

Determination by Fluorescence Spectroscopy of Cadmium at the Subnanomolar Level: Application to Seawater

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Received September 28, 1999; accepted December 22, 1999

This paper reports the development of a molecular fluorescence spectroscopy-based approach for the determination of cadmium in seawater. Anthrylazamacrocycle derivatives—the fluorescence of which is enhanced when chelated to zinc or cadmium—are used as chemosensors. A detection limit of 5 nM has been found at pH 10 for both metals, and spectral shifts allow simultaneous Cd(II)/Zn(II) determination using multiwavelength analysis. While cadmium emission behavior is similar at pH 13, zinc is not detected anymore. This enables the selective detection of cadmium even at a high Zn(II)/Cd(II) ratio. The detection limit is 1 nM. Interferent removal and preconcentration have been developed using a Dowex resin, with a view to determine cadmium in seawater. A global procedure including interferent elimination, cadmium preconcentration (30 fold), and fluorescence detection at pH 13 has been evaluated on certified reference material SLEW-2.

KEY WORDS: Cadmium; zinc; fluorescence; anthrylazamacrocycle; seawater.

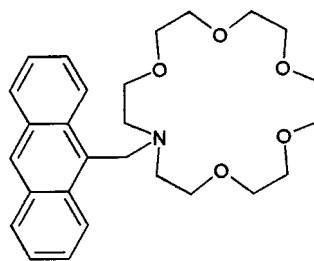
INTRODUCTION

The need for monitoring trace metals in marine environment is well established. Analytical techniques with the necessary sensitivity and accuracy are available, but they are often time-consuming off-line methods. Due to their size and weight (ICP-MS/GF-AAS), these analytical tools are often fragile and their application in a stand-alone system is not realistic.

Optical techniques based on molecular spectroscopy offer the distinct advantages of simplicity and absence of physical contact between the detection systems and the analytes. In particular, luminescence methods offer potential advantages for ion determination: high sensitivity and selectivity, ease of automation, and straightfor-

ward application to fiber optics-based remote sensing. Furthermore, the treatment of optical signals is nowadays in a stage of fast development and one can foresee that their importance will continue to grow in the future.

With the pioneering work of de Silva and de Silva [1] on the effect of alkali metal salts on the fluorescence of anthracene-crown ethers, a novel approach to the detection of cations in methanol has been developed. It is based on the luminescence modification resulting from the binding of metals to azacrown **1** covalently linked to a luminophore via one methylene group.



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In 1990, Czarnik and his co-workers [2,3] reported the modification of the fluorescence intensities of anthrylazamacrocycles (**4**) in the presence of various metal cations (cadmium, zinc, and mercury) in aqueous solutions. Under alkaline conditions, the fluorescence yields of the chemosensors are very small. This is ascribed to an intramolecular quenching by electron transfer from the amines to the excited fluorophore. When chelated to nonquenching metal ions, such as cadmium or zinc, the amine lone pairs become involved in bonding and unable to donate an electron to the excited state of the anthryl group. As a result chelation-enhanced fluorescence (CHEF) is observed, which depends on the nature of the azamacrocycle, the pH, the emission wavelength, and the nature of the metal. The aim of the work presented here was to develop this molecular fluorescence-based approach for cadmium and zinc determination at the nanomolar level. Cadmium analysis was also performed on seawater samples at the subnanomolar level, using a matrix separation column to preconcentrate and to remove interferents.

EXPERIMENTAL

Instrumentation

A Bruker WM 250 spectrometer was used for obtaining FT-NMR spectra. UV spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer. For this application the pH was adjusted at 7 with a phosphate buffer (0.01 M). Fluorescence measurements were recorded on a Shimadzu RF-5001 PC spectrometer. The excitation source was a 150-W steady-state Xenon lamp. Fluorescence excitation was performed at 368 nm (absorption maximum) and emission was collected from 380 to 550 nm. Methacrylate fluorescence cells ($1 \times 1 \times 4 \text{ cm}^3$) were purchased from Aldrich Chemical Co..

Reagents

Labware was thoroughly rinsed with 18 M Ω -cm water produced by a Milli-Q water system. The pH of the solutions was measured with an Ankersmit 420A pH meter calibrated at pH 7.00 and pH 10.01 (buffer solutions purchased from Aldrich Chemical Co.). The fluorescence measurements were made at pH 10.0 (0.001 M borate buffer) and at pH 13 (0.1 M NaOH).

Synthesis

9-(Chloromethyl)anthracene, 1,4,7,10-tetraazacyc-

lodoodecane tetrahydrochloride, 1,4,7,10,13,16-hexaaza-

cyclooctadecane trisulfate, and metal salts were purchased from Aldrich Chemical Co.. 1,4,7,10,13-Pentaazacyclopentadecane was synthesized according to a literature procedure [11,12]. Anthrylazamacrocycles (**4**) were synthesized by the reaction of 9-(chloromethyl)anthracene (**2**) with an excess of azamacrocycle (**3**) ($n = 3-5$) by following the literature procedure [2] (Scheme I).

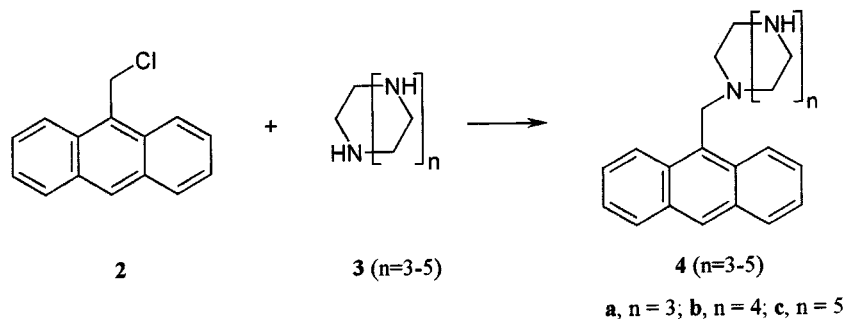
Toluene (99%) and acetonitrile (99.5+%; spectrophotometric grade), purchased from Aldrich Chemical Co. and used in the last step of the synthesis, were not purified further. Chloroform and ethanol used in the purification step were distilled before use. Hydrochloric acid 32% pro analysi (Merck) was used for precipitation of the chemosensors.

Matrix Separation

The resin used for the separation of cadmium from the seawater matrix was Dowex 1X2-400, purchased from Supelco. The column, $100 \times 10 \text{ mm}$, was purchased from Omnifit. A peristaltic pump (Masterflex L/S) was used to inject the solutions (carrier, samples, wash solution) in the column (flow rate, $7 \text{ ml} \cdot \text{min}^{-1}$). Tygon Lab peristaltic tubing was used (0.8-mm i.d. and 1.6-mm o.d.). The eluent solution (0.6 M HCl) was prepared by diluting hydrochloric acid (37% Trace Select; Fluka) with 18 M $\Omega \cdot \text{cm}$ water. The resin was conditioned with 0.6 M HCl. Cadmium was then successfully separated from a mixture of heavy metals by injecting 200 ml of sample (acidified to pH 0.2) and by eluting the column with successively 20 ml of 0.6 M HCl and 10 ml of 18 M $\Omega \cdot \text{cm}$ water. Cadmium was collected in the last 6 ml and its concentration was determined with a 100 nM solution of **4c** at pH 13.

RESULTS AND DISCUSSION

It was observed during our preliminary analytical measurements that purification of the chemosensors is of crucial importance. Performing only one extraction and precipitation during the purification step led to a product contaminated by the chloromethylated aromatic (or its substitution derivative). This contamination induced a background fluorescence, which was not modified by the presence of metals. As a consequence, a decrease in the CHEF factor was observed. Moreover traces of unreacted azamacrocycles (**3**) led to unwanted analyte chelation. The CHEF factor could be enhanced (six fold) by performing several extractions and precipitations of the



Scheme I.

chemosensors with high-quality solvents. Under these conditions the fraction of unreacted material became sufficiently low, and the blank reached its weakest intensity.

Emission Behavior at pH 10

Preliminary measurements were performed using glassware. Samples were prepared in 10-ml pyrex flasks using different range of micropipettes. The sequence of sampling was (1) addition of chemosensor solution, (2) addition of a metal standard solution, (3) addition of the buffer, (4) filling to 10 ml with ultrapure water, (5) thermostating at 20°C during 20 min, and (6) steady-state fluorescence measurement in a pyrex fluorescence cell. This sequence led to poor measurement repeatability, probably due to adsorption of the metals on pyrex [4]. Repeatability has been largely improved by modifying the sequence in the following way: 3-ml samples are now

prepared directly in a disposable methacrylate fluorescence cell using the following sequence: (1) addition of chemosensor solution, (2) addition of a metal standard solution, (3) filling to 2.7 ml with ultrapure water, (4) addition of 300 μ l buffer, and (5) recording of the steady-state fluorescence emission without delays.

The emission spectra of the three chemosensors (**4**) were measured in the presence of various concentrations of Cd(II) or Zn(II) at pH 10. See Fig. 1 for an example of the fluorescence spectrum of anthrylpentaazamacrocyclic (**4b**). The variation of intensity at 450 nm as a function of cadmium concentration is given in Fig. 2.

The fluorescence intensity of a sample containing cadmium, zinc, and an excess of chemosensor can be expressed as the sum of several contributions:

$$R_T(\lambda) = R_{Zn}(\lambda) + R_{Cd}(\lambda) + R_{Ch}(\lambda) + r(\lambda) \quad (1)$$

In this equation R_T is the response of the sample, and

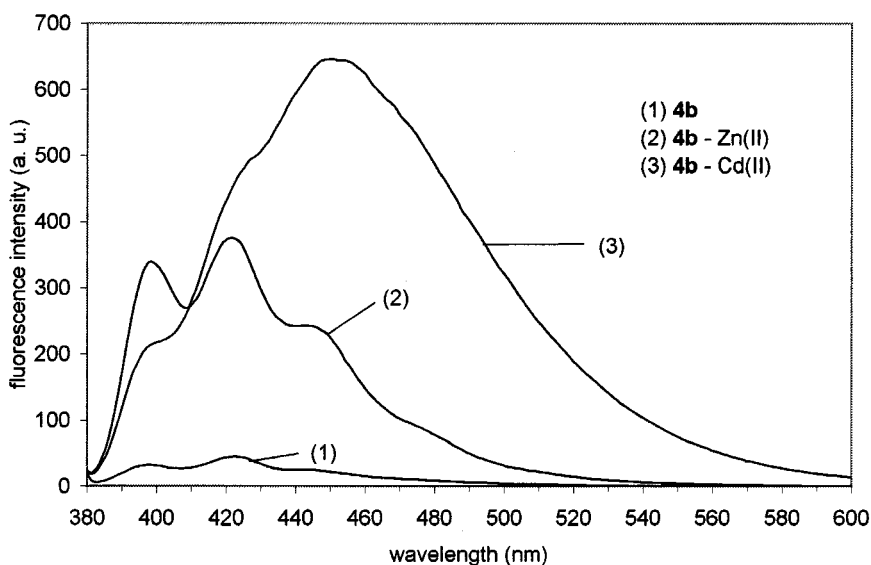


Fig. 1. Steady-state fluorescence spectrum of 5 μ M **4b** at pH 10 in the presence of cadmium or zinc (10 μ M).

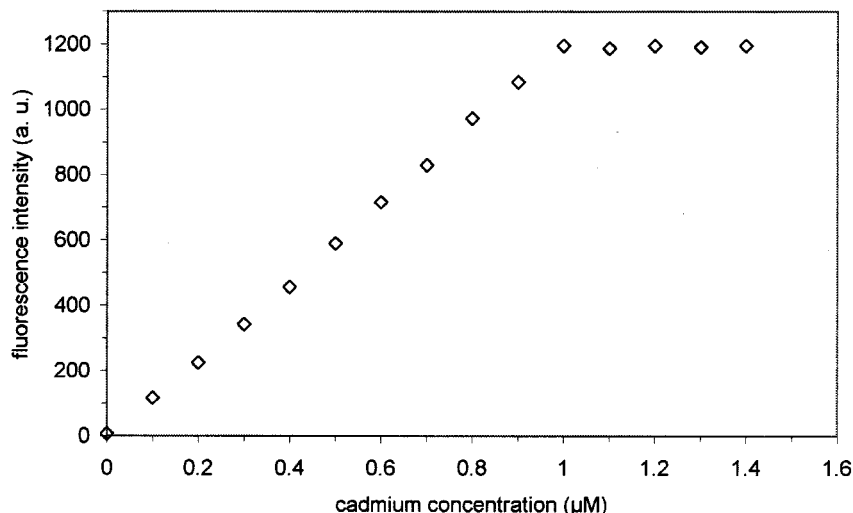


Fig. 2. Titration of cadmium at 450 nm by 1 μM **4b** at pH 10.

R_{Zn} , R_{Cd} , and R_{Ch} are the responses of the chemosensor chelated to zinc, the chemosensor chelated to cadmium, and the free chemosensor, respectively. r takes into account the other contributions, such as water Raman emission, scattered light, and instrumental response. Equation (1) can be rewritten in terms of fractions of the species present in solution:

$$I_{\text{T}}(\lambda) = x \times I_{\text{Zn}}(\lambda) + y \times I_{\text{Cd}}(\lambda) + z \times I_{\text{Ch}}(\lambda) \quad (2)$$

$$x + y + z = 1 \quad (3)$$

In these equations x , y , and z represent the fraction of the chemosensor chelated to zinc, the fraction of chemosensor chelated to cadmium, and the fraction of remaining free chemosensor, respectively. $I_{\text{T}}(\lambda)$ represents the measured signal emitted by the sample; $I_{\text{Zn}}(\lambda)$, $I_{\text{Cd}}(\lambda)$, and $I_{\text{Ch}}(\lambda)$ represent the uncorrected emission intensity of the chemosensor saturated by zinc, the chemosensor saturated by cadmium, and the free chemosensor, respectively. Thus I_{Zn} , I_{Cd} , and I_{Ch} values may include a

possible contribution of scattered light and Raman emission of water.

Equations (2) and (3) can be solved by using fluorescence intensities either at two wavelengths or at more than two wavelengths. In the latter case the system becomes overdetermined. The resolution of overdetermined equation systems leads to a better result, particularly when noisy data lead to important uncertainties when only fluorescence intensities at two wavelengths are used [5].

Several softwares using a matrix computation approach can achieve this operation [6]. A program has been built using Matlab software. Table I reports some experimental data obtained by solving this type of system on steady-state fluorescence data of samples containing cadmium, zinc, and an excess of anthrylpentaazamacrocyclic (**4b**).

This approach has also been evaluated for **4a** and **4c**. The fluorescence spectra of these chemosensors dis-

Table I. Simultaneous Determination of Cadmium and Zinc Concentrations with **4b** Using the Multiwavelength Approach (221 nm, from 380 to 600 nm); Relative Errors (%) in Parentheses

Analytical concentration (nM) in the mixture		Measured concentration (nM)	
Zinc	Cadmium	Zinc	Cadmium
125	125	120 (4)	130 (4)
95	30	100 (5)	30 (0)
45	15	50 (11)	15 (0)
30	95	30 (0)	85 (11)
15	45	15 (0)	40 (11)

Table II. Comparison of the Two Approaches with **4a**; Relative Errors (%) in Parentheses

Analytical concentration (nM) in the mixture	Measured concentration (nM)				
	Two wavelengths (416 and 460 nm)		Multiwavelengths (171 nm, from 380 to 550 nm)		
Zinc	Cadmium	Zinc	Cadmium	Zinc	Cadmium
300	300	380 (27)	231 (23)	307 (2)	297 (1)
300	200	288 (4)	225 (13)	297 (1)	174 (13)
400	100	433 (8)	69 (31)	415 (4)	88 (12)
100	800	112 (12)	760 (5)	104 (4)	786 (2)
800	100	810 (1)	247 (147)	855 (7)	64 (36)

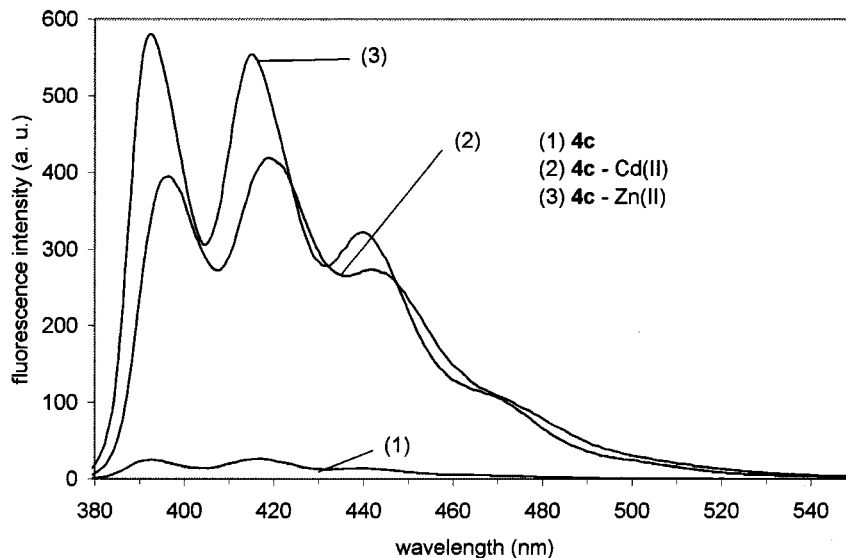


Fig. 3. Steady-state fluorescence spectrum of $10 \mu\text{M}$ **4c** at pH 10 in the presence of cadmium or zinc ($20 \mu\text{M}$).

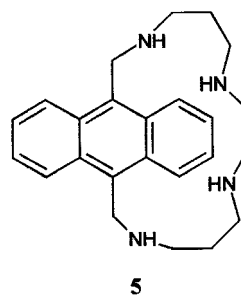
play only minor differences when chelated to zinc or cadmium. This is shown in Fig. 3 for **4c**. The data obtained with **4a** and **4c** using the two approaches (two wavelengths and multiwavelengths) are given in Tables II and III. These results illustrate the improvement of accuracy using the multiwavelength approach and show that the small shifts between the emission spectra of cadmium and zinc chelates are large enough to ensure reliable data.

Analytical performances have been evaluated at pH 10. The detection limit—defined as the metal concentration necessary to produce an analytical signal three times larger than the standard deviation of the blank signal (free chemo-sensor)—reaches 5 nM for both cadmium and zinc. The relative standard deviation (RSD) of 10 independent measurements at 250 nM metal concentrations has been evaluated to 2% for both metals.

Emission Behavior at pH 13

Analytical performances have been evaluated at pH 13. The calibration for cadmium is linear in the concentration range $1\text{--}90 \text{ nM}$ and the relative standard deviation is 1% ($n = 5$) for a cadmium solution of 50 nM . The 3σ limit of detection is 1 nM ($n = 5$).

At this pH, while cadmium induces an increase in fluorescence intensity similar to the one observed at pH 10, zinc is not detected anymore. This particular behavior of zinc under strong alkaline conditions has recently been described by Bernardo *et al.* [7] for another chemo-sensor: 2,6,9,13-tetraaza[14](9,10)anthracenophane (**5**). A CHEF effect is observed for **5** in the presence of zinc and cadmium at pH 10, but the formation of hydroxo-complexes of zinc at a higher pH would, according to the authors, induce a nonradiative deactivation path in the zinc-chelate.



Our similar observation can probably be understood on the same grounds. Yet one can also argue that at pH 13 aqueous zinc complex is in the ZnO_2^{2-} and HZnO_2^-

Table III. Comparison of the Two Approaches with **4c**; Relative Errors (%) in Parentheses

Analytical concentration (nM) in the mixture		Measured concentration (nM)			
		Two wavelengths (395 and 417 nm)		Multiwavelengths (171 nm from 380 to 550 nm)	
Zinc	Cadmium	Zinc	Cadmium	Zinc	Cadmium
25	25	18 (28)	30 (20)	22 (12)	25 (0)
50	25	42 (16)	25 (0)	46 (8)	22 (12)
25	50	21 (16)	51 (2)	23 (8)	50 (0)
150	30	129 (14)	37 (23)	139 (7)	30 (0)
350	35	336 (4)	51 (46)	359 (3)	38 (9)

Table IV. Determination of Cadmium Concentrations in the Presence of Zinc with **4c** at pH 13; Relative Errors (%) in Parentheses

Analytical concentration (nM)		Fluorescence intensity at 420 nm (a.u.)	Measured concentration (nM) of cadmium
Zinc	Cadmium		
0	100	133	102 (2)
1000	100	138	107 (7)
0	25	43	24 (4)
2500	25	48	29 (16)

forms [8], which would not be complexed by the chemosensors.

As a consequence, it should be possible to determine selectively cadmium in strongly alkaline media. Table IV reports some cadmium concentrations determined at various Zn(II)/Cd(II) ratios. These data illustrate an original way to determine cadmium concentrations at the nanomolar level even at a high Zn(II)/Cd(II) ratio, which is normally the case in seawater.

Cadmium Determination in Seawater

The selective detection of cadmium at pH 13 could be used to analyze seawater samples. This implies the identification of the other species that could be complexed by the chemosensors under strongly alkaline conditions. Figure 4 illustrates the competition between cadmium and some heavy metals at pH 13. Heavy metals such as Cu(II) and Hg(II)—present in variable amounts in

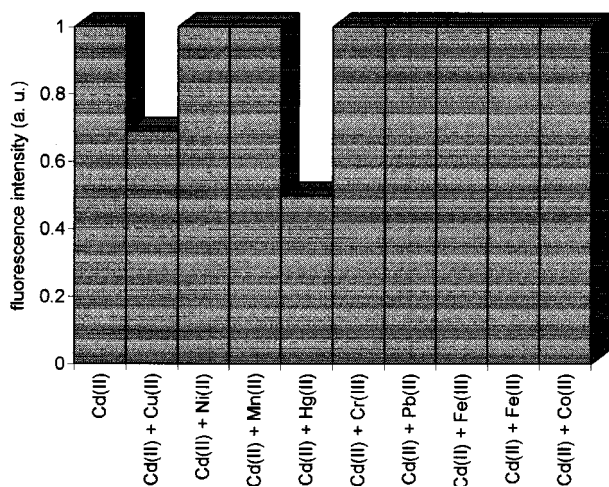


Fig. 4. Comparison of the relative fluorescence intensity of 1 μM **4c** chelated to cadmium in the presence of various heavy metals ($c = 1 \mu\text{M}$) at pH 13.

seawater—interfere with the fluorescence of the cadmium-chelated chemosensor.

Consequently, a separation technique had to be developed to prevent fluorescence quenching by these interferents. In 1953, Kraus and Moore [9] successfully separated Ni(II), Mn(II), Co(II), Cu(II), Fe(III), and Zn(II) at the milligram scale, using Dowex 1 resin. The development of some particular aspects of this study was made during our investigations: (i) the extension of the separation technique to a wider sequence of heavy metals, (ii) the extension to lower concentration levels, and (iii) the preconcentration of cadmium. Using a procedure similar to the one described in the literature [9] (see Experimental), cadmium has been successfully isolated starting from subnanomolar-level samples. Using this procedure an enrichment factor of 30 was obtained.

The fluorescence-based approach has been evaluated on seawater samples collected from Hoek van Holland (The Netherlands) and analyzed without any treatment other than filtration through a 0.45- μm filter (Millipore) and acidification to pH 2. Organic matter, such as humic acids—present in large amounts and having been shown to interfere with the emission of the chemosensors [10]—were not retained during the matrix separation step and were eluted with the heavy-metal interferents. The concentration of cadmium, determined by calibration and confirmed by the standard addition technique, has been determined to be 2.3 nM.

The accuracy of the preconcentration-fluorescence detection technique was examined by the determination of cadmium concentration in water certified reference material obtained from the National Research Council of Canada, Marine Analytical Chemistry Standards Programme. Using our method, we found a cadmium concentration of $169 \pm 13 \text{ pM}$ in estuarine water SLEW-2. This result shows a good agreement with certified values ($170 \pm 20 \text{ pM}$).

CONCLUSIONS

The chemosensors studied in the work presented here seem to suit all required analytical performances necessary to determine nanomolar levels of cadmium and zinc by molecular fluorescence spectroscopy. The detection limit is 1 nM at pH 13 for cadmium and 5 nM at pH 10 for both metals. Selective determination of cadmium or zinc is possible at pH 10 using the multiwavelength approach. The use of matrix separation column was found to be necessary for seawater analysis since fluorescence is strongly modified in the presence of several heavy metals. Incorporating this separation and pre-

concentration technique to fluorescence detection, the concentration of cadmium has been determined for certified seawater samples at a sampling frequency of 3 h^{-1} . The same approach will be used in the near-future to determine the concentration of zinc at the nanomolar level in seawater.

ACKNOWLEDGMENTS

We thank the E.C. Commission for sponsoring this project (Contract MAS 3-CT 97-0143) and the "Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture" (Belgium) for awarding a grant to one of us (S. Yunus).

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