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N-PERMETHYLATION OF POLYAMINES AT TRACE LEVELS FOR GAS CHROMATOGRAPHIC ANALYSIS

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SUMMARY

A method for the trace analysis of polyamines is presented. It involves permethylation of the amines to the corresponding tertiary amines, extraction of the latter into an organic solvent and subsequent analysis by gas–liquid chromatography using nitrogen-selective detection.

The chromatographic behaviour was considerably improved compared with direct analysis of underivatized polyamines, and gave detection limits of the order of 0.1 ng/μl and linear calibration plots in the 0.1–100 ng range. Amines of interest from work environmental health aspects were included as model compounds.

INTRODUCTION

Trace analysis of polyamines, *i.e.* amines with two or more amine groups, is of interest in many fields. They find use as hardeners in the epoxy industry, as commercial fabric softeners, as corrosion inhibitors, as dispersant-detergent additives for motor oils, *etc.*[1]. Since most of them are poisonous and some are suspected to be carcinogenic, it is important from work environmental health aspects to be able to assay them in low concentrations. Similar compounds, such as putrescine, spermidine, spermine etc., are of importance in clinical diagnostics as growth regulators and tumor markers[2,3].

Gas chromatographic (GC) analysis of polyamines in low concentrations is difficult because of the marked tendency for adsorption to occur in the analytical system. The methods for trace analysis of amines by direct injection of free amines using gas–liquid chromatography (GLC), previously presented by us[4–7], are not suitable in this instance.

Analysis of polyamines by derivatization with perfluorofatty acid anhydride followed by electrochemical detection[8] or by flame ionization detection and mass spectrometry[9] has been described. Also, conversion of the amines into their N-iso-BOC derivatives followed by flame ionization detection[10] is possible. Some potential

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TABLE I

LIST OF AMINES INVESTIGATED

Name	Abbreviation	Formula
Ethylenediamine	EDA	$H_2N(CH_2)_2NH_2$
N,N,N',N'-Tetramethylethylenediamine	MEDA	$(CH_3)_2N(CH_2)_2N(CH_3)_2$
Diethylenetriamine	DETA	$H_2N(CH_2)_2NH(CH_2)_2NH_2$
N,N,N',N',N''-Pentamethyldiethylenetriamine	MDETA	$(CH_3)_2N(CH_2)_2NCH_3(CH_2)_2N(CH_3)_2$
1,4-Diaminobutane	BUDA	$H_2N(CH_2)_4NH_2$
N,N,N',N'-Tetramethyl-1,4-butanediamine	MBUDA	$(CH_3)_2N(CH_2)_4NH_3)_2$
Triethylenetetraamine	TETA	$H_2N(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$
1,1,4,7,10,10-Hexamethyltriethylenetetraamine	MTETA	$(CH_3)_2N(CH_2)_2NCH_3(CH_2)_2NCH_3(CH_2)_2N(CH_3)_2$

problems with the derivatization procedures include formation of unwanted derivatives and the presence of unchanged derivatization reagents. In addition, most derivatization methods demand non-aqueous conditions. A permethylation reaction, which can be performed in aqueous solution and which converts primary and secondary amine groups into tertiary groups, offers advantages especially in combination with nitrogen-selective detection. The boiling points of the amines are reduced, adsorption in the chromatographic system is expected to decrease markedly, and any derivatization reagents present do not interfere because the detection is based on the nitrogen content of the original molecule. Furthermore, the kinetics of the permethylation favours the final step where tertiary amines are formed, so problems with mixtures of derivatives are not expected to occur.

In the present paper we describe the application of the permethylation method to trace analysis of polyamines in which the reaction step is followed by extraction and subsequent GLC analysis.

EXPERIMENTAL

Apparatus

A Varian Model 3700 gas chromatograph, equipped with a Varian thermionic specific detector, was used. Typical settings for the detector were: gas flow-rates, 4 ml/min and 180 ml/min for hydrogen and air, respectively; bead-heating current, 7.0 scale divisions; bias voltage, -10 V; detector temperature, 250°C. Nitrogen was used as carrier gas at a flow-rate of 20 ml/min. The column was a glass (190 cm × 3 mm I.D.) packed with 28% Pennwalt 223 with 4% potassium hydroxide on Gas-Chrom R (80–100 mesh). An integrator (Hewlett-Packard Model 3390 A) was used for peak evaluation. Peristaltic pumps were used for addition of the reagents to the amine solution.

Materials

The amines investigated, their abbreviations and formulae, are listed in Table I. EDA (p.a.), MEDA (p.a.) and DETA (purum) were obtained from E. Merck (Darmstadt, F.R.G.), MDETA (99% by GC) from Alfa Products (Danvers, MA, U.S.A.), MBUDA (97% by GC) and MTETA from Janssen Chimica (Beerse, Belgium), and BUDA (puriss) and TETA (purum) from Fluka (Buchs, Switzerland).

Concentrated sulphuric acid, 37% aqueous formaldehyde, sodium borohydride and diisopropyl ether were all p.a. grade and were obtained from E. Merck.

The 28% Pennwalt 223 with 4% potassium hydroxide on Gas-Chrom R (80–100 mesh) was obtained from Alltech (Arlington Heights, IL, U.S.A.).

Permethylation procedure

Standard solutions of the amines were prepared by dissolving accurately weighed amounts of each amine in 0.05 M sulphuric acid followed by dilution to the appropriate concentrations. Then 5 ml of amine solution were thoroughly stirred in an open beaker and cooled with ice to a temperature below 10°C. The reagents, 2 ml of formaldehyde, 1 ml of 3 M sulphuric acid and 10 ml of sodium borohydride solution were simultaneously pumped to the beaker at 0.1, 0.05 and 0.5 ml/min, respectively, for 20 min, using a peristaltic pump.

The solution of sodium borohydride was made by dissolving 180 mg in 10 ml of water. It was cooled with ice in order to minimize the decomposition of sodium borohydride.

After the permethylation procedure was completed the pH of the solution was checked. In a few cases it was necessary to add concentrated sulphuric acid to make the solution sufficiently acidic (pH < 1) to destroy excess sodium borohydride.

Extraction

The extraction efficiency was investigated by adding different amounts of sodium hydroxide to 5 ml of water. The solutions were shaken with 5 ml of diisopropyl ether solution containing 1 ng/μl of the amines investigated. About 4 g of sodium hydroxide, which gave a saturated solution, were needed to ensure that the amines remained entirely in the organic phase.

In the extraction step following permethylation we used 10 ml of diisopropyl ether. According to the results above, 20 g of sodium hydroxide were added to the aqueous solution which had a volume of *ca.* 18 ml. During the addition of solid sodium hydroxide the solution was cooled in an ice-bath. After shaking, the phases separated easily and aliquots for analysis were taken directly from the organic phase.

Detection

The thermionic specific detector was optimized for maximum selectivity for nitrogen-containing compounds by the injection of solutions of MEDA in diisopropyl ether.

Quantitative analysis

Quantitative analysis was based on peak area measurements. The linear response range was established by plotting peak area against concentration for injected standard solutions. Yields were calculated by comparison of the peaks from the permethylated amines and the corresponding standards. Standards were subjected to the same treatment as polyamine solutions to reduce matrix effects.

RESULTS AND DISCUSSION

Permethylation procedure

The basic reaction mechanism proposed by Giumanini *et al.*[11] is the reaction between formaldehyde and amine in acidic solution giving a double bond between nitrogen and carbon, which is reduced by sodium borohydride. Considering the reaction scheme, it is obvious that all reagents used for the permethylation must be present simultaneously in the reaction mixture. This is best achieved by using a peristaltic pump for addition of all necessary reagents in different channels. Such a system is easily operated and several samples can be methylated simultaneously.

Table II shows the yields obtained with the methylation procedure as described in the Experimental section. They compare favourably with those found by Giumanini *et al.*[11], especially for EDA. No significant difference in yield was observed for concentrations of 0.1 ng/μl, 1 ng/μl or 100 ng/μl. The MTETA standard contained 19.5% impurities (determined by GC). For calculation of the yield from the methylation of TETA, these impurities in the corresponding permethylated amine standard were taken into account.

TABLE II

PERMETHYLATION YIELDS OF SOLUTIONS CONTAINING ONLY ONE POLYAMINE IN DIFFERENT CONCENTRATIONS

The yield is calculated from ten permethylations. Average percentage relative standard deviation *ca.* 4% for all amines except TETA, which is *ca.* 6%.

Amine	Yield (%)		
	0.1 ng/μl	*1 ng/μl*	*100 ng/μl*
EDA	102	99	103
BUDA	90	97	99
DETA	95	91	93
TETA	90*	101	94

* Concentration 0.5 ng/μl.

The GC analysis of the standards (2 μl injection) was performed with a standard deviation of *ca.* 2%. The relative standard deviation (R.S.D.), including the permethylation procedure and injection on the column, was on average *ca.* 4% for all amines, but TETA which elutes late in the chromatogram and gives a R.S.D. of *ca.* 6%. There is no significant difference in R.S.D. between the higher and the lower amine concentrations and between different amines.

A variation of the sulphuric acid concentration between 1 and 3 *M* in a volume of 1 ml, a variation of 1.5 to 3 ml of 37% formaldehyde solution and a variation between 180 and 360 mg of sodium borohydride in 10 ml of water solution gave the same yields within experimental errors. However, for concentrated amine solutions (above 100 ng/μl), 300 mg of sodium borohydride in 10 ml was needed for satisfactory yields. Variation of the reagent concentrations influenced the yields of all the different amines investigated in a similar way.

Solutions containing equal amounts (1 ng/μl) of EDA, BUDA, DETA and TETA were also permethylated. The results are presented in Table III. As can be seen, satisfactory yields for all the amines were obtained, which means that the yield is independent of the number of amine groups in the molecule as long as the reagents are in excess.

Another test was carried out with one of the amines, EDA, in great excess. The solution contained 1000 ng/μl EDA and 1 ng/μl BUDA, DETA and TETA. We

TABLE III

PERMETHYLATION YIELDS OF A SOLUTION CONTAINING 1 ng/μl OF ALL THE AMINES INVESTIGATED

The yield is calculated from six permethylations.

Amine	Yield (%)	R.S.D. (%)
EDA	103	2.9
BUDA	98	3.7
DETA	99	3.2
TETA	86	5.3

TABLE IV

PERMETHYLATION YIELDS OF A SOLUTION CONTAINING 1000 ng/μl EDA AND 1 ng/μl DETA, BUDA AND TETA

The yield is calculated from six permethylations.

Amine	Yield (%)	R.S.D. (%)
EDA	96	2.5
BUDA	78	4.8
DETA	98	3.5
TETA	95	4.1

assumed that impurities of other polyamines could be present in EDA, and solutions with EDA concentrations of 1000 and 10 000 ng/μl were methylated (in the last case with still higher concentrations of the reagents). MTETA was only seen when methylating 10 000 ng/μl of EDA, because of its higher detection limit. In the last case, with a very high concentration of MEDA the adsorption in the chromatographic system for later eluting solutes may be somewhat reduced, resulting in an overestimation of the impurities in EDA, especially for MTETA. However, we estimate the uncertainty in the determination to be less than 25 relative %. The chromatograms showed that the methylated EDA contained 0.17% MDETA and 0.006% MTETA, determined from EDA solutions of 1000 and 10 000 ng/μl, respectively, most probably originating from corresponding polyamines DETA and TETA.

When the yields presented in Table IV were calculated the impurities of MDETA and MTETA in MEDA were corrected for. The table shows satisfactory results. However, BUDA has a yield ca. 15% lower than those in Tables II and III; the reason for this is not clear.

Gas chromatography
Fig. 1 shows a chromatogram of a solution containing 1 ng/μl of the permeth-
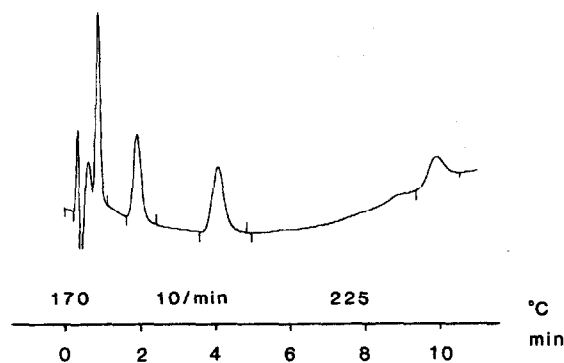

Fig. 1. Chromatogram of MEDA (0.9 min), MBUDA (2 min), MDETA (4 min) and MTETA (9.9 min). Injection of 2 μl of a 1 ng/μl solution. Column, 190 cm × 3 mm I.D. packed with 28% Pennwalt 223 with 4% KOH on Gas-Chrom R (80–100 mesh). Temperature programming and attenuation as shown. Carrier gas, nitrogen at a flow-rate of 20 ml/min. Thermionic specific detector setting: bead-heating current, 5.5 scale divisions; bias voltage, −10 V; temperature, 250°C; hydrogen flow-rate, 4 ml/min; air flow-rate, 180 ml/min.

ylated amines MEDA, MBUDA, MDETA and MTETA in diisopropyl ether. All these amines are easily separated on a 28% Pennwalt 223 column using temperature programming. The baseline drift is caused by column bleeding.

Detection limits

The detection limit for the amines investigated is set by the column bleeding and the tendency of the amines to adsorb in the chromatographic system. The limit for EDA, BUDA and DETA is 100 pg/μl and for TETA it is 300 pg/μl. The detection limit can be decreased at least five times by decreasing the volume of diisopropyl ether in the extraction step.

Quantitative analysis

Linear calibration plots were obtained in a broad range from 150 pg (MEDA, MBUDA and MDETA) and 300 pg (MTETA) to 100 ng. The low concentration range is shown in Fig. 2. The intercepts for all amines except MTETA were close to the origin. MTETA is somewhat adsorbed in the chromatographic system. The baseline drift seen in Fig. 1 does not interfere with the accuracy of the quantitative measurements.
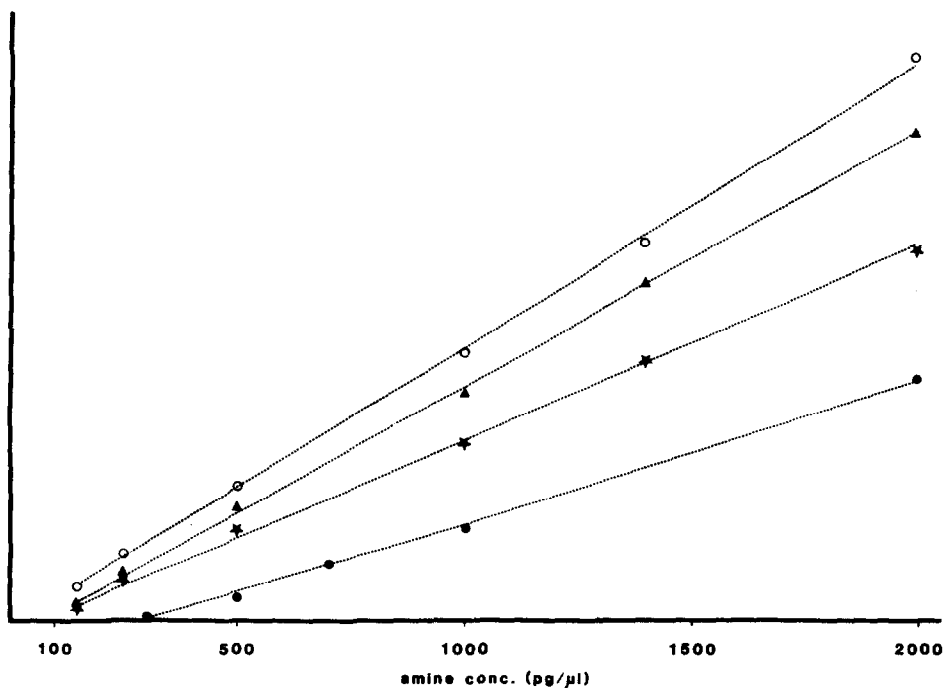

Fig. 2. Calibration curves for MEDA (O), MBUDA (▲), MDETA (★) and MTETA (●). Chromatographic conditions as in Fig. 1.

Stability

Standard solutions of the methylated polyamines in diisopropyl ether in contact with alkaline water solutions were stored at room temperature for one week. No noticeable degradation of the amines was observed when they were compared with a freshly made standard solution.

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