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GAS CHROMATOGRAPHIC STUDIES ON BASIC NITROGENOUS DRUGS

I. ADDITION OF BASIC COMPOUNDS OR STEAM TO THE CARRIER GAS*

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SUMMARY

The addition of basic compounds or steam to the carrier gas has been found to facilitate the on-column release of the free bases from basic nitrogenous drugs injected as the salts. Three relatively volatile basic organic additives were evaluated, as well as ammonia gas and steam. The extent to which each of the five additives will overcome the adsorption effects associated with polar compounds in general has been assessed both qualitatively and quantitatively, with respect to five local anaesthetics. These methods of assessment are discussed in detail, and may be applied during the evaluation of other gas chromatographic systems.

INTRODUCTION

Various on-column techniques[2–12] have been employed as a means of overcoming the adsorption problems[1–14] which may arise during the gas chromatographic analysis of basic nitrogenous compounds in general. Such techniques have included the use of inert or highly silanised supports[2,8,13–15]; supports modified with basic material, often potassium hydroxide[2,5,9]; alkaline pre-columns[2,10]; or low loadings of stationary phase on glass microbeads[8,11,12]. These procedures have been reviewed in detail elsewhere[1,2,4].

The less volatile nitrogenous compounds present additional difficulties, which may be overcome by the preparation of volatile derivatives[1,16,17], although the need to use elevated injection zone temperatures is well recognised[1,4,7,11,13,14] whether or not derivatives are being examined.

* Presented[1] in part at a meeting of the Joint Pharmaceutical Analysis Group of the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry, Nottingham, June 1973.

The incorporation of some basic material into the system is of particular relevance to the analysis of basic nitrogenous drugs, which are normally present in pharmaceutical preparations as the stable salt of a mineral acid. The free base is liberated on-column by the alkali[10], thus avoiding the extraction of the base from alkaline solution into an organic solvent prior to analysis by gas chromatography.

The addition of formic acid vapour to the carrier gas in the analysis of acids[18,19] has become an established technique in gas chromatography, and similar approaches may be adopted with other classes of compounds. Some time ago, Knight[3] suggested the addition of *n*-hexylamine to the carrier gas in order to facilitate the analysis of basic nitrogenous compounds, but, although this technique has been subsequently applied, the full potential of the method does not appear to have been exploited. More recently, hydrazine hydrate in steam has been employed as the carrier gas in the separation of a range of aliphatic amines[20].

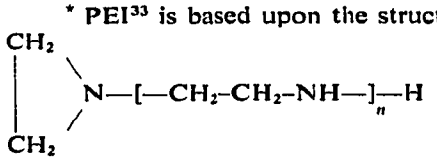
The four basic additives detailed in Table I have been evaluated in the present

TABLE I

BASIC ADDITIVES TO THE CARRIER GAS

Additive	*Designation*	*Supplier*
Ammonia gas (from ammonium carbonate)	—	Evans Medical (Speke, Liverpool, Great Britain)
Poly(ethylenimine) (50% aqueous solution: "Polymin P")	PEI*	BDH (Poole, Dorset, Great Britain)
Tetraethylenepentamine	TEPA**	Phase Separations (Queensferry, Flints., Great Britain)
Tetra(hydroxyethyl)ethylenediamine	THEED***	Phase Separations

* PEI[33] is based upon the structure shown, but it may also include side chains:



** TEPA[34]: $NH_2-CH_2-CH_2-NH-CH_2-CH_2$ $\diagdown$ NH $\diagup$ $NH_2-CH_2-CH_2-NH-CH_2-CH_2$

*** THEED[35]: $HO-CH_2-CH_2$ $\diagdown$ $N-CH_2-CH_2-N$ $\diagup$ CH_2-CH_2-OH; $\diagup$ $HO-CH_2-CH_2$, $\diagdown$ CH_2-CH_2-OH

study, both qualitatively from the effects upon peak shape (sharpness and symmetry), and quantitatively from the reproducibility of peak height ratios in replicate injections of the same solution. The effects of the additives were also assessed by calculation of the plate number and the height equivalent to a theoretical plate (HETP) for each test drug with each additive.

In the past, steam has been added to carrier gas in order to facilitate the gas

chromatographic analysis of a range of compounds[3,21] and hence it has been included in this study.

EXPERIMENTAL

Gas chromatography conditions

A Pye Series 104, Model 64 gas chromatograph (Pye Unicam, Cambridge, Great Britain) was used in conjunction with a 10-mV input Honeywell Class 19 potentiometric recorder (Honeywell, Brentford, Great Britain). The glass column, 1 m in length × 4 mm I.D. and packed with 2.5% w/w Silicone OV-1 on AW-DMCS Chromosorb G, 80–100 mesh (Perkin-Elmer, Beaconsfield, Great Britain), was maintained at 235°. The injection zone was at *ca.* 285°, and the flame ionisation detector at 265°. High-purity nitrogen was used as the carrier gas at a flow-rate of 50 ml/min, and the flame gases were hydrogen at 50 ml/min and air at 600 ml/min.

Introduction of additives into the carrier gas

The additives were introduced into the carrier gas line by means of the device illustrated in Fig. 1.

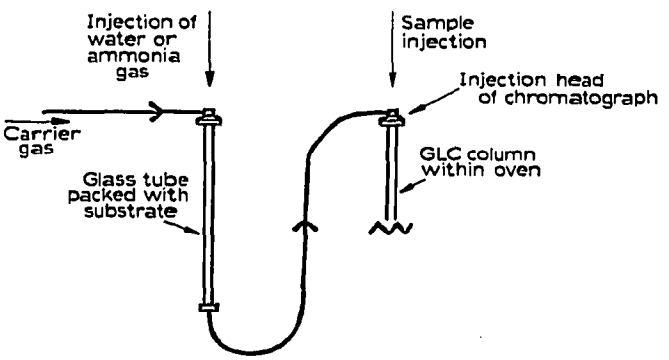


Fig. 1. Modifications to the carrier gas line to permit the introduction of additives.

Ammonia was incorporated by the gradual decomposition of ammonium carbonate, packed into the glass tube. The removal of carbon dioxide by soda lime, packed into the tube after the ammonium carbonate, did not cause any obvious improvement in peak symmetry or in the degree of tailing, and this system was not investigated further. As an alternative way of adding ammonia, *ca.* 500 μl of the headspace above 0.880 ammonia solution was injected through the preliminary assembly (Fig. 1) onto Celite (BDH, Poole, Dorset, Great Britain) packed into the glass tube. A gas-tight syringe (S.G.E. Ltd.) was used to inject the ammonia into the assembly immediately prior to the injection of the test solution through the normal injection head. Again this technique did not lead to any obvious improvement and so was not investigated further.

The three organic additives were coated onto Celite from methanolic solution by rotary evaporation at 100°. In each case, the resultant material was packed into

the glass tube, and the system conditioned under the operating parameters for at least 2 h before use.

When evaluating the four basic additives, the column effluent was checked with a test paper for alkalinity before the flame was ignited. The column was purged with pure carrier gas for several hours when changing additives. It was necessary to lag the glass tube during the evaluation of TEPA only, in order to protect it from the heat rising from the chromatograph; otherwise there was excessive bleed of this comparatively volatile compound[22] onto the column.

Steam was introduced into the carrier gas by injecting aliquots of water (100–500 μl) onto Celite, packed into the glass tube, until it was visibly wet. The carrier gas was allowed to become saturated, and a further quantity of water (*ca.* 500 μl) was added prior to the series of injections of the test solution. The introduction of these comparatively large volumes of water tended to extinguish the flame, which was re-ignited and allowed to stabilise before injection of the sample.

The test solution of the five local anaesthetics detailed in Table II was prepared by dissolving the salts in distilled water. This solution was stored in the dark at room temperature, conditions under which similar solutions had been found previously to remain stable for at least one month[4]. The free bases were extracted into ether from an aliquot of the aqueous solution rendered alkaline with sodium carbonate. This extract was stored under identical conditions.

The concentration of each drug in the test solution was such that "on-scale" peaks of appropriate size for measurement were obtained from each compound at one attenuation setting, which was normally 1×10^4 for the salts and 50×10^2 for the ether extract of the bases.

In order to ascertain that the column system had stabilised, the data arising from at least the first injection of the test solution of the salts were rejected. Five replicate injections (*ca.* 1 μl) of this solution were then made, together with one or more injections (*ca.* 4 μl) of the ethereal solution of the bases. In the absence of any additive, the drugs all showed an unacceptable degree of peak tailing; such a system was therefore not considered to be of any practical value.

ASSESSMENT OF DATA

Qualitative assessment

The symmetry of the peaks arising from the five injections of the test solution was assessed for each drug independently for each system, using the scale: +++, symmetrical peak —no tailing; ++, symmetrical peak —slight tailing; +, symmetrical peak —pronounced tailing; —, asymmetrical peak —excessive tailing.

Since the on-column liberation of the free bases from the salts is, in itself, a potential source of peak tailing, an injection of the bases was included for comparison. The symmetry of a peak arising from the injection of a salt was assessed against that from the corresponding free base for all the five test drugs, as follows: 0, no difference in peak symmetry between the salt and base; —, slight tailing evident from the salt as compared with the base.

Numerical assessment of peak symmetry

In addition to the qualitative methods, the peak symmetry was also assessed

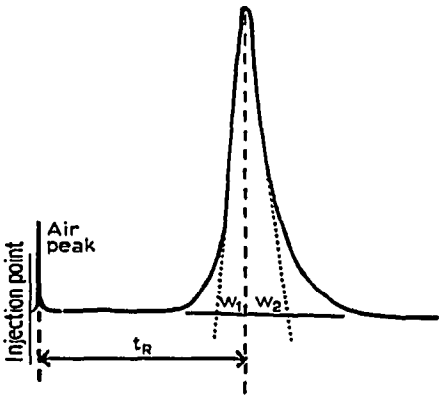


Fig. 2. Constructions used to measure numerical ratios, plate numbers, and HETP values.

numerically[23] by calculation of the ratio w_2/w_1 from the construction shown in Fig. 2. This ratio will be unity for a perfectly symmetrical peak, and will increase with the degree of asymmetry. It is recognised that this measurement does not necessarily take into account the full extent of peak tailing which may occur.

Quantitative assessment

The chromatogram illustrated in Fig. 3 is typical of all the systems, except for different degrees of peak symmetry and tailing. The height of each peak was measured

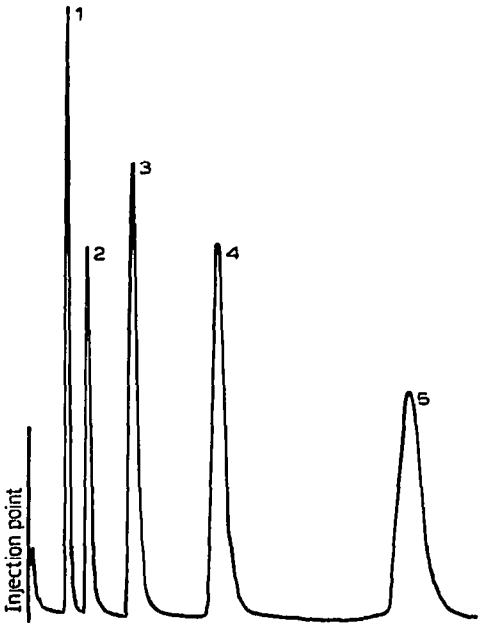


Fig. 3. Typical chromatogram obtained from the injection of five local anaesthetics as the salts (additive = PEI). 1 = Lignocaine hydrochloride monohydrate; 2 = procaine hydrochloride; 3 = amethocaine hydrochloride; 4 = butacaine sulphate; 5 = cinchocaine hydrochloride.

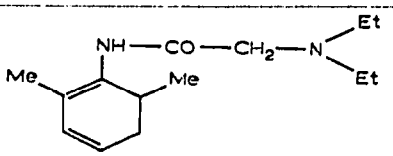
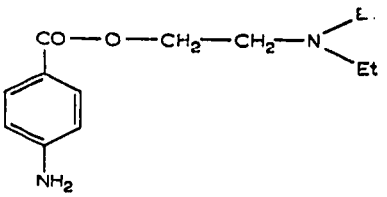
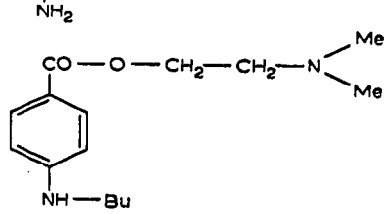
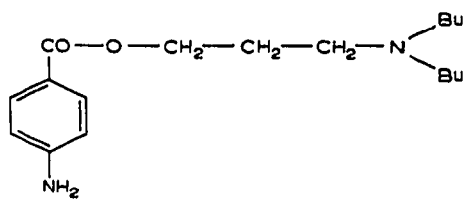
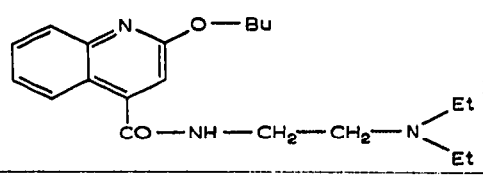
from the baseline across each chromatogram as a whole, and the ratio of the height of each peak to every other peak was calculated, giving the ratios:

L/P	L/A	L/B	L/C
	P/A	P/B	P/C
		A/B	A/C
			B/C

the designations L, P, A, B and C being as defined in Table II. Thus for each column system, the series of five injections of the test solution of the salts led to five values for each of the ten ratios. The standard deviation (σ) and hence the coefficient of

TABLE II

LOCAL ANAESTHETICS USED IN THE EVALUATION OF ADDITIVES TO THE CARRIER GA

Drug (and salt injected)	*Desig-nation*	*Structure*	*Free base**		*Concentration in the test solution (% w/v)*
			Mol. wt.	*M.p. (°C)*	
Lignocaine (hydrochloride monohydrate)	L		234.3	66–69	0.275
Procaine (hydrochloride)	P		236.3	61[37]	0.277
Amethocaine (hydrochloride)	A		264.4	41–46	0.444
Butacaine (sulphate)	B		306.4[38]	liquid[38]	0.696
Cinchocaine (hydrochloride)	C		343.5	63–66	0.873

* See the monographs on individual drugs in ref. 36.

variation (C_v) was calculated for each ratio, using an Olivetti Programma 101 desk computer as follows:

$$C_v = \pm \frac{100\,\sigma}{M}\ \%$$

where M is the mean peak height ratio.

A low value for C_v is indicative of good quantitative reproducibility between successive injections. This is of great importance when applying a routine assay procedure based upon the internal marker technique.

Calculation of plate number and HETP

One individual chromatogram, taken at random from the series of injections of salts with each additive, was used to calculate the plate number (n) and the HETP (H) for each drug[24]. The calculations are outlined below, and the measurements are based upon the constructions illustrated in Fig. 2. For non-Gaussian peaks

$$n = 16\left(\frac{V}{W}\right)^2$$

where V is the retention volume of the peak and W is the peak width across the baseline ($w_1 + w_2$ in Fig. 2).

Under constant operating conditions, t'_R (the retention time measured from the air peak) may be substituted for V, provided W is measured in the same units. Therefore

$$n = 16\left(\frac{t'_R}{W}\right)^2$$

n may be used to calculate the approximate HETP from the equation:

$$H = \frac{L}{n}$$

where L is the length of the column. The terms L, t'_R and W were measured in cm.

RESULTS AND DISCUSSION

The results from the qualitative assessments of the chromatograms are summarised in Tables III and IV. The differences in peak symmetry between the injections of salts and bases are only minor. The numerical assessments of peak symmetry (Table V) did not reveal any gross differences between ammonia, steam, PEI, or THEED, although TEPA gave high ratios for the four drugs eluted after lignocaine. Thus, by either of the criteria applied in the assessments of peak symmetry, TEPA cannot be regarded as satisfactory. The best overall peak symmetry was obtained by the addition of PEI or steam.

The data arising from the quantitative assessments are summarised in Table

TABLE III

ASSESSMENT OF PEAK SYMMETRY FROM INJECTIONS OF THE SALTS IN AQUEOUS SOLUTION

The scale used has been described in the text.

Additive	*Test drug*					*Overall rating**
	Lignocaine	*Procaine*	*Amethocaine*	*Butacaine*	*Cinchocaine*	
NH_3 gas	++	—	—	—	—	2—
PEI	++	++	+	+	++	8+
TEPA	++	+	+	+	—	4+
THEED	++	+	+	+	+	6+
Steam	++	++	++	+	+	8+

* Based upon the convention that 1+ cancels 1— to give zero.

TABLE IV

COMPARISON OF PEAK SYMMETRY BETWEEN INJECTIONS OF THE SALTS AND THE CORRESPONDING FREE BASES

The scale used has been described in the text.

Additive	*Test drug*				
	Lignocaine	*Procaine*	*Amethocaine*	*Butacaine*	*Cinchocaine*
NH_3 gas	0	0	0	0	—
PEI	0	0	0	0	0
TEPA	—	—	0	0	0
THEED	0	—	—	0	—
Steam	—	0	0	0	0

VI, and these show that the most reproducible results were obtained by the incorporation of PEI, THEED, or steam into the carrier gas. The values for plate number and HETP, given in Table V, follow the normal pattern in that the values for plate number increase with an increase in retention time, and the rate of increase tends to decrease with an increase in retention time until it levels out to an asymptotic value. When a series of "*t*" tests was applied between the various combinations of the five sets of data, no statistically significant differences were detected.

One criterion by which a "good" column may be assessed is that it should possess an HETP value for any given compound of 0.1 cm or less[24], and consequently none of the systems can be regarded as being satisfactory for lignocaine at this temperature. The best overall HETP values were obtained from steam.

It is evident from a broad view of the various methods of assessment that the best overall results were obtained following the inclusion of water vapour (*i.e.* steam) into the carrier gas, with good results from PEI and THEED. These results from steam were rather unexpected when dealing with basic nitrogenous compounds, and are not readily explained. The natural basicity of the support may, however, exert an enhanced effect under moist conditions, which may also assist the dissociation of the salts as in aqueous solution.

It is not clear why PEI should yield better results than either of the other two organic additives or ammonia, although the effect of hydrogen bonding has been dis-

TABLE V

SUMMARY OF RESULTS FROM THE NUMERICAL ASSESSMENTS OF PEAK SYMMETRY, PLATE NUMBER AND HETP

Designations as in Tables I and II.

Additive	*Peak*	t'_R *(cm)*	*Ratio* w_2/w_1	*Plate number*	*HETP (cm)*
NH_3 gas	L	1.10	1.09	366.0	0.27
	P	1.60	2.57	655.4	0.15
	A	2.82	2.08	795.2	0.13
	B	5.17	1.61	1188.0	0.08
	C	10.35	1.25	1252.1	0.08
PEI	L	1.02	1.50	416.2	0.24
	P	1.55	1.44	794.2	0.13
	A	2.75	1.69	987.8	0.10
	B	5.05	1.40	1133.4	0.09
	C	10.12	1.20	1306.3	0 08
TEPA	L	1.02	1.44	343.9	0.29
	P	1.57	5.40	385.1	0.26
	A	2.75	3.38	987.8	0.10
	B	4.97	2.00	1097.8	0.09
	C	9.92	1.62	1301.2	0.08
THEED	L	1.02	1.50	416.2	0.24
	P	1.57	2.57	631.0	0.16
	A	2.75	2.20	1181.6	0.08
	B	5.10	1.28	1280.9	0.08
	C	10.17	1.24	1319.2	0.08
Steam	L	1.02	1.50	416.2	0.24
	P	1.52	1.50	924.2	0.11
	A	2.70	2.20	1139.1	0.09
	B	4.92	1.60	1432.3	0.07
	C	9.92	1.28	1375.2	0.07

cussed in this context[25,26]. PEI is the least volatile[22] of the additives examined here. It has been employed in the gas chromatographic analysis of a range of aliphatic compounds[25,28,30,32].

The terminal hydroxyl groups on the THEED molecule may tend to react with active sites on the test drugs, giving rise to a certain degree of peak tailing. TEPA, by far the most volatile of the organic additives[22], gave results unsatisfactory by any of the assessments. The results from ammonia were disappointing, but are consistent with those previously encountered from inorganic compounds when used to modify the support. Experience has shown that such compounds (*e.g.* KOH) tend to cause an irreversible adsorption of less volatile drugs, or at least give rise to excessive peak tailing[27]. This is particularly true of drugs injected as the salts.

The three organic compounds which were evaluated as additives to the carrier gas are all relatively volatile[22], and have documented applications in gas chromatography[25,26,28–32]. Although they have been previously employed as stationary phases or as a means of modifying the support, at the oven temperature of 235° used in our

TABLE VI

STATISTICAL ASSESSMENT OF THE REPRODUCIBILITY OF PEAK HEIGHT RATIOS BETWEEN REPLICATE INJECTIONS OF THE TEST SOLUTION OF THE SALTS

Designations as in Tables I and II.

Peak ratio	*Coefficient of variation (± per cent of mean peak height ratio)*				
	NH_3	*PEI*	*TEPA*	*THEED*	*Steam*
L/P	7.0	1.9	16.7	2.7	3.5
L/A	5.7	2.9	11.9	1.9	4.0
L/B	7.8	3.8	11.4	2.5	4.7
L/C	8.6	4.3	11.7	3.5	5.6
P/A	4.4	1.3	5.2	1.9	0.9
P/B	5.2	2.2	8.9	4.1	2.1
P/C	6.5	2.7	9.9	5.5	3.5
A/B	2.5	1.8	4.5	2.8	1.7
A/C	3.2	1.9	5.6	4.1	3.4
B/C	1.7	0.8	1.6	1.8	1.8
Mean	5.3	2.4	8.7	3.1	3.1

present study they would be expected to pass through the column in the vapour phase[22]. Their role in the gas–liquid partition process may be complex, although a comparison of retention times for any given drug between the five additives reveals only minor differences, suggesting that their effect upon the partition is minimal. This hypothesis is supported by the fact that the differences in retention times with and without each additive are also minor.

The important function of the basic additives is probably twofold, (1) to liberate the bases from the salts on-column, and (2) to overcome the adsorption effects by being, themselves, preferentially adsorbed upon any active sites in the support. Any such sites which are acidic in nature will be neutralised by the base, and the amount of base present in the carrier gas needs to exceed that required for their neutralisation. That this was so was confirmed by the alkaline pH of the column effluent. It has been previously noted[9] that an excess of alkalinity is required in order to satisfactorily overcome adsorption effects.

CONCLUSIONS

In general, good correlation was found to exist between the various methods of assessment. The best results were obtained from steam or PEI, which enabled mixtures of a series of less volatile basic nitrogenous drugs to be analysed successfully when injected as their salts.

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