopted, the salinities being measured *in situ* with a portable salinometer. Samples collected by car would be cooled in an ice box and brought to the laboratory. To avoid having to measure the salinity of these samples prior to adding the reagents, since there was not a second salinometer and titration with silver nitrate is expensive, the citrate method would be adopted. The salinity could be measured later with the portable salinometer in an aliquot of these samples saved for that purpose. The correcting «salt effect factors» could then be obtained from Grasshoff's table [5].

Since two alternative methods would have to be used simultaneously an intercalibration exercise was performed to access their equivalence.

In this paper the results of that exercise are reported and it is shown that the two methods are not always equivalent and that, if the indophenol blue technique is to be used, the magnesium sulphate method should be chosen for the analysis of ammonia in brackish waters.

## 2 — EXPERIMENTAL

### EQUIPMENT AND INSTRUMENTS

All absorbance measurements were made by using a Perkin Elmer 200 UV spectrophotometer with matched quartz cells of 10, 20, 50 and 100 mm path-length. A Orion 901 pH meter, equiped with a glass-silver chloride combination electrode, was used for all pH measurements. Calibration of the system was performed with Carlo Erba pH standard solutions of pH 7.00 and 9.00. Salinities were measured by using a Yellow Springs Instruments C-S-T meter, model 33. Water was purified by distillation in a borosilicate glass still, Fissons model Fi-Stream 4, followed by deionization in a mixed bed column, Elgastat model 104 HR. Water samples for the CM were filtered, under reduced pressure, through 0.45  $\mu\text{m}$  membrane filters supported on an all glass filtering system. Glassware, set apart exclusively for this work, was soaked in hydrochloric acid for about one hour, rinsed several times with purified water

and drip dried in an inverted position inside a closed cupboard. The colour developing reaction was carried out in 100 ml all glass, cilindrical, stoppered reagent flasks.

### REAGENTS

Analytical quality reagents were used throughout without further purification. The single exception was the hypochlorite solutions which were diluted from successive bottles of a commercial bleaching preparation after titrating the hypochlorite contents with standardized thiosulphate solution. Dilution water for preparing all reagent solutions and ammonia standards was purified, as described above, on the same day of the experiment, a single batch of water being used in each case. Reagent and standard solutions were prepared and stored as prescribed by GRASSHOFF [6]. In short these solutions are:

- a) Alkaline aqueous suspension of  $\text{Mg}(\text{OH})_2$  being approximately 50% m/v in  $\text{MgSO}_4$ .
- b) 38 g phenol plus 400 mg disodium nitroprusside dissolved in enough water to make 1 liter.
- c) Hypochlorite in 0.5 mol  $\text{dm}^{-3}$  aqueous NaOH having 0.15% m/v available chlorine.
- d) 0.02 ml  $\text{dm}^{-3}$  NaOH containing 480 g per liter of trisodium citrate dihydrate.
- e) 0.1 mol  $\text{dm}^{-3}$   $\text{NH}_4\text{Cl}$  aqueous standard.

### SAMPLING

Water samples were collected by bucket immersion along three branches of the lagoon of Aveiro, two of them polluted by sewage. In the landward extremes of those branches salinities were very low at low tide. The samples were transfered to flasks, kept in an ice box and brought to the laboratory. The time elapsed between collection and the beginning of the analytical procedure was always less than one hour.

### PROCEDURE[7]

70 ml of purified water (for blanks), standard solutions and samples were measured with a 100 ml graduated cilinder into 100 ml reaction flasks. Using repetitive dispensers the reagents were added in succession, with gentle swirling between additions, by

the following order: for the citrate method 2 ml each of solutions d) (citrate), b) (phenol + nitroprusside) and c) (hypochlorite); for the sulphate method appropriate volumes of suspension a) ( $\text{MgSO}_4$ ) to correct for the different salinities, this is, 1.4 ml to blanks, standards and samples of salinities below  $5^\circ_{00}$ , 1.1 ml to samples of salinities between  $5^\circ_{00}$  and  $15^\circ_{00}$  and 0.7 ml to samples with salinities between  $15^\circ_{00}$  and  $25^\circ_{00}$  followed by 2 ml of solutions b) and c), as above.

Spiking was made by adding to the initial 70 ml of the samples 1 or 2 ml of standards with convenient concentrations.

The flasks were put on trays and kept in a light-proof cupboard, at room temperature, until the next day. The trays were then carefully moved to the vicinity of the spectrophotometer to avoid resuspension of the precipitate. In all cases the samples treated by the citrate method were clear while those treated by the sulphate method had an abundant residue. The undisturbed supernatant liquid, however, was devoid of any visible traces of turbidity and was used directly for taking absorbance measurements.

As the spectrophotometer cells were washed twice with the samples prior to filling, the inhouse built glass device shown in fig. 1 was used to pour the samples into the cells, thus avoiding any disturbance of the solid residue. It was the size and shape of the device that imposed a bigger sample volume than indicated in the original procedure [6].

### 3 — RESULTS AND DISCUSSION

Early work done with ten to thirty replicates of standard solutions showed that the within batch standard deviation,  $s$ , of any of the methods was virtually independent of the concentration,  $c$ , and was of the order of magnitude of only a few  $\mu\text{g dm}^{-3}$ , as nitrogen. However, the between batch standard deviation was very high and the absorbance of the blanks varied excessively from day to day. These difficulties are attributed to changes in the air quality of the research laboratory under the influence of a nearby teaching laboratory. The work was repeated during the Summer recess and it was found that the average value of  $s$  for both methods,  $s_{\text{CM}}$  and  $s_{\text{SM}}$ , calculated from ten or more replicates of standard solutions in each batch, had

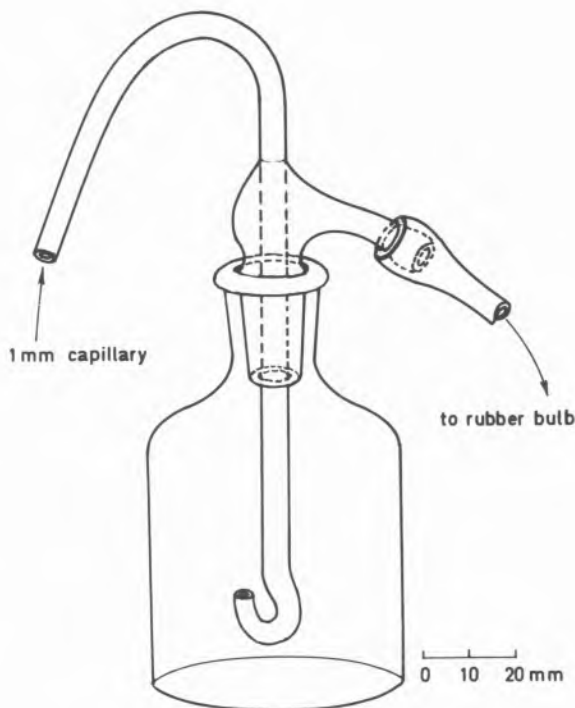


Fig. 1

*Schematic representation of the apparatus used to fill the spectrophotometer cells without disturbing the precipitate*

average values of respectively  $2.4 \mu\text{g dm}^{-3}$  and  $4.4 \mu\text{g dm}^{-3}$ , as nitrogen.

Also the day to day change in the absorbance of blanks, measured against water, never exceeded 16%. The value of  $2.4 \mu\text{g dm}^{-3}$  agrees well with those reported by CROWTHER and EVANS [8] who used an automated procedure for a method equivalent to the CM.

Although  $s$  and  $c$  are positively correlated ( $r_{\text{CM}} = 0.477$  and  $r_{\text{SM}} = 0.306$ , against a tabled critical value of  $r = 0.282$  for 47 points),  $s$  varies so slowly with  $c$  that it may be considered constant over large concentration ranges.

Indeed, splitting the concentration range covered into two parts, one up to  $150 \mu\text{g dm}^{-3}$  with 30 points and the other from 150 to  $371 \mu\text{g dm}^{-3}$  with 27 points, and calculating the corresponding average values of  $s$ , it is observed that these values are statistically undistinguishable at the 95% confidence level (calculated values of  $t_{\text{CM}} = 0.44$  and  $t_{\text{SM}} = 1.49$  against a tabled value of 2.95).

The higher standard deviation of the SM is not due to the presence of any residual turbidity in the samples since it did not improve after centrifugation at



3000 rpm for 5 minutes or filtration through Whatman GF/C fiberglass filters. The slopes of the calibration curves for the CM were systematically higher than those for the SM by, on average, 9%.

For comparing the two methods, the difference  $D = c_{SM} - c_{CM}$  for each sample was calculated and its statistical significance at the 95% confidence level evaluated.

Since the values of 2.4 and 4.4  $\mu\text{g dm}^{-3}$  for  $s_{CM}$  and  $s_{SM}$ , referred above, were calculated from a very large number of experiments they may be considered very close to the respective true standard deviations. Therefore a value of 5.0  $\mu\text{g dm}^{-3}$ ,  $(2.4^2 + 4.4^2)^{1/2}$ , certainly is a good estimate,  $s_D$ , for the standard deviation of D when calculated from a single measurement by each method.

At this stage a decision had to be taken on what difference between the two methods one should attempt to get statistical significance for. A number of factors had to be taken into account; on one side consideration had to be given to the work load, the cost of chemicals and glassware and the time and the space required for doing replicate analysis on a routine basis. On another side the order of magnitude of the ammonia concentration normally found in estuarine waters had to be considered, since there would be little interest in trying to get statistical significance to ammonia concentrations so low that they would be seldom found in natural estuarine environments. Still on another side attention had to be given to the fact that when changing from ammonia standard solutions to real samples some increase was to be expected in the standard deviation estimate of each method,  $s$ , and that, acting in the opposite direction, the effect of  $n$  replicates would be a decrease of  $s$  by a factor of  $n^{-1/2}$ .

Since  $s$  was found to be virtually independent of  $c$  along the concentration range of interest it was on the low concentration side of this range that  $s$  would become relatively more important (higher variation coefficient). The limit of detection of the citrate and sulphate methods, defined as  $4.65 s$  [9], would be respectively 11.2 and 20.4  $\mu\text{g dm}^{-3}$ , these being the concentrations that could be claimed to be greater than the blank with a confidence level of a 95%. Therefore the decision was taken that one should not attempt to get statistical significance to a difference smaller than the larger of those two values.

Using Table VIII.I of reference [10], a value of 20  $\mu\text{g dm}^{-3}$  for the minimum significant D and a value of 5.0  $\mu\text{g dm}^{-3}$  for  $s_D$ , the number of replicates needed to know D at the 95% confidence level is found to be four. However, since the statistically outlying results would be eliminated by the Q test [11], all the experiments were performed with five replicates of each solution so that the power of the experiment would always be sufficient to allow a difference of 20  $\mu\text{g dm}^{-3}$  or bigger to be detected.

A total of 47 samples, with salinities ranging from 2‰ to 35‰, were analysed by the citrate and sulphate methods, together with blanks, standards, spiked samples and running standards for analytical quality control.

The estimates for the standard deviations of a single determination by the two methods, obtained under those conditions, were  $s_{CM} = 4.0$  and  $s_{SM} = 4.9$   $\mu\text{g dm}^{-3}$ , as nitrogen, bigger than the earlier determinations, as expected.

The value for  $s_D$  was 2.9  $\mu\text{g dm}^{-3}$ , as nitrogen, this value being the standard deviation of a single difference calculated from the average of five replicates with each method. The effect of replication is to bring the value of  $s_D$  down to 2.9 from the value of 6.3  $\mu\text{g dm}^{-3}$   $(4.0^2 + 4.9^2)^{1/2}$  that would correspond to a difference between two single measurements. With a value of 2.9 for  $s_D$ , differences of 8.7 and 11.6  $\mu\text{g dm}^{-3}$  can be detected with five and four replicates respectively [10]. A value of about 10  $\mu\text{g dm}^{-3}$  is a reasonable lower limit for the concentration of ammonia in estuarine waters. Individual differences were assessed both by comparing them with that value and by calculating the corresponding value of the Student  $t$  parameter.

Eleven sets of recovery tests were done with both methods. The average percent spike recoveries obtained were  $\bar{r}_{CM} = 82.8$  and  $\bar{r}_{SM} = 99.5$  and their respective 95% confidence limits were 76.1-89.5 and 96.6-102.4  $\mu\text{g dm}^{-3}$ . Therefore, the sulphate method gives recoveries that are both more complete and more reproducible than those obtained with the citrate method.

Differences between the concentration values obtained by the SM and CM for real samples were always positive and statistically significant whenever  $c_{SM} > 60$   $\mu\text{g dm}^{-3}$ . Below this concentration level D was positive in five, and statistically significant in only two, out of sixteen cases. However the corres-

ponding differences for the running control standards were always statistically undistinguishable.

Two aspects of these results need further consideration. Firstly, the sulphate method, despite being the least precise and the least sensitive of two, (higher and lower slopes for the calibration curves), was the most accurate (best recoveries). Secondly, the systematically higher concentration values obtained with the SM for real samples above  $60 \mu\text{g dm}^{-3}$  suggest the existence of some fundamental difference between the two methods which shows up with samples and not with standards. Therefore the two methods can not always be considered as being equivalent.

In an attempt to discover the reasons underling our observations a close scrutiny was made of all the variables susceptible of affecting the methods response, namely light intensity, temperature, reagent concentration and pH.

Light has been recognized as being a very important factor on colour development rate and intensity. Visible light, in particular, originates a side reaction that interferes with indophenol blue development [12]. Some authors [13] have even advised the irradiation of the reaction vessels with UV light for better and faster colour formation. However, when time is not a problem, the normal procedure is to carry out the indophenol blue formation in the dark for not less than 3 hours and preferably overnight. This indeed was the procedure we strictly adhered to. The samples were properly randomized prior to adding the reactants. These additions were done rather quickly and the flasks immediately closed in a light proof cupboard where they stayed until the beginning of absorbance reading, on the following day. Since all the flasks had exactly the same exposure to light this factor can not be made responsible for the differences observed between them.

Temperature does influence the rate of colour formation and the rate of hydrolysis of interfering substances. However it seems to be more important when the indophenol dye is formed from salicylate than from phenolate [14, 15]. In all experiments reported here flasks were kept at room temperature and all were exactly in the same conditions. No record is kept of laboratory temperatures but over the course of this work (May to October) they may have spanned, in both directions, a range of some  $5^{\circ}\text{C}$ . Nevertheless no correlation was observed between the slopes of the calibration curves and the

date on which they were determined. Therefore, changes in temperature do not account for the difference between the two methods.

It is the chemical nature and the concentration of reagents that determine both the particular set of colour developing reactions that occur and their specific kinetics. Throughout our experiments the concentrations of phenol, nitroprusside, and hypochlorite were constant but the concentrations of hydroxide, citrate and magnesium ions changed considerably. Since magnesium ions do not catalyse the reaction and citrate only participates in the chelation of metal ions their influence upon the reaction may come only through their impact on the pH.

The effect of pH upon the rate of colour formation and the colour intensity has been extensively studied both for methods using phenol [16] and phenol derivatives [17-19]. There is no agreement about the optimum pH for carrying out the reaction. Recommended values range from 9.9 to 13.1 [16-19], the individual values chosen varying with the reagents used and their concentration.

Accepting the statement made by PYM and MILHAM [17] that adjusting the pH after colour development has the same effect as adjusting it in the beginning of the reaction, we studied the change in colour intensity with pH for the systems we used. The various pH values were obtained by adding NaOH or HCl to samples or standards where the indophenol reaction had been carried out in the usual way. In each case the pH was changed only in one direction. Solutions treated by the SM could only have their pH decreased due to precipitation at higher pH while sample solutions treated by the CM could not have their pH raised above 11.7 for the same reason. Therefore all measurements at pH values higher than 11.7 had to be done with standard solutions treated by the CM. The results of this study, illustrated in fig 2, clearly show that a plateau of colour intensity exists between pH values of about 10 and 12. This is in disagreement with the results of HARWOOD and HUYSER [16] that have noted a peak at a pH value of 12.3. However they worked with phenol concentrations about 45 times bigger than those used in this work and their solutions also had different proportions of the reactants.

Following the observation of the change in colour intensity with pH we measured the pH of a very lar-

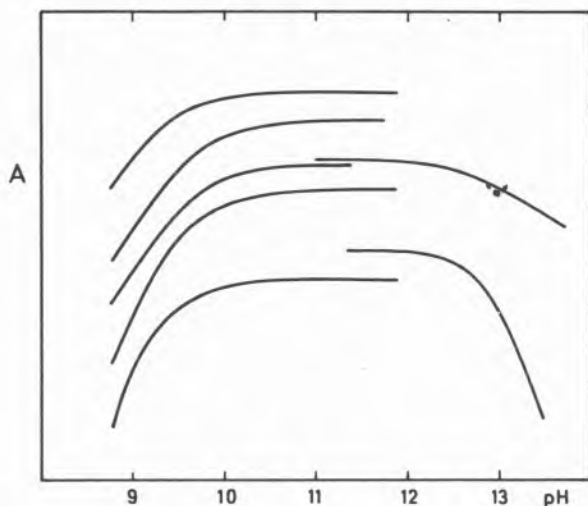


Fig. 2

Variation of absorbance readings with pH for samples and standard solutions of  $\text{NH}_3$  treated by the indophenol blue technique. The successive curves are displaced vertically for better display. Absorbances of plateaus are typically of 0.3

ge number of samples and standards treated either by the CM or the SM. Samples had a wide range of salinities. pH measurements were made immediately after adding the reagents and also on the following day. The reported values are uncorrected for the electrode sodium error. A few interesting observations come out of this work.

The pH of the solutions did not change overnight. This means that the large excess of NaOH present buffers the effect of the pH dependent steps in the mechanism of indophenol formation [18, 19] and explains why small variations in the initial pH are of little importance for the final colour intensity. This observation supports the statement of PYM and MILHAM [17] referred above.

The pH of blanks and standards treated by the CM ranged between 11.30 and 11.32 while the pH of those treated by the SM ranged from 10.08 to 10.18 (the instrument allowed readings with three decimal figures, but only two were completely stable). The considerable pH difference between the two methods results from the precipitation of  $\text{Mg}(\text{OH})_2$  in one case, which lowers the pH, and the presence of citrate ion in the other which increases the buffering capacity of the solution.

The pH of samples treated by the CM ranged from 9.63 to 11.40 while those treated by the SM ranged from 9.87 to 10.08. The higher upper pH limit of CM samples (11.40) in relation to that of standards

(11.32) may be due to the larger electrode sodium error. What surprises most in these values is the large drop in the pH of samples treated by the CM with respect to the corresponding standards and blanks. This drop is as high as nearly 1.7 pH units while in the SM it is only of 0.21 pH units. Most likely, it is the alkalinity of the sample that causes this pH drop in the CM and it is the presence of excess magnesium ions that prevents it in the SM.

These observations shine some light on the problem of the CM and SM giving different results with samples but not with control standards, as mentioned earlier. Indeed, the CM samples have a pH below the region maximum colour development while the standards have a pH right in the middle of that region. The absorbance values for the samples are therefore depressed and their calculated concentrations are too low. With the SM that problem does not exist since samples and standards have virtually the same pH. The small pH variations among SM samples may account for the higher  $s_{\text{SM}}$  value in relation to  $s_{\text{CM}}$ .

GRASSHOFF [5], by directly correlating alkalinity and salinity, is able to calculate a table of compensating coefficients which are supposed to take into account the change in alkalinity occurring with the dilution of seawater. This may be true in pristine coastal and estuarine waters but it seldom applies to antropogenically occupied mixing areas where very important sources of alkalinity, other than seawater, may be present. In the lagoon of Aveiro, for instance, we have the effluent of a kraft paper mill and several point sources of untreated sewage altogether corresponding to a population equivalent of some 280,000, in BOD terms.

The results reported in this paper certainly support the recommendation that if a choice must be done between the CM and SM of the indophenol technique, the second should be selected for the manual analysis of  $\text{NH}_3$  in brackish waters of changing salinity. The method is not amenable to be used in automated systems, due to the presence of a precipitate. However, if an automated system is available for the analysis, it should rather be modified, as recommended by CROWTHER and EVANS [20], to introduce a distillation step, than run employing the citrate method.

Received 23.Sptember.1981

## ACKNOWLEDGEMENTS

We are very grateful to Prof. José S. Redinha for his most pertinent comments.

## REFERENCES

- [1] J. D. H. STRICKLAND, T. R. PARSONS, "A Practical Handbook of Seawater Analysis", Fisheries Research Board of Canada Bulletin n.º 167, 2<sup>nd</sup> Ed., Ottawa, 1972.
- [2] K. GRASSHÖFF, "Methods of Seawater Analysis", Verlag Chemie, 1976.
- [3] M. CHAUSSEPIED, "Manuel des Methodes de Prelevements et d'Analysis, vol. 1: Caracteristiques Physicochimiques et Hydrobiologiques", Réseau National d'Observation de la Qualité du Milieu Marine, Brest, 1977.
- [4] L. SOLORZANO, *Limnol. & Oceanogr.*, **14**, 799 (1969).
- [5] Ref. [2], p. 131.
- [6] Ref. [2], pp. 129-130.
- [7] Ref. [2], pp. 131 and 133.
- [8] J. CROWTHER, J. EVANS, *Analyst*, **105**, 849 (1980).
- [9] R. V. CHEESEMAN, A. L. WILSON, "Manual on Analytical Quality — Control for the Water Industry", Water Research Center TR66, Medmenham, p. 47, 1978.
- [10] Ref. [9], p. 129.
- [11] G. D. CHRISTIAN, "Analytical Chemistry", 2<sup>nd</sup> Ed., Wiley, p. 69, 1977.
- [12] N. GRAVITZ, L. GLEYE, *Limnol. & Oceanogr.*, **20**, 1015, (1975).
- [13] M. I. LIDDICOAT, S. TIBBITTS, E. I. BUTTLER, *Limnol. & Oceanogr.*, **20**, 131 (1975).
- [14] B. L. HAMPSON, *Water Research*, **11**, 305 (1977).
- [15] H. VERDOUW, C. J. A. VAN ECHTELD, E. M. J. DEKKERS, *Water Research*, **12**, 399 (1978).
- [16] J. E. HARWOOD, D. J. HUYSER, *Water Research*, **4**, 501 (1970).
- [17] R. V. E. PYM, P. J. MILHAM, *Anal. Chem.*, **48**, 1413 (1976).
- [18] C. J. PATTON, S. R. CROUCH, *Anal. Chem.*, **49**, 464 (1977).
- [19] M. D. KROM, *Analyst*, **105**, 305 (1980).
- [20] J. CROWTHER, J. EVANS, *Analyst*, **105**, 841 (1980).