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Note

Analysis of trace quantities of ammonia and amines in aqueous solutions by reversed-phase high-performance liquid chromatography using m-toluoyl derivatives

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The simultaneous determination of small amounts of both ammonia and amines in aqueous solutions is an important environmental analytical problem because of the similar chemical nature of these compounds. Ammonia is typically separated by distillation and then can be determined by colorimetry or specific ion electrodes[1–6]. However, with trace quantities, the recoveries and the separations are poor and the process is time consuming. Ammonia has also been determined by the use of "ion chromatography", an ion-exchange separation technique, but sodium ions, which are always present in environmental samples interfere[7]. An ideal procedure for this problem is the use of high-performance liquid chromatography (HPLC) but the usual detectors do not respond well to aliphatic amines and ammonia.

Wellons and Carey[8] have solved this problem for amines by forming the *m*-toluoyl derivatives, using pyridine and extracting the derivatives with dichloromethane, then separation of the products by HPLC and detection by UV absorption. Ammonia and water were considered to be interfering agents. In this work, this procedure has been modified by using 1 *M* NaOH rather than pyridine as the reaction medium and by showing that quantitative results can be obtained for ammonia. In order to optimize the reaction conditions, the temperature, reaction time, pH, and molar ratio were varied systematically for NH_3 and ethylenediamine (EDA). The chemical structures of the products of the reactions with NH_3, EDA, and diethylamine were established by HPLC, gas chromatography–mass spectrometry (GC–MS), and proton magnetic resonance (PMR) techniques. These results and other quantitative data characterizing this reaction with other classes of amines are the subject of this note.

EXPERIMENTAL

A Waters Assoc. Model 6000 solvent pump, a Model U6K Universal injector, and a Model 440 UV detector constituted the chromatographic system and were used

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with a Houston Instruments Omniscribe 5000 recorder. A Varian Model T-60A NMR, A Hewlett-Packard Model 5700/5930A GC–MS–DS System and a Burrell Wrist-Action Shaker were used in these studies. The chromatographic conditions were maintained constant and are given in the captions for Figs. 1 and 2.

EDA, piperazine, diethylenetriamine, and triethylenetetramine were obtained from the Dow Chemical (Freeport, Texas, U.S.A.) and all, except piperazine, were redistilled before use. Spectrometric grade acetonitrile and analytical-reagent grade NaOH, NH$_4$OH, and K$_2$CO$_3$ were purchased from Mallinckrodt (St. Louis, Mo., U.S.A.). Spectrometric grade dichloromethane was obtained from J. T. Baker (Phillipsburg, N.J., U.S.A.). Benzylamine, p-phenylenediamine, and aniline were obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.). The m-toluoyl chloride was purchased from Aldrich (Milwaukee, Wisc., U.S.A.).

The standard reaction procedure developed in this study is as follows. Standards of 500 ppm of the pure compounds were prepared and diluted for analysis. Volumes of 20 ml of sample or standard were taken; 5 ml of 20% NaOH, and 2 ml of toluoyl chloride were added and the sample shaken for 10 min. Then 2 ml of concentrated NH$_4$OH or, if ammonia is to be determined, 1 ml of EDA was added to destroy the excess reagent and the sample was shaken for another 10 min. The samples were then extracted with 20 ml of dichloromethane and washed with 10% K$_2$CO$_3$ and distilled water. For maximum sensitivity, the dichloromethane can be evaporated and the derivatives redissolved in mobile phase.

RESULTS AND DISCUSSION

Reaction conditions

The derivatization of ammonia and EDA was studied as a function of pH, reaction time, temperature and molar ratio in order to determine the optimum conditions for reaction. A standard solution of each of these was allowed to react under various conditions and the response was measured by HPLC. To the maximum response in a series of experiments is given the arbitrary value of 100 and other results are expressed relative to this. This does not imply that the reaction is complete. The starting conditions were NaOH (1 M) pH = 14, 23°, and 10 min reaction time. The molar ratio was varied from 10:1 to 100:1. The range of the other variables and the results of these studies are given in Table I. The optimum conditions for ammonia were pH 14, 23°, molar ratio 50–100:1 and 10 min reaction time. From Table II, it can be seen that for EDA, the optimum is pH 10, room temperature, molar ratio 3:1, and reaction time 5 min. However, the EDA conversion at pH 14 is only slightly lower than at pH 10 so that a single procedure can be used for determining both ammonia and amines.

Quantitative aspects of the procedure

Purchased samples of m-toluamide and N,N-diethyl-m-toluamide were used as primary standards for determining the efficiency of the method for ammonia and diethylamine. The overall efficiency was 33% for NH$_3$ and 99% for DEA. The low efficiency for ammonia is due to an extraction efficiency of only 60%, determined independently and a 50% reaction efficiency. However, for analytical procedures, repeatability, not efficiency, is the criterion. To determine this, ten samples of 100

TABLE I

REACTION CONDITIONS FOR AMMONIA AND EDA

Molar ratio	Time (min)	pH	Temp. (°C)	Relative response ammonia	Relative response EDA
10	10	14	23	33	96
25	10	14	23	90	100
50	10	14	23	100	98
70	10	14	23	98	100
100	10	14	23	100	100
100	5	14	23	75	93
100	10	14	23	100	100
100	15	14	23	98	99
100	20	14	23	100	100
100	10	8	23	25	10
100	10	10	23	40	100
100	10	14	23	100	92
100	10	14	0	75	95
100	10	14	23	100	100
100	10	14	37	80	99
100	10	14	80	5	100

TABLE I₁

REACTION CONDITIONS FOR EDA AT pH 10

Molar ratio	Time (min)	Temp. (°C)	Relative response EDA
1	10	23	55
2	10	23	100
5	10	23	100
10	10	23	100
5	0.5	23	88
5	1	23	100
5	2	23	100
5	5	23	100
5	10	0	100
5	10	23	100
5	10	37	98
5	10	80	72

ppm NH_3 were run and a standard deviation of 3.8% of the observed value was obtained. For comparison Wellons and Carey[8] reported a pooled variation of 4% for their results. A different procedure was used to determine the efficiency for EDA. A sample of EDA was subjected to the procedure and the aqueous phase reacted with additional reagent to obtain two samples for chromatography. The efficiency was calculated as the response for the first sample divided by the sum of the responses for the first and second samples and was found to be 99%.

The sensitivity of the method was established for ammonia and EDA. For EDA one ppm gave a peak height of 1.6 cm for a 20-μl sample while ammonia gave

a peak of 0.90 cm measured at 0.005 a.u.f.s. The noise level is about 0.5 cm so that the minimum detectable quantity is about 1 ppm for these compounds. Fig. 1 shows a chromatogram of 10 l of a 20 ppm NH_3 sample. If the dichloromethane is evaporated and the derivative is taken up in 1 ml of mobile phase, then the minimum detectable amount can be decreased by a factor of 20. This was done experimentally for ammonia and a minimum detectable amount of 0.04 ppm was obtained. At this level, contamination becomes extremely important.
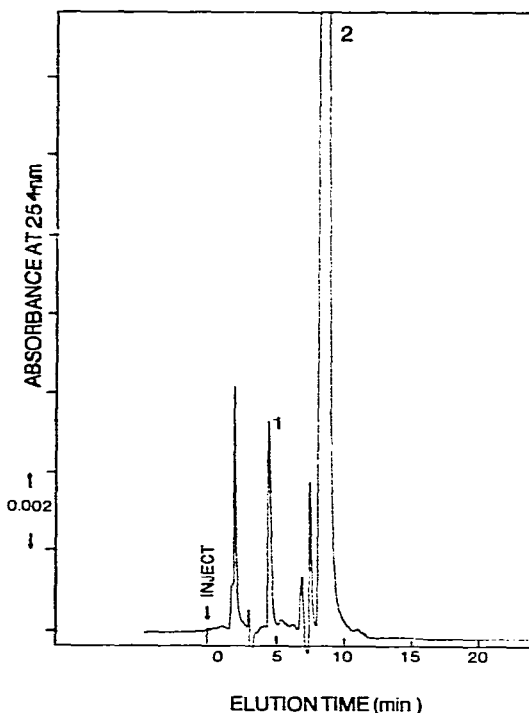

Fig. 1. Chromatogram showing the toluoyl derivatives of (1) ammonia (20 ppm), and (2) EDA (excess). Conditions: sample size, 10 μl; flow-rate, 0.8 ml/min; column temperature, ambient; mobile phase, water–acetonitrile (2:1.5); elution mode, isocratic; separation mode, reversed-phase; column, stainless-steel tube, 4 × 300 mm; column packing, μBondapak C_{18}, particle size, 10 μm.

The linear range has been determined for NH_3 and EDA to be from 500 ppm to at least 1 ppm so that calibration curves can be used in this range to quantitate. Alternatively internal standards may be used if accurate response factors are available. Fig. 2 shows a chromatogram used to determine the response factors for the four compounds, EDA, piperazine, diethylenetriamine, and triethylenetetramine. The internal standard is benzophenone, which would be dissolved in the dichloromethane. An amine standard that would be carried through the total procedure could also be used. The accurate molar response factors established for the above compounds, approximate response factors for other amines, derivatized to show the versatility of the method, and the chromatographic retention times relative to *m*-toluamide are given in Table III.
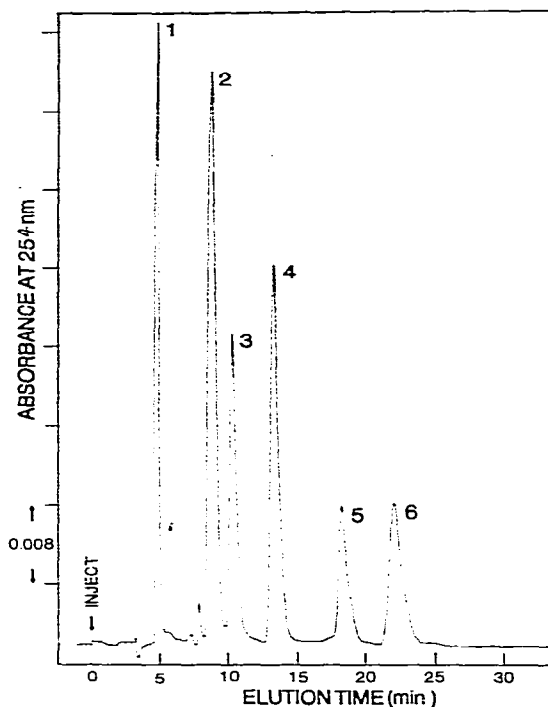
Fig. 2. Chromatogram of the following toluoyl derivatives plus an internal standard; (1) ammonia (excess), (2) EDA (300 ppm), (3) piperazine (103 ppm), (4) diethylenetriamine (152 ppm), (5) benzophenone (80 ppm), and (6) triethylenetetramine (190 ppm). Conditions: sample size, 10 μl; flow-rate, 0.8 ml/min; column temperature, ambient; mobile phase, water–acetonitrile (2:1.5); elution mode, isocratic; separation mode, reversed-phase; column, stainless-steel tube, 4 × 300 mm; column packing, μBondapak C_{18}, particle size, 10 μm.

TABLE III

RELATIVE RETENTION TIMES FOR m-TOLUOYL DERIVATIVE OF CERTAIN AMINES

Amine	Relative retention time	Weight response factor
Ammonia	1.00	≈0.8
EDA	1.82	1.01
Diethylamine	2.02	5
1,4-Butanediamine	2.07	2
Piperazine	2.11	1.38
1,5-Pentanediamine	2.43	2
Benzylamine	2.68	2
Diethylenetriamine	2.73	1.59
Aniline	3.02	0.5
Monoethanolamine	3.70	2.5
1,7-Heptanediamine	4.14	2
p-Phenylenediamine	4.77	1
Triethylenetetramine	4.89	2.46
1,8-Octanediamine	5.89	2

Product characterization

The derivatives of NH_3, EDA, and diethylamine were characterized by HPLC, PMR, and GC–MS. The retention times of the primary standards, toluamide and N,N-diethyltoluamide and the products of the reaction with NH_3 and diethylamine were identical and no spurious peaks were detected, giving supportive but not conclusive evidence for the structure. The chromatograms for the EDA derivative showed no extraneous peaks. The mass spectra and PMR spectra for the primary standards and the reaction products for NH_3 and diethylamine were identical. The mass spectrum for the EDA derivatives did not show a parent peak but the fragmentation pattern was quite logical for the product 1,2-bis(*m*-toluoylamino)ethane. The PMR spectrum for this derivative indicates that it is disubstituted.

CONCLUSIONS

The analytical method developed during this research, a modification of the procedure given by Wellons and Carey[8] is a rapid, viable procedure for determining trace amounts of NH_3 and various amines and is being used to monitor these compounds in industrial processes and waste water. A major advantage of this procedure is that trace amounts of these materials can be rendered non-volatile in the field by reaction and extraction and can be quantitated chromatographically later. Another important feature of the method is that increased sensitivities can be obtained by reacting relatively large volumes of the sample and concentrating the product by evaporation.

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