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Continuous solid-phase extraction and dansylation of low-molecular-mass amines coupled on-line with liquid chromatography and peroxyoxalate chemiluminescence-based detection

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Abstract

An on-line liquid chromatographic system involving solid-phase extraction, dansylation and peroxyoxalate chemiluminescence-based detection was used to determine low-molecular-mass amines in environmental water samples. Sample volumes of 50 ml were enriched in the amines by passage through an IRC-50 cation-exchange column, elution and on-line derivatization with 0.16 *M* borate buffer (pH 11) and dansyl chloride, respectively, to a C_{18} analytical column, and determined by using an integrated derivatization-chemiluminescence detection unit based on the bis(2,4,6-trichlorophenyl)oxalate–hydrogen peroxide system. Dynamic ranges from 60 ng l^{-1} to 400 μg l^{-1}, limits of detection at the nanogram-per-litre level and relative standard deviations from 2.2 to 5.0% were thus obtained for the amines. The proposed method surpasses other chromatographic alternatives based on different detection techniques in terms of limits of detection. Also, it affords routine analyses of environmental waters containing low-molecular-mass amines at trace levels.

Keywords: Chemiluminescence detection; Detection, LC; Derivatization, LC; Environmental analysis; Sample handling; Water analysis; Amines

1. Introduction

The determination of low-molecular-mass amines, especially by chromatographic techniques, is well documented in various areas including water [1–6], air [7–9], food [10,11] and clinical analysis [12]. However, the low contents of these compounds in environmental samples and, especially, in waters (below the microgram-per-litre level in many cases) call for suitable analytical methods. Highly sensitive chromatographic detection systems such as mass spectrometry and luminescence spectroscopy in gas and high-performance liquid chromatography (HPLC), respectively, coupled to trace enrichment procedures, are the most interesting alternatives in this context. These methods usually derivatize the amine to improve their analytical features.

Trace enrichment by liquid–liquid extraction has traditionally been the technique of choice for the determination of low-molecular-mass amines by gas

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chromatography [1,2]. On the other hand, when the amines are determined by HPLC or capillary electrophoresis, enriching by solid-phase extraction (SPE) has proved a more efficient choice than the classical liquid–liquid extraction by virtue of the wide availability of sorbent materials and of the fact that the need to dispose of organic solvents is avoided [3,4,6]. Despite these advantages, SPE has scarcely been used so far to preconcentrate low-molecular-mass amines from water samples; polystyrene [3], Amberlite CG-120 resin [4], cation-exchange cartridges and cation-exchange extraction discs [6] – mainly in a discrete mode – have been used as sorbents for this purpose.

Liquid chromatography and, more recently capillary electrophoresis, have been more frequently used than gas chromatography for the determination of low-molecular-mass amines in environmental waters over the last decade [13]. This can be ascribed to a trend to using SPE systems with high preconcentration factors which provide similar or even lower limits of detection than gas chromatography–mass spectrometry with more affordable instrumentation. In these methods, fluorescence detection has been widely employed to determine this type amine following batchwise derivatization [4,10]. Although the sensitivity of these determinations can be increased in various ways, a luminescence technique of such great potential in this field as peroxyoxalate chemiluminescence (PO-CL) detection has scarcely been used in this context – apparently only one [14]. PO-CL is a very sensitive detection method for HPLC; in fact, it provides sensitivities often 10–100 times higher than that of conventional fluorescence detection [15].

This work reports a rapid, straightforward method for the continuous preconcentration and derivatization of low-molecular-mass amines in environmental waters coupled on-line with a liquid chromatograph equipped with a PO-CL detector that provides limits of detection at the nanogram-per-litre level. Ten aliphatic amines of one to seven carbon atoms were systematically studied and various cation exchangers tested in order to establish the best analytical conditions for their determination at low levels. Following continuous derivatization with dansyl chloride (Dns-Cl), the amines were determined by using a post-column zero-dead volume PO-CL detection system based on the bis(2,4,6-trichlorophenyl)oxalate (TCPO)–hydrogen peroxide system. The special features of this detector, which circumvents the shortcomings of PO-CL reactions in liquid chromatography [16,17], and the use of a sample-enrichment procedure, provide lower limits of detection than do existing chromatographic alternatives.

2. Experimental

2.1. Standards and reagents

All chemicals used were of analytical-reagent grade. The amines studied were methylamine, ethylamine, diethylamine, propylamine, butylamine, isobutylamine, pentylamine, isopentylamine, hexylamine and heptylamine, and all purchased from Aldrich. A 1.000 g l^{-1} standard solution of each was prepared in Milli-Q water and stored at 4°C in a refrigerator. Working-strength solutions were prepared by diluting to 100 ml appropriate volumes of the stocks with 0.16 *M* borate buffer (pH 11) or 10^{-2} *M* hydrochloric acid, as required. Amberlite cation-exchange materials (IR-120 PLUS, IRC-50 and CG-120) were supplied by Sigma in a particle size of ca 35 μm the first two and 150 μm the last. A 2.6×10^{-3} *M* dansyl chloride (Dns-Cl; 5-dimethylaminonaphthalene-1-sulphonyl chloride, Sigma) solution was prepared in acetone and stored refrigerated. A 3.5×10^{-3} *M* TCPO solution was made by dissolving 157 mg of the chemical (Aldrich) in 100 ml of ethyl acetate (Merck). The buffered oxidant solution was prepared by mixing 40 ml of concentrated hydrogen peroxide (Merck) and 1.0 ml of Tris (Merck) buffer, and making up to 100 ml with 2-propanol (Merck). A 0.15 *M* Tris [tris(hydroxymethyl)methyl amine, Merck] buffer solution was prepared by dissolving 1.8 g of product in water and adding enough hydrochloric acid to adjust the pH to 9.5 in a final volume of 100 ml. 0.16 *M* borate buffer solution was made by dissolving 1.0 g of boric acid (Merck) in water and adjusting the pH to 11.0 with sodium hydroxide in a final volume of 100 ml.

2.2. Apparatus

The instrumental setup used is depicted in Fig. 1. The continuous-flow system used for preconcentration and dansylation of amines consisted of a Gilson

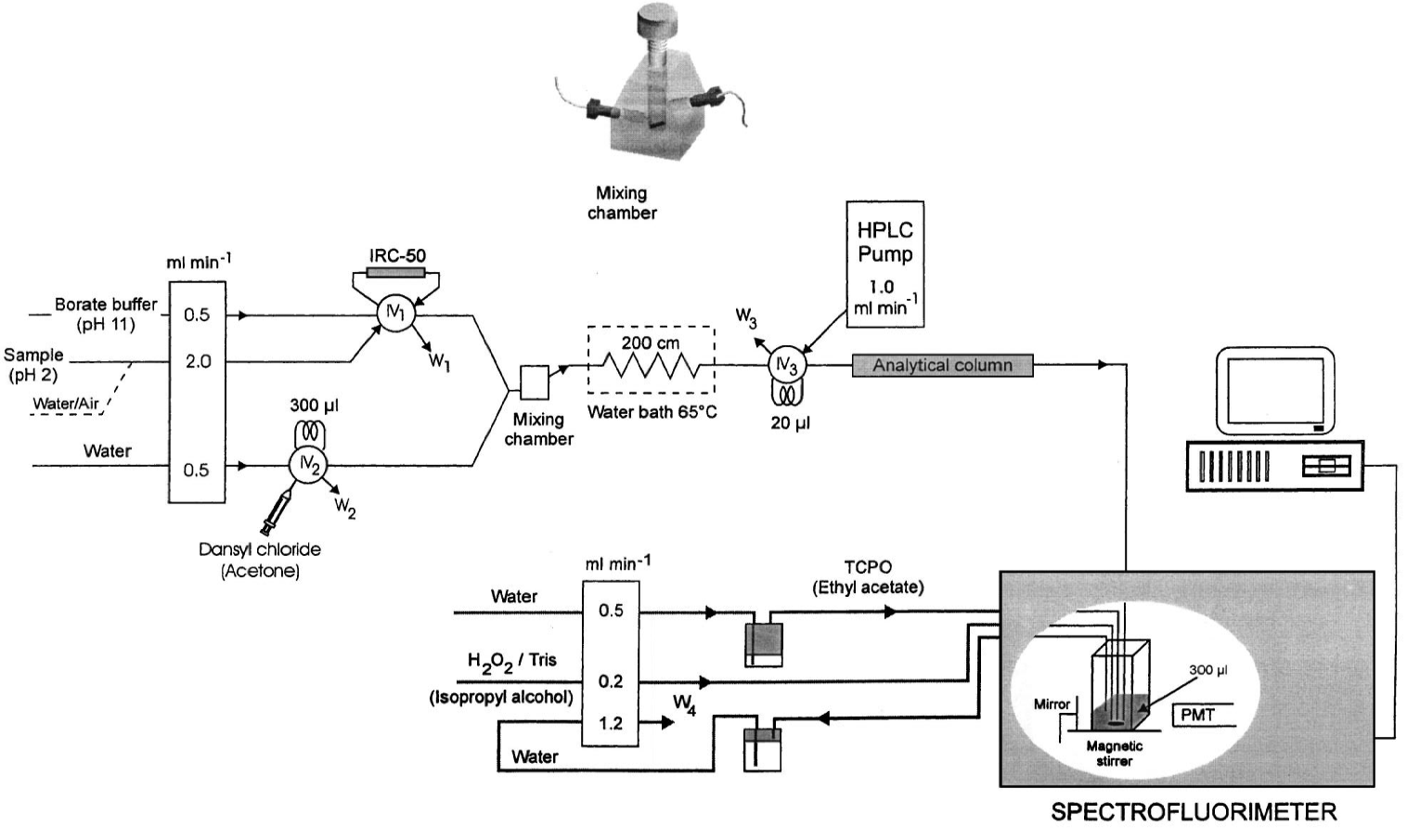

Fig. 1. Experimental setup used for the continuous solid-phase extraction and dansylation of amines, and their determination by HPLC-CL. IV, Injection valve; W, waste.

Minipuls-3 peristaltic pump fitted with poly(vinyl chloride) pumping tubes, two Rheodyne 5041 injection valves, a customized poly(tetrafluoroethylene) (PTFE) mixing chamber of 1 ml inner volume that included a PTFE-covered magnetic stirring bar for efficient mixing, a Selecta thermostat and PTFE tubing of 0.5 mm I.D. for coils. Laboratory-made exchange columns packed with different materials were constructed for solid-phase extraction. The columns were made from PTFE capillaries (3 mm I.D.). The end-caps were formed by fitting 30× 0.5 mm I.D. PTFE tubing into a 10×1-mm I.D. PTFE tube, which facilitates insertion into the continuous system, and both ends were sealed with a small plug of glass wool to prevent material losses.

The HPLC system consisted of a Waters W-600E multisolvent pump, a Rheodyne 7010 injector (20 μl loop) and 4-μm C_{18} Nova-Pak (15 cm×3.9 mm) cartridge columns. Retention times, peak heights and peak areas were provided by a Waters Maxima 820 chromatographic workstation interfaced to a NEC PC-AT 33-MHz compatible computer.

The CL detection system consisted of a Gilson Minipuls-3 peristaltic pump and two displacement bottles, one for pumping the TCPO solution and the other leading to waste, which ensured constancy in the volume of the reaction mixture in the cell. Poly(vinyl chloride) pumping tubes were used for carrying all reactants except hydrogen peroxide, which contained some isopropanol and must, therefore, be pumped through Solvaflex tubing. All other connections were established through stainless steel tubes. The CL signal was monitored with a Hitachi F2000 spectrofluorimeter with its light source off. The spectrofluorimeter's sample compartment was replaced with a small magnetic stirrer that supported the 1.0-cm spectrofluorimeter quartz cell and an Oriel 441321 1-in diameter mirror in order to acquire as much emitted light as possible. As can be seen in Fig. 1, the reactant solutions (TCPO and hydrogen peroxide–Tris) delivered by the peristaltic pump and the eluate from the column were mixed in the 1.0-cm quartz cell, the resulting PO-CL signal being simultaneously monitored by the photomultiplier. One additional channel was used to keep the volume of the reaction mixture (300 μl) constant in the cell. The optimum experimental conditions for this CL detection system were described elsewhere [16].

2.3. Continuous solid-phase extraction and dansylation of amines

For the continuous preconcentration and dansylation of amines, the weakly acidic cation-exchange column IRC-50 (200 mg) was conditioned by passing 10^{-2} *M* hydrochloric acid through it at a flow rate of 2.0 ml min^{-1}. A volume up to 50 ml of standard or water sample, at pH 2.0 (adjusted with hydrochloric acid), was then continuously introduced into the system at 2.0 ml min^{-1} and propelled through the column located inside the loop of the injection valve (IV_1). Amines were retained and the sample matrix was sent to waste (W_1); then, the column was washed with 5.0 ml of water and air-dried. As IV_1 was switched ($t=0$), a 0.16 *M* borate buffer (pH 11) was passed through the column in order to elute the analytes; simultaneously, the Dns-Cl solution was injected into the water carrier stream. After merging of both streams in the 1.0-ml PTFE mixing chamber, dansylation was performed in a PTFE reaction coil (200 cm×0.8 mm I.D.) immersed in a thermostated water bath at 65°C for 10 min, the pump being stopped at $t=120$ s. Finally, 20 s after the flow was resumed, a 20-μl aliquot was continuously injected into the liquid chromatograph via IV_3.

2.4. Chromatographic analysis

In the above-described HPLC system, amines were chromatographically separated by using two coupled 4-μm C_{18} Nova-Pak (15 cm×3.9 mm) cartridge columns with a linear gradient mobile phase of acetonitrile–water (25–50% acetonitrile for 0–5 min, 50% 5–30 min, 50–75% 30–35 min, 75% 35–50 min) circulated at 1.0 ml min^{-1}. Under these conditions, all amines were eluted within about 45 min.

3. Results and discussion

3.1. Continuous dansylation of amines on-line to HPLC

There are many available fluorescent probes for derivatizing the amine group, one of the most widely

used of which is dansyl chloride [18]. However, there is no general consensus as regards the optimal conditions for dansylation of amines in general or those studied in this work in particular [8,10,19–21]. In addition, amine dansylation reactions are performed batchwise and no reference to continuous dansylation systems appears to exist.

In view of the situation, we established the optimal reaction conditions for batchwise dansylation of the amines with a view to the subsequent optimization of the continuous dansylation process. The optimum conditions for the batch procedure were as follows: 5.0 ml of a solution containing an amine concentration of ca 100 μg l^{-1} in 0.16 *M* borate buffer (pH 11) was mixed with 3.0 ml of 2.5×10^{-3} *M* Dns-Cl in acetone. Following immersion in a water bath at 65°C for 25 min, a 20-μl aliquot was directly analysed by HPLC. Subsequently, the optimum experimental conditions for continuous dansylation of the amines were established by using a flow system similar to that depicted in Fig. 1 (less the exchange column and mixing chamber), off-line to the HPLC equipment. For this purpose, 400-μl fractions were collected in glass vials and 20-μl aliquots from them were analysed by HPLC–CL.

In order to boost CL signals, Dns-Cl must be dissolved in acetone; because this solvent attacks pumping tubes, it had to be inserted via an injection valve. For this purpose, the loop of the injection valve (IV_2 in Fig. 1) was filled with Dns-Cl solution in acetone by means of a syringe. The influence of the concentration of derivatized reagent was studied at levels up to 3.0×10^{-3} *M* (its solubility limit in acetone). The signal increased with increasing concentration up to ca 2.5×10^{-3} *M* and then levelled off up to the maximum concentration tested. A concentration of 2.6×10^{-3} *M* (0.8 mg ml^{-1}) was selected as optimal. Then, the effect of the injected Dns-Cl volume was studied between 50 and 500 μl. The CL signal peaked at about 250 μl and then remained constant. A loop of 300 μl was thus adopted as optimum.

The dansylation temperature and time, two other important variables in this process, were investigated over the ranges 30–70°C (using a thermostated water bath) and 5–25 min (after stopping the pump), respectively. Their influence is illustrated for methylamine with the response surface of Fig. 2. As can be seen, the effect of both variables on the dansylation reaction was similar; the signal peaked at about 65°C and 10 min, respectively, which were thus selected for further experiments. It is worth noting the substantially reduced dansylation time achieved relative to batch procedures [8,10,19–21].

The length of the reaction coil and the pump speed were both influential on the residence time of the sample–Dns-Cl mixture in the system. However, the fact that the stopped-flow mode was used (10 min), made it redundant to determine the effect of either variable (reaction coil dimensions and flow-rates). A reaction coil of 200 cm (0.8 mm I.D.) was selected because it ensured that, even if the sample/Dns-Cl plug was dispersed, it would not be swept out of the thermostated water bath. Flow-rates were set at 0.5 ml min^{-1}. Under these conditions, the pump was stopped about 1 min after Dns-Cl injection. The sample introduction device (viz. the interface between the continuous system and the liquid chromatograph) was an injection valve with a 20-μl loop (IV_3 in Fig. 1). The only variable affecting the performance of this coupling device was the time at which injection was carried out after the flow was resumed, which was optimized in order to maximize sensitivity in the analytical signal. For this purpose, several fractions were collected at 5-s intervals from the end of the loop into glass vials after the pump was started. The fraction containing the highest concentration of dansylamines was that obtained at 20 s. This time coincided with that at which the injection valve should be switched.

3.2. Solid-phase extraction system

The initial experiments for preconcentration of the aliphatic amines were devoted to finding the most suitable sorbent material. For this purpose, and because of the cationic character of amines in an acid medium, three substantially different cation exchangers on polystyrene were assayed, namely: two strongly acidic cation exchangers of different particle size (IR-120PLUS, ca 35 μm; and CG-120, 150 μm) and a weakly acidic one (IRC-50, ca 35 μm). Based on the pK_a values for the amines studied, aqueous standard solutions of the amines were adjusted at pH 2.0 with hydrochloric acid, which ensured protonation of the amine group in all cases. In order to

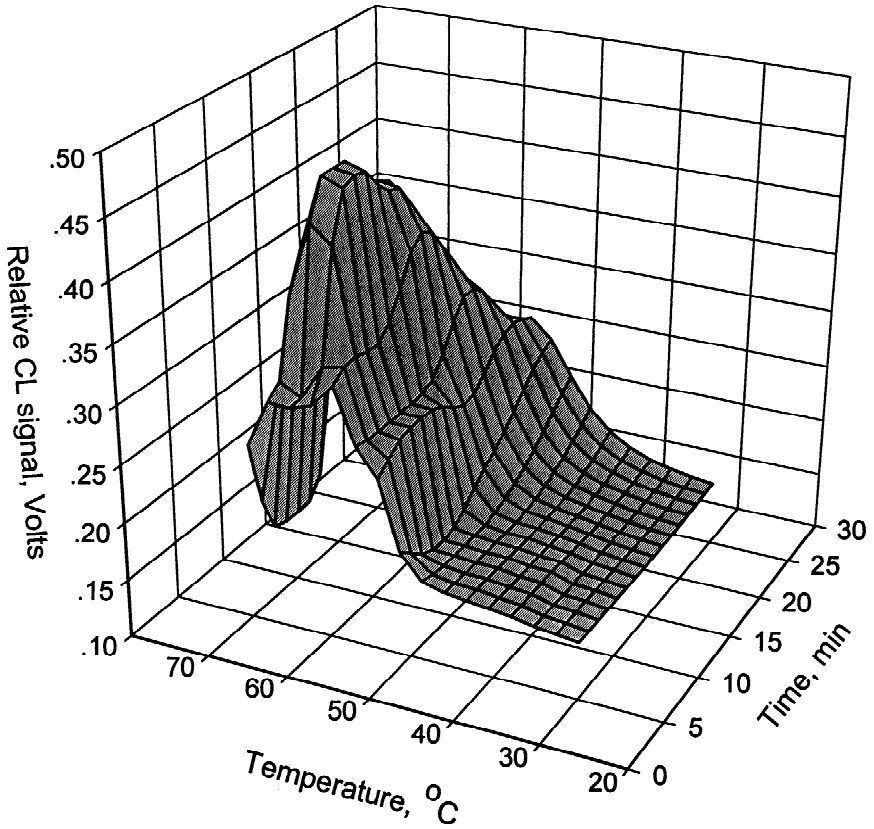

Fig. 2. Response surface for the influence of temperature and time on the continuous dansylation of 100 μg l^{-1} methylamine (for conditions, see Section 2).

simplify coupling of the SPE unit to the continuous dansylation of amines, only one eluent was tested, viz. 0.16 *M* borate buffer solution (pH 11), which provided a suitable pH for the dansylation reaction.

Initially, the study was conducted by using the same amount of exchanger (200 mg), packed as described in Section 2. The exchange-retention and overall efficiency of the SPE process were assayed in three individual experiments as follows: (1) as reference, 1.0 ml of aqueous standard solution containing ca 100 μg l^{-1} of each amine at pH 2.0 was diluted to 5.0 ml with 0.16 *M* borate buffer (pH 11) following neutralization with dilute sodium hydroxide; (2) to test the exchange-retention process, the same aqueous standard amine solution was passed through the exchange columns at 0.5 ml min^{-1} and 1.0-ml portions were collected after the columns. These fractions were diluted to 5.0 ml as stated above; and (3) to evaluate the elution process, 1.0 ml of the standard amine solution was passed through the columns at the same flow-rate. Then, the amines were eluted by continuously pumping 5.0 ml of the borate buffer solution. These solutions were dansylated by using the above-described continuous procedure and analysed by HPLC–CL to obtain the following analytical signals: S_1, S_2 and S_3, respectively. It is worth noting that, after each sample was processed, the column was flushed with 1.0 ml of 1.0 *M* sodium hydroxide and then with 5.0 ml of water, both at a flow-rate of 1.0 ml min^{-1}.

The exchange-retention efficiency was evaluated by comparing the signals S_1 and S_2, which revealed that all the amines studied were retained by the three exchangers with similar efficiency (higher than 95%). On the other hand, the overall SPE efficiency was determined by comparing S_1 and S_3. Table 1

Table 1
Overall amine solid-phase extraction efficiency[a] for various exchange materials as determined for 5.0-ml volumes of sample solutions containing amines at a concentration about 20 μg l^{-1} each

Amine	IR-120PLUS	CG-120	IRC-50
Methylamine	76 (5.3)	73 (5.5)	98 (5.1)
Ethylamine	81 (3.7)	78 (6.4)	91 (4.4)
Diethylamine	90 (4.4)	88 (3.5)	100 (4.0)
Propylamine	82 (3.6)	79 (5.0)	99 (4.7)
Butylamine	64 (6.2)	74 (6.7)	99 (5.6)
Isobutylamine	48 (8.3)	83 (4.8)	100 (4.0)
Pentylamine	41 (7.3)	73 (5.5)	91 (4.4)
Isopentylamine	49 (6.1)	79 (4.4)	93 (3.8)
Hexylamine	30 (8.8)	69 (7.2)	86 (5.8)
Heptylamine	27 (9.7)	68 (6.6)	83 (4.8)

[a] Average percent recovery for three determinations (relative standard deviation).

shows the overall efficiencies obtained (triplicate analyses) for the different amines by using the exchanger materials assayed. The following conclusions can be drawn from the results. (1) The overall SPE efficiency of the weakly acidic cation exchanger was in most cases higher than 95%. The lower efficiency of the strongly acidic cation exchangers can be attributed to their higher retention capacity, which probably hindered elution. In fact, some carry-over was observed if additional fractions of eluate were collected. (2) With the strongly acidic cation exchangers, elution was favoured at the higher particle size, which resulted in an increased interstitial volume in the column. This effect became more prominent as the length of the aliphatic chain of the amine increased. (3) The SPE efficiency decreased with increasing molecular weight of the amines (from pentylamine). Again, this can be related to the stronger retention of these compounds onto the column, with no effect on the collateral adsorption process, which might hinder elution. Based on these results, the IRC-50 (weakly acidic) cation exchanger was selected for preconcentration of the amines.

Various exchange columns packed with variable amounts of IRC-50 between 50 and 300 mg were prepared as described in Section 2.2 in order to establish the optimal amount of exchanger to be used to retain the amines. Increasing the amount of exchanger increased the analytical signal and hence the overall SPE efficiency of a column packed with 150 mg relative to another packed with 50 mg. However, no significant differences were found among columns packed with greater amounts of exchanger up to 300 mg. A column packed with 200 mg of IRC-50 was thus adopted for further investigations.

The coupling of the proposed SPE unit to the dansylation one is depicted in Fig. 1. As can be seen, a mixing chamber was included to favour homogenization of the eluted fraction and reagent prior to the reaction coil. In addition, both injection valves (IV_1 and IV_2) were simultaneously switched, and, about 2 min later, the pump was stopped allow the reaction to complete in the water bath.

Finally, the maximum sample volume that the SPE system could handle while maintaining low limits of detection (LODs) for the amines was determined. For this purpose, variable volumes (1–100 ml) of aqueous standards containing the same amount of each amine were adjusted to pH 2.0 and propelled through the exchanger column at a flow-rate of 0.5 ml min^{-1}. The results were quantitative up to 50 ml of sample. This volume could be increased with a view to lowering the LODs by using amounts of exchanger exceeding that chosen as optimal (200 mg). However, any improvement in the LODs thus obtained was found to be offset by the irreproducibility resulting from the ensuing hydrodynamic problems in the flow system.

In order to boost sample throughput, the influence of the sample flow-rate was studied over the range 0.5–3.0 ml min^{-1}. Very small changes were observed up to 2.0 ml min^{-1}, above which the analytical signal decreased through decreased residence time in the column. A sample flow-rate of 2.0 ml min^{-1} was chosen.

3.3. Chromatographic conditions

The composition of the mobile phase used to provide the most suitable conditions for the HPLC determination of the dansylamines was selected according to the following criteria: (1) maximal resolution for the amines; and (2) compatibility of the mobile phase used for separation with the post-column PO-CL detection system in order to ensure efficient CL production. In fact, the detector signal decreased with increasing water content in the

reaction medium [16]. In general, the dansylated amines studied were all efficiently separated by the C_{18} column provided gradient elution with acetonitrile in water was used [10,21].

The composition of the mobile phase (acetonitrile–water) and its flow-rate were thus optimized for a compromise between resolution and efficient PO-CL production. Based on the experimental results, two coupled 4-μm prepacked analytical reversed-phase C_{18} columns (15 cm×3.9 mm) were used to separate of dansylamines, using an overall linear gradient mobile phase from 25 to 75% in acetonitrile, which comprised several steps (see Fig. 3), at a flow-rate of 1.0 ml min^{-1}. This gradient, and the subsequent elution order for dansylamines, were both consistent with the polar character of these compounds, which decreased with increasing length of the aliphatic chain.

As can be seen from Fig. 3, some peaks (e.g. Nos. 3 and 9) are ill-defined and exhibit a shoulder. Because the chromatogram corresponds to standards, the effect cannot be ascribed to the presence of impurities, but rather to irreproducibility in the mixing of the three streams that converge on the 1.0-cm quartz cell (see Fig. 1) resulting from small oscillations in their flow-rates. In any case, the influence on peak height, the parameter used to construct the calibration graphs, is negligible.

3.4. Analytical figures of merit

Analytical curves for aqueous samples containing variable amounts of amines prepared as described in Section 2.3 were obtained by plotting peak height against analyte concentration. The analytical figures of merit for the maximum sample volume (50 ml) are summarized in Table 2 As can be seen, the sensitivity (slope of the calibration graph) decreased with increasing molecular mass of the amine up to propylamine, beyond which it remained virtually constant. LODs for the maximum sample volume used were calculated as the minimum concentrations providing a chromatographic signal twice the peak-to-peak noise [22]. The precision, expressed as relative standard deviation (RSD), was checked on 11 samples of 50 ml spiked with each amine at a

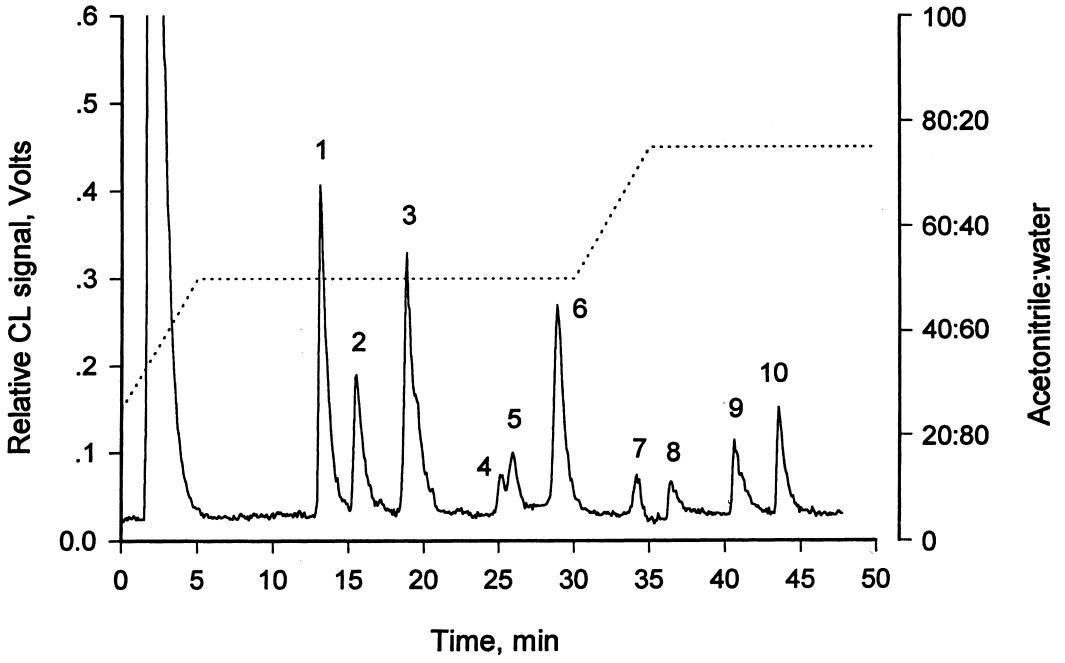

Fig. 3. Optimum chromatographic separation of the dansylamine standard solutions: 1 methyl-; 2 ethyl-, 3 propyl-, 4 isobutyl-, 5 butyl-, 6 diethyl-, 7 isopentyl-, 8 pentyl-, 9 hexyl- and 10 heptylamine; 20 pmol of amine injected in all cases (for conditions, see Section 2).

Table 2
Features of the calibration plots and analytical figures of merit of the CL determination of low-molecular-mass amines in 50-ml sample volumes[a]

Amine	Dynamic range ($\mu g\ l^{-1}$)	Linear regression equation	Correlation coefficient ($n=12$)	Limit of detection ($ng\ l^{-1}$)	RSD (%)
Methylamine	0.06–120	$H=6.1\times10^{-4}+7.7\times10^{-2}C$	0.999	15	4.8
Ethylamine	0.15–150	$H=-2.3\times10^{-3}+4.9\times10^{-2}C$	0.992	45	4.2
Diethylamine	0.45–300	$H=-8.5\times10^{-3}+3.0\times10^{-2}C$	0.997	60	3.8
Propylamine	0.50–250	$H=-1.2\times10^{-2}+3.4\times10^{-2}C$	0.996	62	3.3
Butylamine	2.5–300	$H=-4.9\times10^{-3}+5.4\times10^{-3}C$	0.998	340	5.0
Isobutylamine	2.0–250	$H=-7.3\times10^{-3}+6.2\times10^{-3}C$	0.999	325	2.6
Pentylamine	2.5–350	$H=-4.8\times10^{-3}+6.8\times10^{-3}C$	0.995	300	3.3
Isopentylamine	2.5–350	$H=-1.2\times10^{-2}+5.8\times10^{-3}C$	0.993	320	3.0
Hexylamine	3.0–350	$H=-2.2\times10^{-2}+6.7\times10^{-3}C$	0.995	300	3.3
Heptylamine	3.5– 400	$H=-2.0\times10^{-2}+6.8\times10^{-3}C$	0.996	300	2.2

[a] H, peak height (V); C, analyte concentration ($\mu g\ l^{-1}$); RSD, relative standard deviation.

concentration within the range of the calibration graph. The relative standard deviation ranged from 2.2 to 5.0%.

At this point, it may be interesting to compare the proposed chromatographic method with existing alternatives for the chromatographic determination of low-molecular-mass amines. However, such methods have only been applied to some of the aliphatic amines studied in this work–those with the lower molecular masses – so they preclude systematic comparison. The following conclusions can be drawn.

1. Gas chromatography has scarcely been used for this purpose. Mass spectrometry [1,2] provides lower LODs (0.01–0.5 $\mu g\ l^{-1}$) than does thermospecific [12] detection (20 $\mu g\ ml^{-1}$). In all cases, amines were derivatized with different reagents, such as benzenesulphonyl chloride [1], isobutyl- [12] or trichlorethyl- [2] chloroformate.
2. Similar LODs are obtained by using HPLC [3,23,24] or CE [25] with fluorescence or laser-induced fluorescence detection, respectively, and even with UV detection [6,10,26]; the high sensitivity achieved, however, relies on the use of solid-phase extraction. Again, amines are derivatized prior to chromatographic separation.
3. Poorer LODs ($\mu g\ ml^{-1}$ level) are achieved by using ion chromatography with either conductimetric [5,27] or amperometric [28] detection.
4. As stated above, there is only one reference to the use of the peroxyoxalate-based CL detection system for the chromatographic determination of these amines following derivatization with Dns-Cl, and then only from butyl- to decylamine [14]. Although the LODs provided by this method are slightly lower than those achieved in this work, the differences can be ascribed to instrumental features (the method in question uses a photon-counting system as detector instead of the simple spectrofluorimeter employed in this work). However, we believe this slightly higher sensitivity does not warrant use of more expensive equipment.

In summary the proposed chromatographic method is a useful choice for the determination of low-molecular-mass amines with lower limits of detection than existing chromatographic alternatives.

3.5. Analysis of real water samples

The proposed trace enrichment method was used to determine the amines most frequently found in water, viz. methyl-, ethyl-, propyl- and butylamine. All samples (tap, surface, ground and river water) were filtered through 0.45-μm mesh (4 mm diameter, Micron Separations, Westboro, MA, USA) to remove particulates. A volume of 25 ml of filtered water was adjusted to pH 2 with hydrochloric acid and diluted to 50 ml prior to analysis. Preliminary tests on 50-ml volumes of water revealed the absence of the amines. Consequently, the water samples were spiked with the four amines at concentrations over

Table 3
Average recoveries and RSD values obtained following the on-line preconcentration of 50 ml of environmental water samples spiked with a mixture of low-molecular-mass amines at different concentration levels

Amine	Tap water		Surface water		Ground water		River water	
	Spiked ($\mu g\ l^{-1}$)	Recovery (RSD)[a] (%)	Spiked ($\mu g\ l^{-1}$)	Recovery (RSD)[a] (%)	Spiked ($\mu g\ l^{-1}$)	Recovery (RSD)[a] (%)	Spiked ($\mu g\ l^{-1}$)	Recovery (RSD)[a] (%)
Methylamine	6.5	96 (5.5)	7.5	99 (6.0)	5.0	103 (4.8)	5.0	97 (6.5)
Ethylamine	5.0	99 (3.4)	6.5	101 (4.9)	10	100 (5.3)	5.0	98 (4.7)
Propylamine	7.5	93 (5.3)	10	98 (4.6)	7.5	99 (5.4)	7.5	96 (3.7)
Butylamine	150	95 (5.9)	75	96 (7.3)	150	94 (6.4)	150	93 (6.8)

[a] Relative standard deviation ($n=6$).

the range 5–10 $\mu g\ l^{-1}$ (by exception, butylamine was added at a concentration between 75 and 150 $\mu g\ l^{-1}$ on account of its lower sensitivity); following filtration, the samples were analysed by inserting a volume of 50 ml into the SPE system. Reproducibility was tested by using two replicate water samples over a 3-day period. The results (Table 3) were quite satisfactory as regards both recovery and relative standard deviation.

The good recoveries obtained testify to the absence of matrix effects, a result of the high selectivity of the proposed method for the determination of the four amines studied in environmental samples. Such a high selectivity relies on two facts, namely: (a) the prior SPE step not only increases the sensitivity (through preconcentration), but also removes most of the sample matrix, as only those species that are positively charged in the acid medium can be retained by the column and thus be potential interferents for the target amines; (b) following elution, any potential interferents will only actually disturb the PO-CL analytical signal if they are amenable to dansylation. Obviously, the PO-CL detector is more selective than the traditional UV detector used in HPLC. From these considerations and the recoveries and RSD values of Table 3, the dynamic ranges and LODs for the amines in real water samples can be deemed similar to those of Table 2. This was experimental confirmed for one of the amines (methylamine).

4. Conclusions

An on-line solid-phase extraction–dansylation system coupled to a liquid chromatograph was developed for the preconcentration/determination of low-molecular-mass amines in waters by use a zero-dead-volume peroxyoxalate-chemiluminescence detector. The simplicity of the proposed method probably makes it suitable for monitoring amine compounds in environmental waters. Assessment of the proposed method leads to the following conclusions:

1. As stated by Worsfold and co-workers [29], expanding the use of chemiluminescence detection for liquid separations requires facilitating automation (via on-line derivatization systems) and interfacing sample clean-up (e.g. solid-phase extraction) with analytical separation and detection. All have been accomplished for the first time in this work.
2. The continuous dansylation system provides two major advantages over reported batch counterparts including an appreciably shortened reaction time and avoidance of the additional step involving removal excess dansyl chloride with proline required by some methods.
3. The limits of detection obtained (at the nanogram-per-litre level and lower than those of existing chromatographic alternative) make the proposed method a useful choice for the determination of low-molecular-mass amines in environmental waters. Such low LODs result from the sample enrichment procedure employed and from the high sensitivity of the zero-dead volume peroxyoxalate-chemiluminescence detector [16,17] used to monitor the analytical signal.
4. Finally, for fully automatic functioning of the proposed on-line solid-phase extraction-dansylation system coupled to a liquid chromatograph, the pump and valves, must be governed by a computer – but not the post-column PO-CL

detector. Although this is fairly simple, we opted for having the system controlled by an operator in this work.

In summary, the most salient contributions of this work are the proposed continuous solid-phase extraction–dansylation system and the high sensitivity to low-molecular-mass amines achieved in combination with the zero-dead volume PO-CL detector used.

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