Chromatographic Behavior of Open-Chain Polyamines NH_2-$(CH_2)_2$-[NH-$(CH_2)_2$]$_n$-NH_2 and Their Quantitative Determination in Sea Water by High-Performance Ion-Exchange Chromatography

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Abstract

The results are reported of a study using high-performance liquid chromatography for the chromatographic behavior of a homologous series of open-chain polyamines having the general molecular formula NH_2-$(CH_2)_2$-[NH-$(CH_2)_2$]$_n$-NH_2 (n = 0–4). The exchange column is a carboxylate-functionalized resin. Amines are eluted with mixtures of $HClO_4$ 0.200 mol/L, $NaClO_4$ from 0.100 to 1.40 mol/L, and acetonitrile (ACN) from 0% to 40% (v/v) and detected amperometrically. The dependence of the retention factor k' on sodium and ACN concentrations in the eluent and the total number of nitrogen atoms in the amines are considered and discussed. Suitable relationships are derived. Examples of polyamines separation are given, and a quantitative determination of analytes in spiked sea water is shown.

Introduction

The chromatographic behavior of some classes of amines in relation to the composition of the mobile phase has been reported in previous papers (1–3), in which linear monoamines and linear- and *N*-alkyl-substituted diamines were taken into account. It was thus deemed appropriate to extend this research to polyamines because they are both widely found in the animal and plant kingdoms (bioamines) and extensively employed as raw materials and intermediates in the production of other chemicals, polymers, pesticides, and dyestuffs. Inspection of the literature in this area reveals there to be a large number of papers dealing with the determination of monoamines and diamines, with separation usually by reversed-phase liquid chromatography (LC), previous derivatization, and detection by UV measurements (4–8). Only a few studies have delt with the determination of polyamines, such as the paper of Duong et al. (9,10), which describes the determination of pentaethylenehexamine, and of Henriks-Eckerman et al. (11), which examines the separation of ethylenediamine, diethylenetriamine, triethylenetetramine, and tetraethylenepentamine (in this paper the authors affirm that the subsequent UV detection was excellent for all polyamines in the 1–30-µg/mL range). Finally, it is underlined that in all papers the analytical procedures suggested for the determination of polyamines in real matrices were very time consuming (direct introduction of the sample into the chromatographic column was not possible) and elution times were often long.

Herein is presented an investigation into the chromatographic behavior of the amine homologous series NH_2-$(CH_2)_2$-[NH-$(CH_2)_2$]$_n$-NH_2 (n = 0–4), which can be proposed as a model for polyalkylamines, containing two primary and n secondary amino groups intercalated among them at regular intervals by (n + 1) ethylenic groups. Preliminary elution tests were carried out using the same experimental approach described in previous papers (i.e., sulphonic cationic exchanger; mixtures of $HClO_4$, $NaClO_4$, and acetonitrile (ACN) in variable concentrations as the mobile phase; and amperometric detection) (1,2). These tests gave disappointing results, especially towards polyamines with more than three nitrogen atoms, because the retention times (t_R) were high and the peaks were broad. To overcome these problems, the replacement of the strong exchanger with a column containing a carboxylic cationic exchanger was undertaken. The qualitative composition of the mobile phase remained unchanged so that the carboxylic sites were dynamically and reversibly saturated by the protons of strong acid $HClO_4$, allowing $NaClO_4$ and ACN to modulate the retention times. The choice of appropriate experimental conditions allowed for the simultaneously separation of all components of the homogeneous series with elution times lower than those reported in literature (6,7,9–11). Amperometric detection (unchanged with respect to previous papers) was independent of possible inorganic species and did not require the chemical

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pretreatment of natural samples (such as sea water), because it was necessary for UV or conductometric detection, which are generally used in polyamine analysis.

The aim of this work is to: (*i*) identify the mechanisms involved in chromatographic processes by studying the relationships between the concentrations of the components of the mobile phase and the t_R of polyamines; (*ii*) predict optimum conditions for their separation; (*iii*) propose reliable separation methods; and (*iv*) propose reliable methods for the quantitative determination of polyamines in natural matrices such as sea water.

Experimental

Chemicals

Standard stock solutions of polyamines [ethylenediamine (EDA), diethylenetriamine (DETA), triethylenetetramine (TETA), tetraethylenepentamine (TEPA), and pentaethylenehexamine (PEHA)] were prepared by dissolving the appropriate amount of their purified hydrochlorides in water. The $HClO_4$ solution was prepared from the corresponding concentrate and standardized against Na_2CO_3. The $NaClO_4$ solution was prepared from the corresponding solid product previously dried under vacuum. NaOH was prepared by diluting a standard 10 mol/L solution. High-performance liquid chromatography-grade ACN was used. All the reagents were Fluka (Buchs, Switzerland) or Aldrich (St. Louis, MO). All solutions and eluents were prepared using grade A glassware and ultrapure water (conductivity < 0.1 µS) ultrasonicated for 30 min and then filtered on Millipore 0.45 µm (Pall Corp., Ann Arbor, MI). The eluent solutions were introduced into the delivery systems under helium pressure.

Instrumentation

The chromatographic system was a DX500 ion chromatographic analyzer (Dionex, Sunnyvale, CA) incorporating a gradient pump. The post-column addition of NaOH was carried out using a Dionex PC10 pneumatic controller. The detector was a thin-layer Dionex ED40 amperometric cell, and the potential was applied according to Integrated Square-Wave Detection rules (12). The system was controlled by Dionex Peaknet 4.10 chromatographic software. The Dionex IonPac CS14 cation-exchange column (4- × 250-mm i.d.) was used as the analytical column, and the IonPac CG14 (4- × 50-mm i.d.) was used as the guard column (Dionex). The exchanger in the IonPac CS14 and CG14 columns is a ethylvinylbenzene-divinylbenzene, carboxylate-functionalized copolymer that is generally used for the isocratic separation of aliphatic monoamines and of alkali and alkaline–earth cations (13). Mixtures of $HClO_4$ 0.200 mol/L, $NaClO_4$ from 0.100 to 1.40 mol/L, and ACN from 0% to 40% (v/v) having a flow rate of 1.0 mL/min were used as the mobile phase. The choice of eluents and perchlorate anion, in particular, has already been discussed (1).

Results

Procedure

To estimate the dependence of the elution of polyamines on the composition of the mobile phase, the t_R of analytes was measured using three different eluents that have the following concentrations [concentrations (*C*) of H^+ and Na^+ are reported on a molar concentration scale: mol/L, and as a percent (v/v) for ACN; they will be omitted for simplicity in the following text). For the first eluent, $C_H = 0.200$, $0.100 \le C_{Na} \le 1.00$, and ACN = 0. For the second eluent, $C_H = 0.200$, ACN = 9, and $0.100 \le C_{Na} \le 1.40$. For the third eluent, $C_H = 0.200$, $C_{Na} = 0.600$, and $0 \le ACN \le 40$. For every amine and experimental condition at least four trials were carried out, with a relative standard deviation (RSD) of 2–3% in

Table I. Mean k' Values as a Function of C_{Na}, pT, and ACN

		EDA		DETA		TETA		TEPA		PEHA	
		ACN†		ACN†		ACN†		ACN†		ACN†	
		0	9	0	9	0	9	0	9	0	9
C_{Na}*	pT	k'									
0.100	0.523	0.46	0.38	1.42	1.19	6.53	4.26	–	–	–	–
0.200	0.398	0.41	0.31	1.15	0.86	4.13	2.80	18.03	11.48	–	–
0.300	0.301	0.37	–	0.93	–	2.93	–	10.97	–	–	
0.400	0.222	0.34	0.25	0.80	0.64	2.25	1.66	7.47	5.10	22.90	14.38
0.600	0.097	0.30	0.24	0.69	0.52	1.64	1.14	4.50	2.89	11.29	7.49
0.800	0	0.27	0.19	0.55	0.42	1.16	0.83	2.77	1.87	6.97	4.36
1.00	–0.079	0.24	0.17	0.44	0.32	0.86	0.62	1.86	1.25	4.21	2.58
1.20	–0.145	–	0.16	–	0.29	–	0.51	–	0.91	–	1.73
1.40	–0.204	–	0.14	–	0.22	–	0.38	–	0.63	–	1.08

* C_{Na} in mol/L.
† ACN (%, v/v).

Table II. Polyamines Eluted with $C_H = 0.200$, ACN% = 0, and $0.100 \le C_{Na} \le 1.00$

	Parameters of equations 2 and 3			
	Equation 2		Equation 3	
Amine	*y*	log *I*	log *I**	log *I*†
EDA	0.432 ± 0.007‡	–0.566 ± 0.002	–0.558 ± 0.009	–0.560 ± 0.004
DETA	0.82 ± 0.03	–0.276 ± 0.012	–0.296 ± 0.011	–0.296 ± 0.012
TETA	1.42 ± 0.03	0.056 ± 0.010	0.055 ± 0.009	0.057 ± 0.011
TEPA	2.04 ± 0.05	0.436 ± 0.008	0.451 ± 0.012	0.454 ± 0.014
PEHA	2.41 ± 0.05	0.824 ± 0.011	0.822 ± 0.012	0.822 ± 0.005

* $a_1 = 0.52$, $a_2 = 0.64$.
† $a'_1 = 0.51$, $a'_2 = 0.61$.
‡ ± 3 times SD.

t_R. From t_R values, the retention factors k' were calculated by means of the simple expression:

$$k' = (t_R - t_0)/t_0 \quad \text{Eq. 1}$$

where t_0 indicates the hold-up time of the system, which is equal to 2.16 min in the experimental conditions. Because the Na^+ and H^+ present in the eluent have equal charges, the total cationic concentration in the mobile phase was $C_H + C_{Na} = C_T$. The total cationic concentration (C_T) is expressed, successively, as pT, where pT = –log C_T.

k'–pT relationship

Table I reports the mean k' values for the elution of polyamines with mobile phases having $C_H = 0.200$, $0.100 \le C_{Na} \le 1.00$, and ACN = 0; some k' values for TEPA and PEHA are missing because the t_R obtained with low C_{Na} are over 60 min and the peaks are too broad. If log k' values are reported as a function of pT (Figure 1), a linear relationship between log k' and pT ($R^2 = 0.993 - 0.998$) for all polyamines is observed. Slopes of the straight lines are > 0 and not parallel with each other. This trend proves that log k' values depend on both the ionic contribution to elution associated with the increase in C_{Na} in the mobile phase (a decrease in t_R is observed) and the charge of the fully protonated polyamines (an increase in t_R is observed). When the charge is low (as in EDA), the increase in C_{Na} provokes small variations in t_R. On the other hand, when the charge is high (as in PEHA), the increase in C_{Na} depresses t_R considerably.

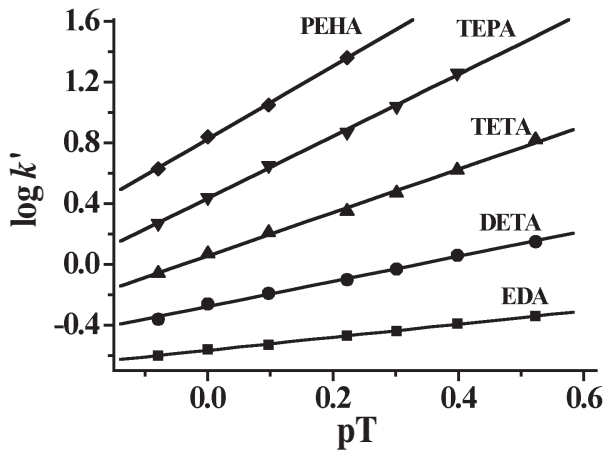

Figure 1. log k' versus pT in the elution of polyamines with a mixture containing $C_H = 0.200$, ACN = 0, and $0.100 \le C_{Na} \le 1.00$.

To determine the extent of the ionic contribution to the elution of polyamines, the following equation was used, as suggested by Haddad et al. (14):

$$\log k' = (y/x)\ \text{pT} + \log I \quad \text{Eq. 2}$$

where x is the eluent charge ($x = 1$, in this case), y is the analyte charge, and I is the isocratic constant ($\log k' = \log I$ when pT = 0). In order to calculate y and log I values for the five straight lines, the data in Table I (ACN = 0) was processed using the LIANA computer program (15). The results (Table II, 1st and 2nd columns) show that the values of y for every polyamine (i.e., their charges) are not equal to the number of protons in the amine, but are somewhat lower. In fact, the high degree of protonation achieved by the carboxylic sites allows the resin to interact dynamically and reversibly only with a fraction of the total charges of the amine, thus lowering the value. Therefore, the possibility of separating the polyamines quickly, even with high n_{tot} (n_{tot} represents the total number of nitrogen atoms), is dependent on the coupling of a carboxylic resin with a sufficiently acidic eluent. To quantitate the dependence of log k' on n_{tot}, the term y in equation 1 was replaced with $[(n_{tot} \times a_1) - a_2]$ (the analyte charge is equal to the number of nitrogen atoms (i.e., $y = n_{tot}$) and considered x to be 1:

$$\log k' = [(n_{tot} \times a_1) - a_2]\ \text{pT} + \log I \quad \text{Eq. 3}$$

Using LIANA, the value of the three empirical parameters were calculated: a_1 and a_2 are equal for all the polyamines and are, ± 3 times the standard deviation (SD), $a_1 = 0.52 \pm 0.02$ and $a_2 = 0.64 \pm 0.07$. Values for log I, which are very close to those calculated using equation 2, are reported in Table II (3rd column). Parameter a_1 halves the value of polyamine charges, and a_2 subtracts a fixed quantity.

k'–pT–ACN relationship

The results of the analysis carried out with the addition of ACN to the eluent were then considered. The t_R values obtained for two different mobile phases were measured, the first one contained $C_H = 0.200$, $0.100 \le C_{Na} \le 1.40$, and ACN = 9; the second contained $C_H = 0.200$, $C_{Na} = 0.600$, and $0 \le$ ACN ≤ 40. The results obtained by eluting with the first mobile phase are reported in Table I. Their analysis confirms the linear relationship between pT and log k' previously observed when ACN = 0 and shows that, for the same amine and C_{Na}, the value of k' at ACN = 9 decreases approximately 30% in comparison with the corresponding values at ACN = 0. The experimental data fit equation 2, and the y and log I values obtained are reported in Table III (1st and 2nd columns). A comparison of the values reported in

Table III. Polyamines Eluted with $C_H = 0.200$, ACN% = 9 and $0.100 \le C_{Na} \le 1.40$

	Parameters of equations 2 and 3			
	Equation 2		Equation 3	
Amine	y	log I	log I*	log I†
EDA	0.56 ± 0.02‡	–0.727 ± 0.009	–0.722 ± 0.009	–0.721 ± 0.005
DETA	0.94 ± 0.03	–0.41 ± 0.01	–0.42 ± 0.01	–0.418 ± 0.011
TETA	1.40 ± 0.03	0.098 ± 0.007	–0.11 ± 0.01	–0.110 ± 0.013
TEPA	2.04 ± 0.03	0.256 ± 0.011	0.258 ± 0.011	0.256 ± 0.007
PEHA	2.60 ± 0.09	0.61 ± 0.01	0.603 ± 0.004	0.606 ± 0.009

* $a_1 = 0.50$, $a_2 = 0.48$.
† $a'_1 = 0.51$, $a'_2 = 0.51$.
‡ ± 3 times SD.

the 1st and 2nd columns of Tables II and III shows that comparable y values were obtained for the same polyamines, though all log k' were 0.17 ± 0.03 units lower because an equal addition of ACN in the mobile phase gave an equal improvement in the affinity between polyamines and eluent. The dependence of log k' on n_{tot} was also verified in these experimental conditions. The experimental data fit equation 3, and the values of parameters (± 3 times SD) were $a_1 = 0.50 \pm 0.01$ and $a_2 = 0.48 \pm 0.06$. The log I values, which were very close indeed to those obtained from the fit of equation 2, are reported in Table III (3rd column). Because the a_1 values obtained by calculations based on data from experiments carried out with eluents containing ACN = 0 or 9 are very close, a unique a'_1 common value can be calculated for each experimental condition (ACN = 0, 9), two different a'_2 values common to all the polyamines, and different log I values for each polyamine. The experimental data were reprocessed and the results [$a'_1 = 0.51 \pm 0.01$, a'_2 (ACN = 0) = 0.61 ± 0.03, and a'_2 (ACN = 9) = 0.51 ± 0.05] and values of log I are reported in the last column of Tables II (ACN = 0) and III (ACN = 9). The closeness of all results, as well as the overall statistical parameters of the fit (SD = 0.00852; mean deviation = 0.0165) and those of single parameters are really low and confirm the validity of the model.

The results for the elutions of the five analytes with mobile phases containing $C_H = 0.200$, $C_{Na} = 0.600$, and $0 \le$ ACN ≤ 40 (shown in Figure 2 as k' vs. ACN percentage) were then considered. The t_R values, expressed as k', increased from EDA to PEHA and, for the same amine, decreased when the ACN percentage was increased in the mobile phase. There was a constant 75% decrease in k' for all amines as the ACN concentration in the eluent increased from 0% to 40%. To explain these results, the influence of the three main characteristics of amines on their chromatographic behavior must be considered: (*i*) hydrophilicity—calculated as log P (16) (P is the partition coefficient of a certain substance between two immiscible solvents, such as octanol and water) and equal to –1.62, –2.13, –2.65, –3.16, and –3.67, from EDA to PEHA—increases from EDA to PEHA and involves decreasing values of t_R in the same series; (*ii*) increase in molecular weight; and (*iii*) increase in charges from two to six in their fully protonated form.

All these effects are consistent with the final result, which is that k' values increase consistently (*i*) in progression from EDA to PEHA and (*ii*) when ACN concentration decreases. As an indication, the differences in k' values between EDA and PEHA are $\Delta k' = 10.99$ for ACN = 0 and becomes $\Delta k' = 2.74$ for ACN = 40. It is possible to rationalize the function $k' = f$(ACN) using the fol-

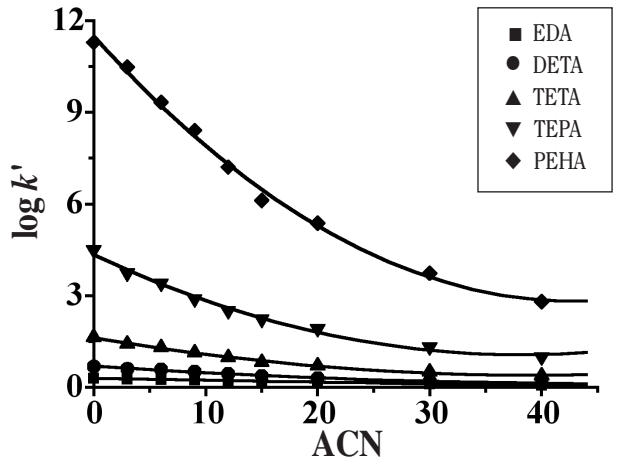

Figure 2. k' versus ACN in the polyamines elution with a mixture containing $C_H = 0.200$ and $C_{Na} = 0.600$.

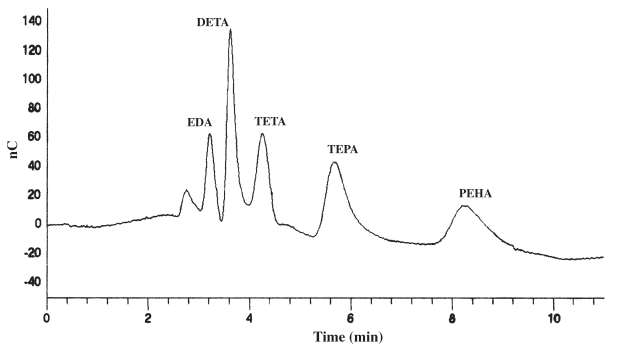

Figure 3. Elution of polyamines. Analysis conditions: $C_H = 0.200$, ACN = 20, and C_{Na} gradient from 1.00 to 1.50 and from 0 to 6 min. Concentrations: C_{EDA} = 1 µmol/L, C_{DETA} = 2 µmol/L, C_{TETA} = 3 µmol/L, C_{TEPA} = 4 µmol/L, and C_{PEHA} = 6 µmol/L.

Table IV. Analytical Data in the Polyamine Calibration Plots

Amine	C_A*	C_A†	S‡	SD%	R^2
EDA	5–60	0.3–3.6	1.170 ± 0.007	0.64	0.99972
	0.12–5	0.007–0.3	1.295 ± 0.028	2.17	0.99738
DETA	5–80	0.5–8.3	1.197 ± 0.009	0.74	0.99936
	0.5–5	0.051–0.5	1.320 ± 0.033	2.47	0.99776
TETA	8–100	1.2–14.6	1.618 ± 0.010	0.61	0.99972
	0.8–8	0.117–1.2	1.814 ± 0.015	0.83	0.99972
TEPA	10–150	1.9–28.4	1.716 ± 0.010	0.60	0.99958
	0.8–10	0.151–1.9	1.735 ± 0.103	5.94	0.97563
PEHA	10–150	2.3–34.9	1.405 ± 0.008	0.56	0.99992
	1.2–10	0.279–2.3	1.582 ± 0.058	3.7	0.99840

* C_A in µmol/L.
† C_A in ppm.
‡ Slope value.

Table V. LOD and LOQ of Polyamines

	LOQ					LOD	
			area × 10^{-6}				
Amines	C_A*	C_A†	plot	exper.	Δ%	C_A*	C_A†
EDA	0.12	7	0.16	0.22	–27	0.03	2
DETA	0.5	51	0.66	0.62	–7	0.1	10
TETA	0.8	117	1.45	1.68	–14	0.2	29
TEPA	0.8	151	1.39	1.53	9	0.2	38
PEHA	1.2	279	1.90	2.36	19	0.3	70

* C_A in µmol/L.
† C_A in ppb.

lowing linear equation (± 3 SD in all the figures):

$$\log k' = -0.0162(\pm 0.0003) \times \mathrm{ACN} + \boldsymbol{a} \qquad \text{Eq. 4}$$

where $\boldsymbol{a}$ = –0.483 ± 0.005 (EDA), –0.171 ± 0.007 (DETA), 0.198 ± 0.009 (TETA), 0.618 ± 0.008 (TEPA), 1.054 ± 0.009 (PEHA), and $\boldsymbol{a} = \log k'$ when ACN = 0.

Discussion

Polyamines separation

Taking into account the experimental data obtained with the different mobile phases, the separation chromatogram (Figure 3) is proposed for the five polyamines studied in this work. The separation of polyamines occured with an eluent containing constant ACN and $HClO_4$ concentrations and a gradient of $NaClO_4$. The analysis was very quick: it lasted 9 min and separation occured in less than 6 min. The peaks were well defined and clearly separated from each other. Different analysis conditions can be tested according to specific needs.

Quantitative determination

The calibration plots report polyamine concentrations versus peak area. Retention times were checked day-to-day and no meaningful variations were observed. The results are reported in Table IV. For each amine, the first row refers to the macro-range, and the second to the micro-range of the concentration. The linear dependence of the area on the analyte concentration (in µmol/L) can be expressed by the relationship:

$$\text{area} = S \times \mu\text{mol/L} \qquad \text{Eq. 5}$$

where the parameter S refers to the slope value of straight line.

The very good statistical parameters (SD% and R^2) allowed for valid determinations to be carried out. For each amine, nine concentration values (besides null) were considered; the lowest four constitute the micro-range (Table IV, second rows), and the highest concentration in the micro-range corresponds to the lowest in the macro-range (Table IV, first rows). The lowest concentration considered corresponds to the limit of quantitation (LOQ). The peak heights were considered when evaluating the limits of detection (LOD) and LOQ. Having verified the noise of the system to be approximately 5 nC and a signal of 10 nC to be clearly distinguishable, the LOD was set at the analyte concentration, giving a peak with a maximum of approximately 10 nC, and likewise a signal of 25 nC was considered to indicate the LOQ. On this premise, the limits were measured (the results are reported in Table V). As can be seen, both LOD and LOQ were very low, and the percentage differences between the experimental measurements and those obtained from the calibration plots (Δ%) in LOQ was acceptable. It can be concluded that the proposed method is suitable for the quantitation of polyamines at low concentrations [around parts per billion (ppb)].

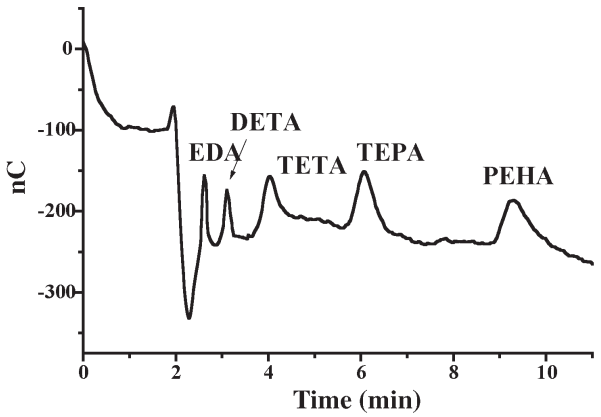

Figure 4. Elution of polyamines in spiked sea water. Analysis conditions as in previous figure. Concentrations: C_{EDA}, C_{DETA} = 0.5 µmol/L, C_{TETA} = 3 µmol/L; and C_{TEPA}, C_{PEHA} = 5 µmol/L.

Table VI. Analysis of Polyamines in Sea Water

Polyamine	Spiked (µmol/L)	Recovery* (µmol/L)	Recovery range (%)	Recovery 5 days† (µmol/L)
EDA	0.52	0.53(6.1)	90–109	0.55(6.3)
DETA	0.55	0.53(5.9)	92–109	0.56(6.4)
TETA	3.1	3.1(4.7)	97–110	3.3(5.5)
TEPA	4.4	4.5(7.5)	91–111	4.4(6.5)
PEHA	8.2	8.1(5.0)	86–109	8.0(4.8)

* Mean values of 5 determinations. RSD values in parentheses.
† Mean values of 25 determinations. RSD values in parentheses.

Analysis in sea water

It was thought that the amperometric detection of polyamines (very selective toward this class of compounds) might allow their determination in sea water by the direct injection of the natural sample into the analytical column without preliminary separation or pretreatment. To this end, a sample of sea water was ultrasonicated, filtered on 0.45-µm cellulose acetate membranes (Pall Corp.), and then analyzed for possible polyamine content. Once the absence of amperometric signals was established, sea water samples were spiked with known amounts of polyamines and, once again, analyzed. Five replicate analyses were carried out on five consecutive days. The results are reported in Table VI, and Figure 4 shows a typical separation. As can be seen, the quantities of polyamines recovered and reported as daily mean values are in good agreement with the quantities added, and the RSD and recovered ranges percentage are also consistent with this type of analysis. Moreover, the mean recovery values obtained from five sets of analyses carried out daily over 5 days (as well as the corresponding RSD), comparable with the daily reading, indicate good reproducibility of the method.

Conclusion

In this study of the chromatographic behavior of a homologous series of polyamines, from EDA to PEHA and as a function of eluent composition, the synergy between analytical and instrumental choices was observed. This allowed for the attainment of

very satisfactory results in the quantitative determination of the polyamines. The strong acid present in the mobile phase protonates the carboxylic anions of the resin, decreasing the reactant sites and allowing Na^+ and ACN to modulate the values of t_R. In this way the number of polyamine charges that interact with the carboxylic groups seems halved, and the t_R values are suitable for determination. Moreover, the amperometric detector, which makes it possible to use eluents (and matrices) with high ionic content, proved to be the best transductor for the detection of amines and did not require the samples to undergo any particular pretreatment, such as extraction from natural matrices or purification, as is necessary when using other detection methods.

Analytical evaluation of the proposed methodology reveals it to be fast and sensitive. The simultaneous separation of the five polyamines took place in 9 min, a drastically lower period of time than any reported in literature (5,9), and sensitivity (ppb) is better than that achieved previously (10). LOD and LOQ values obtained in this work are lower than those reported in literature for every polyamine. For example, Henriks-Eckerman et al. (10) reports a linear response in the range of 1–30 parts per million (30 times). However, for the same analytes, the LOD and LOQ values were herein between 2 and 70 ppb and 7 and 279 ppb, respectively, and a very broad linearity interval (100–500 times). The possibility of analyzing polyamines directly in matrices with high ionic content, such as sea water, is also very interesting.

Acknowledgments

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