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DETERMINATION OF TRACES OF AMINE SALTS IN WATER BY GAS CHROMATOGRAPHY

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SUMMARY

A method has been developed for the direct determination of alkyl amine hydrochlorides in aqueous solution. The method involves conversion of the hydrochloride salts to the free amines in the injection port of a gas chromatograph, followed by chromatographic separation and detection of the free amines. Standard response curves for each of a group of thirteen amine hydrochlorides have been determined. These curves cover a range from approximately 17 to 250 parts per million of the amine hydrochloride in aqueous solution. Primary, secondary and tertiary aliphatic amines, as well as isomeric amines in the series methyl through pentyl have been tested. A single column system used at three different temperatures provides for the independent determination of any or all of the thirteen amine hydrochlorides tested.

INTRODUCTION

Gas chromatography has become almost universally accepted as the analytical method of choice for the determination of mixtures containing volatile organic components. However, in the case of amines and amine mixtures, adequate application of this technique has not been consistently achieved. This fact is largely due to the relatively high reactivity and ease of hydrogen-bond formation with the amines. Many analysts, despite the normal desirability of working with an unmodified sample, have resorted to the formation of less reactive, less polar derivatives to accomplish the desired analyses. Acetates, fluoroacetates, other fluoro- and chloroacyl derivatives and trimethylsilyl derivatives are most commonly suggested[1–6].

Whenever chromatography of the unaltered amines has been attempted a number of difficulties have been encountered. These have involved apparent "loss" of sample during chromatography, badly skewed elution peaks, so-called "ghost peaks" and other evidence of the ready reactivity of these compounds[7–12].

In addition, the lighter aliphatic amines are sufficiently volatile as to present serious difficulties in sampling and likewise do not lend themselves to normal isolation and concentration procedures involving extraction and solvent evaporation. A signifi-

cant proportion of the reported literature concerning gas chromatography of amines is concerned only with qualitative aspects, and the sample quantities used are relatively so large that the problems suggested above are not recognized because of the attenuation or lack of sensitivity of the detection system used[11,13–17].

Where quantitative measurements have been reported, two factors have been common. One is the use of base-loaded columns, usually KOH, to reduce adsorption problems, and the second is the use of relatively concentrated samples. With one exception[12] samples containing no less than 1% of the amine component present in lowest proportion have been reported[7–10,18–22].

In processing biological materials for the isolation of amines, they are customarily obtained by distillation and subsequent trapping in HCl solution. Thus, they are available for analysis as the hydrochloride salts or aqueous solutions of the hydrochloride salts. The objective of the work reported in this paper was to provide an analytical method for such samples, using gas chromatography which avoided or eliminated the problems mentioned previously. This includes the use of the amine hydrochloride salts which permit concentration to dryness, if necessary, without loss due to volatility, the use of water as a solvent for the final solution to be analyzed, and direct conversion of the salts to free bases in the gas chromatograph so that no loss of sample occurs throughout the sample handling processes.

EXPERIMENTAL

A gas chromatograph (F&M Model 400) with an all-glass chromatographic system was used for this work. A 6 ft. × 3 mm I.D. glass column was used packed with 10% Amine 220 + 10% KOH on 80/100 mesh Chromosorb W. The column oven temperatures were 60°, 90°, and 110° isothermal, the detector and injection port temperatures, 250° and 200°, respectively. The gas flow rates of helium, hydrogen, and air were 60, 50, and 350 ml/min, respectively. The sample size was 2.6 μl. The chart speed was 1.5 in./min. All reagent amines or hydrochlorides used were Eastman White Label without further purification. When the reagent was available only as the free amine, standard solutions were prepared by the addition of 10 μl of pure liquid to 10 ml of 0.1 *N* HCl. Reagents available as hydrochlorides were weighed in 10 mg quantities and separately diluted to 10 ml with distilled water. Three standard mixtures were prepared from these solutions and are designated through the text of this paper as Groups I, II and III.

Group I contained methylamine, dimethylamine, trimethylamine, and ethylamine, all originally available as hydrochloride salts. The mixture was prepared using equal volumes of each solution. From this mixture two additional standard solutions were prepared by dilution to one-fifth and one-tenth the initial concentration.

Group II contained trimethylamine, ethylamine, diethylamine, triethylamine, *n*-propylamine, and isopropylamine. The latter two were prepared as hydrochlorides from the free amine. A standard solution of this mixture was prepared, again by taking equal volumes of each separate solution.

Group III contained *n*-propylamine, *n*-butylamine, isobutylamine, *sec.*-butylamine, *tert.*-butylamine, and *n*-pentylamine. The standard mixture was prepared using equal volumes of each solution. Hydrochloride salts of *n*-butylamine and *tert.*-

butylamine were available. Hydrochlorides of the balance of this group were prepared as described above.

Additional standard mixtures of Groups II and III were prepared by serial dilution of the described mixture to one-fifth and one-tenth the initial concentration as with Group I. Aliquots of 2.6 μl (2.0 μl in the barrel and 0.6 μl vaporized from the needle) of these solutions were injected directly into the chromatograph.

The particular instrument used for this study has a U-shaped glass column with an injection heater which wraps around the entry area of the column. Normally this portion of the column remains unpacked and serves as an expansion chamber for injected liquid samples. For the direct analysis of amine hydrochlorides, this area serves as a reaction chamber for the conversion of the salts to the free bases. Because of this, after the column has been packed in its normal fashion, the injection port area is packed with 20% KOH coated on Chromosorb 101. This packing provides sufficient surface for total contact with all of the sample and thus, immediate and total conversion of salts to the free bases. The temperature of this packed portion is maintained at 200–230°.

Preparation of the column and the packing in the injection port is extremely critical because of the nature of the samples used and the very small differences in retention time among members of the various groups. The column packing of 10% Amine 220 and 10% KOH on 80/100 acid-washed Chromosorb W is prepared by dissolving the two phases in methanol and coating on the Chromosorb by a slurry technique or by first coating the support with KOH, drying and repeating the process with the Amine 220. Care must be exercised in stirring or mixing this material while drying to avoid, in so far as possible, crushing of the diatomaceous earth particles. Nitrogen gas should be passed over the preparation during all operations to avoid adsorption and reaction with atmospheric CO_2. Silane-treated support materials should not be used because of their ready hydrolysis, particularly in the presence of base. At best the effects of the silane treatment will have been largely eliminated before the packing preparation is completed, while at worst, detectable silane compounds will be released from the column with each sample injection, giving signals which bear no direct relationship to the sample.

Packing of the injection port to provide both the surface and amount of base necessary to neutralize the hydrochloride salts and release the free amines is equally important. Packing the injection port with base-loaded pyrex wool, coating the interior wall of the injection port area of the column with KOH, packing with base-loaded stainless steel wool and with base-loaded Chromosorb W have all been tested in this investigation. Only the last two showed any promise. However, the best results have been obtained by packing this portion of the column with 20% KOH coated on Chromosorb 101. This is also prepared from a methanol slurry and must be purged with nitrogen during preparation to avoid reaction with CO_2. Columns once prepared in this fashion should be purged with inert gas and capped during storage.

It is important that the packing throughout the column be as uniform in density as possible and that the injection port area containing the base-loaded Chromosorb 101 be kept at a temperature not less than 200° nor more than approximately 230°. This packing should be close enough to the actual point of insertion of the sample that the syringe needle reaches some distance into it (1/4–1/2 in.). If large numbers of samples are to be analyzed, it will be necessary to replace the injection port packing periodically since it will become neutralized.

TABLE I

STANDARD AMINE MIXTURES

The amines are listed in order of elution; the compounds are present as hydrochloride salts.

Amine	*Weight percent in solution*		
	Standard mixture No. 1	*Standard mixture No. 2*	*Standard mixture No. 3*
Group I			
Trimethylamine	0.025	0.005	0.0025
Methylamine	0.025	0.005	0.0025
Dimethylamine	0.025	0.005	0.0025
Ethylamine	0.025	0.005	0.0025
Group II			
Trimethylamine	0.0167	0.00334	0.00167
Ethylamine	0.0167	0.00334	0.00167
Isopropylamine	0.0187	0.00374	0.00187
Diethylamine	0.0167	0.00334	0.00167
n-Propylamine	0.0194	0.00388	0.00194
Triethylamine	0.0167	0.00334	0.00167
Group III			
tert.-Butylamine	0.0167	0.00334	0.00167
n-Propylamine	0.0134	0.00388	0.00194
sec.-Butylamine	0.0179	0.00348	0.00179
Isobutylamine	0.0182	0.00364	0.00182
n-Butylamine	0.0167	0.00334	0.00167
n-Pentylamine	0.0178	0.00356	0.00178

RESULTS

Table I describes the standard mixtures used in this investigation. Table II lists the analytical data obtained from these mixtures. These data are used for the standard response curves of Fig. 1a–c. Typical chromatograms of each group are shown in Fig. 2a–c.

Each group analyzed contains one or more members of the group immediately following or preceding it in numerical sequence in order to be assured that in actual analysis of a sample no overlap between the components measured in a group would occur to confuse an analysis. While the three standard groups were analyzed at three different temperatures, any given sample might require only two different temperature conditions. Under normal circumstances, the analysis of the total mixture would be carried out using temperature programming; however, because of the extreme dilution of these samples, the high concentration of stationary phase used and the relative volatility of the stationary phase, signal due to column bleed would be overwhelming under such circumstances. In addition, the total analysis could be carried out at the lowest isothermal temperature indicated, but the components of Group III would then require excessive time for elution and significant band spreading would occur. This band spreading would result in apparent significant loss of sensitivity for the larger components analyzed. It is for this reason that differing operating conditions are used.

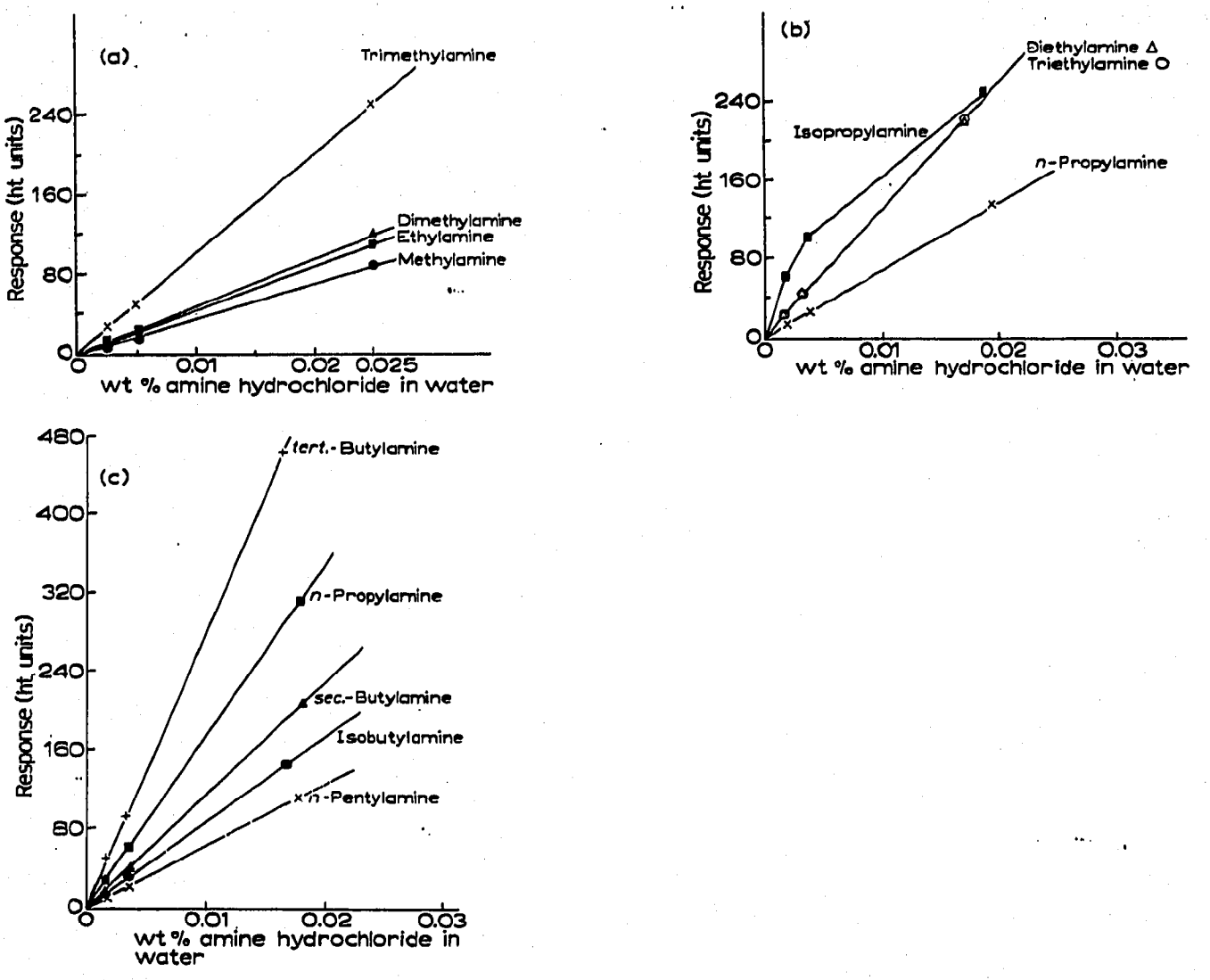

Fig. 1. Standard response curves of (a) amine group I, (b) amine group II, and (c) amine group III.

External standardization (*i.e.* comparison of the sample chromatogram to known standards chromatographed separately) was used in all cases. The use of internal standardization (the addition of a known component to the sample prior to analysis) was precluded in this case because of the complexity of the sample and the need to use different standards for the differing chromatographic conditions. Normally the internal standard method would provide somewhat better accuracy.

DISCUSSION

The analysis of mixtures of amines has always presented significant difficulty because of the high reactivity of the amine group and its dominance of the chemical behavior of the molecule. Further, most of the members of the group of amines under consideration here are significantly volatile when present as free bases at ordinary room temperature.

In theory, gas chromatographic processes should provide the best means for

TABLE II

ANALYSIS OF STANDARD AMINE MIXTURES

The amines are listed in order of elution; the compounds are present as hydrochloride salts.

Amine	*Retention time (min)*	*Peak height*[a]		
		Standard mixture No. 1	*Standard mixture No. 2*	*Standard mixture No. 3*
Group I	*Column temperature 60°*			
Trimethylamine	0.65	252	48	26.5
Methylamine	0.77	88	15	7.5
Dimethylamine	0.89	120	20	10.0
Ethylamine	1.17	108	20	10.0
Group II	*Column temperature 90°*			
Trimethylamine	0.5	432	82	41
Ethylamine	0.74	136	26	14
Isopropylamine	0.87	248	110	61
Diethylamine	1.20	232	44	22
n-Propylamine	1.29	136	24	14
Triethylamine	1.64	232	44	22
Group III	*Column temperature 110°*			
tert.-Butylamine	0.70	464	92	50
n-Propylamine	0.88	240	54	26
sec.-Butylamine	1.05	312	60	27
Isobutylamine	1.17	208	40	16
n-Butylamine	1.59	144	32	14
n-Pentylamine	2.96	112	22	12

[a] Average of triplicate analyses (arbitrary units corrected for sensitivity factors).

the analysis of such mixtures. Because of the reactivity of the amine group, the purity of the chromatographic process as partition only is seldom achieved; thus a combination of chromatographic processes occurs and efficiency suffers as a result. For example, the analysis of amine mixtures such as studied here, when present in 1 to 10% or greater quantities of each component, is relatively straightforward. This is because in the combination of partition and adsorption which occurs during chromatography the proportion of any component involved in adsorption is relatively small compared to the portion involved in partition. When the sample components decrease to 0.1% or significantly less, as in this investigation, the absolute amount of adsorption remains essentially the same as with the larger samples but the proportion of any component involved in adsorption becomes markedly greater with the result that elution bands are badly skewed and components which should separate do not show the expected degree of separation as a result of the "tailing" of one band into the leading portion of a succeeding elution band. Quantitative measurements are also subject to very serious errors under these conditions.

It is necessary to devise a system in which every effort is made to eliminate or minimize adsorption problems. For this reason we have used glass column construction material after having demonstrated that both copper and stainless steel result in serious loss of components during chromatography. We have also added significant quantities of base (KOH) to the column packing material intending that adsorptive sites be saturated by the more basic molecule in preference to the less basic amines.

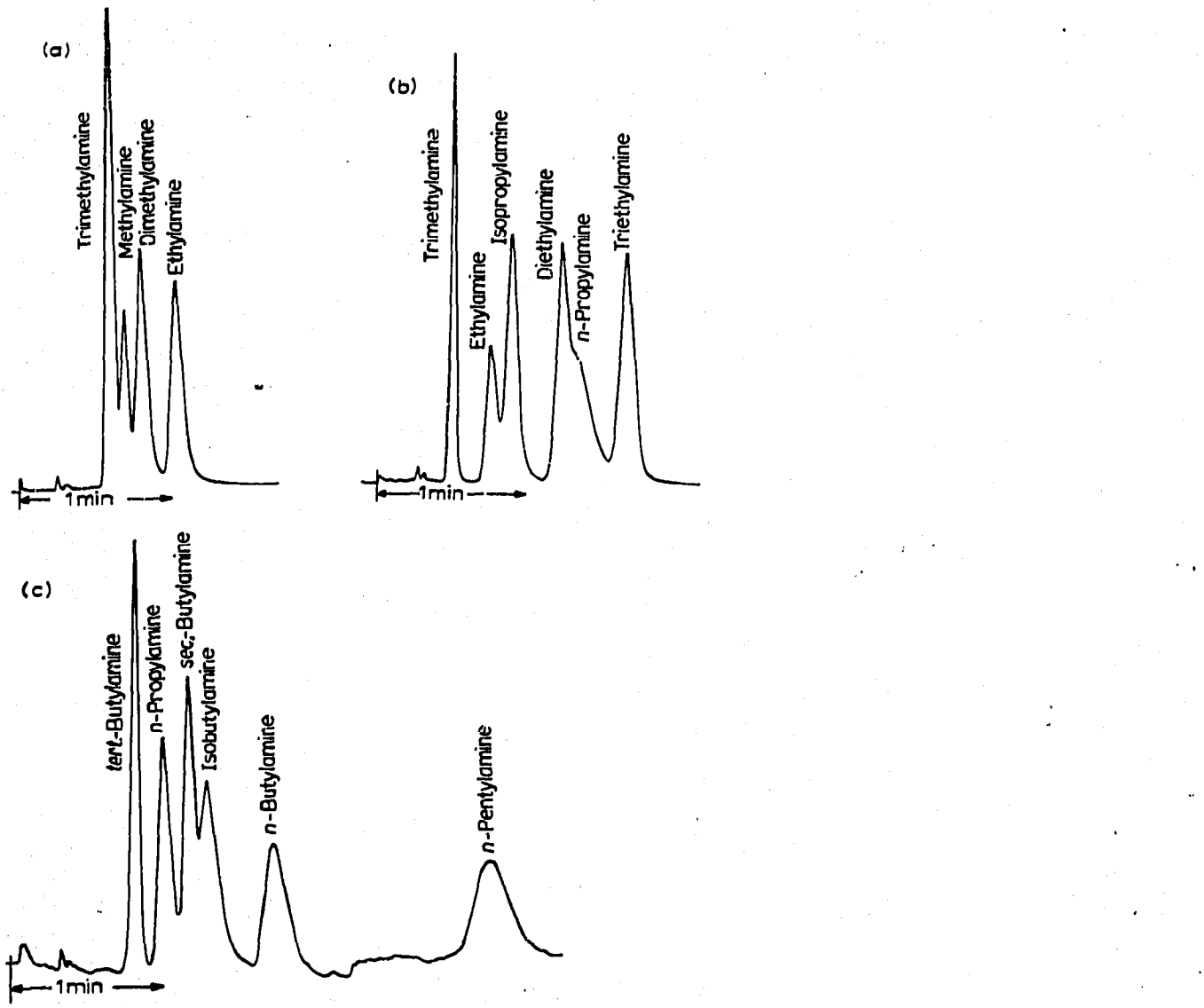

Fig. 2. Typical chromatograms of (a) amine group I (column temperature 60°), (b) amine group II (column temperature 90°), and (c) amine group III (column temperature 110°).

The linearity of the response curves obtained shows that success has been largely achieved.

The one significant deviation from this linearity is shown in the standard response curve for isopropylamine. The deviation of this curve from linearity is in a direction opposite to that expected if adsorption were a problem. The total cause for this deviation is not known at this time. However, information is available which indicates some of the factors which may bear on this problem. The first of these is the fact that water emerges from the column system used with the same retention time as isopropylamine. While water should not be detected by a flame ionization detector, it is apparent at these sensitivities a signal resulting from the injection of water occurs. This appears to be a reasonably reproducible magnitude of signal and the points plotted for isopropylamine have been corrected for signal due to water. The water signal results in a much greater error in the lowest concentration of iso-

propylamine than in the highest simply because the signal is further amplified at the low concentrations. In addition, we have noted that a number of the components in this particular mixture show a small peak with the same retention time as isopropylamine when they are chromatographed separately. Certainly a part of this signal is due to water or a compound released from the column on the injection of water. It is also possible that these additional peaks may be impurities in the other amines. It would be fortuitous if all these impurities were isopropylamine and therefore, unlikely. However, the fact that they add to the isopropylamine signal introduces an additional positive error, though this error should be proportional to the amount present and therefore does not account for the deviation from linearity shown in the response curve for isopropylamine.

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