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A TECHNIQUE FOR THE DETECTION OF NITROGEN-CONTAINING COMPOUNDS IN GAS CHROMATOGRAPHIC ELUATES BY MEANS OF HYDROGENOLYSIS AND COLOUR REACTIONS

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SUMMARY

A simple technique is described whereby compounds containing nitrogen on being eluted from a gas chromatographic column are detected by means of colour reactions. As they emerge from the column, the compounds are cleaved by hydrogenolysis in a small reactor containing a platinum catalyst to produce ammonia, which is subsequently detected either by the indicator phenolphthalein in a glass porous layer open tubular (PLOT) capillary, or by means of the ninhydrin reagent coated on a PLOT capillary containing activated alumina. The limit of detection for a representative number of nitrogen compounds was 0.2–0.4 μg, and in favourable cases 0.1 μg.

INTRODUCTION

The specific detection of compounds containing heteroatoms such as nitrogen and sulphur in gas chromatographic (GC) eluates is an important and now well established procudure for obtaining information on unknown components of complex mixtures. A number of element-specific detectors are currently available, such as the flame photometric detector (FPD) for sulphur and phosphorus, the electron capture detector (ECD) for halogen-containing compounds, and the alkali-sensitised flame detector (AFD) for nitrogen compounds.

Other specific detectors are based on post-column combustion or hydrogenolysis of organic compounds to produce small fragments, such as SO_2, NO_2, NH_3, HCl, etc., which are then detected coulometrically or in an electrolytic conductivity cell. While such methods are in general highly effective, they necessarily involve the purchase of expensive instrumentation additional to the basic chromatograph. For many workers the need to look for the presence of a specific heteroatom such as nitrogen may only arise infrequently. A simple and inexpensive method which could

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be adapted for use with a standard chromatograph equipped with a flame ionisation detector (FID) would be of considerable use.

We have recently described the detection of functional groups in organic compounds eluted from GC columns at the submicrogram level by trapping the components on short lengths of glass porous layer open tubular (PLOT) capillaries containing activated alumina, followed by colour reactions on the trapped bands[1]. In the present paper, a method is presented in which nitrogen-containing compounds eluted from a GC column are split between an FID and a small hydrogenolysis reactor packed with platinum catalyst, where they are cleaved to produce ammonia. The latter is then detected by either of the following methods: (a) In the 'off-line' mode, the ammonia from a suspected nitrogen-containing compound is trapped as a sharp band from the reactor on a short length of a PLOT capillary, packed with activated alumina and coated with the ninhydrin reagent. The blue-violet coloration produced by ammonia is subsequently developed by heating the trap. (b) In the 'on-line' mode the ammonia is swept onto a PLOT trap containing Celite 545, moistened with the indicator phenolphthalein, to produce a red coloration which quickly fades as the ammonia is swept from the tube, thereby allowing a response to be observed for a subsequent nitrogen-containing peak. A limit of detection of 0.1–0.5 μg has been obtained for a representative range of nitrogen compounds by both methods.

EXPERIMENTAL

Preparation of platinum catalyst

Pyrex glass, ground to 100–150 B.S. mesh, was allowed to stand in saturated KOH solution overnight. It was then washed to neutrality with distilled water and dried at 100°. Chloroplatinic acid (0.05 g), dissolved in a little ethanol, was added to the glass support (1 g) to give a loading of 2% w/w Pt on the glass. The mixture was stirred vigorously on a water-bath to ensure an even coating of the acid on the support. When dry, the material was reduced to the metal and activated by heating in a stream of hydrogen for successive 30-min periods at 100°, 150°, 200°, and 250°. The activated catalyst (500 mg) was then packed into a stainless-steel tube (12.5 cm × 3.1 mm O.D.) into one end of which was silver-soldered a short length of stainless-steel capillary tube (15 mm × 0.25 mm I.D.). This end (the gas exit end) also contained a plug of glass wool, presaturated with lithium hydroxide, to remove any acid gases which might be evolved, *e.g.* during the hydrogenolysis of unsuspected halogen-containing compounds in unknown mixtures.

The tube containing the catalyst was heated by a small furnace constructed in a similar manner to one recently described[2], and was mounted in place of the normal fraction collector heating block in the side of a Pye Series 104 temperature-programmed flame ionisation gas chromatograph (Pye Unicam, Cambridge, Great Britain). The column effluent was split between the reactor and the FID in the ratio of about 9:1, and a make-up flow of humidified hydrogen was introduced to reduce the residence time of the ammonia produced by the hydrogenolysis of nitrogen compounds in the reactor. Details of the system are shown in Fig. 1.

Samples were analysed using a 23 m × 0.5 mm glass PLOT column coated with a 0.8% w/v solution of Carbowax 20M in methylene chloride, except for short-chain aliphatic amines, which were analysed on a similar PLOT column, coated first

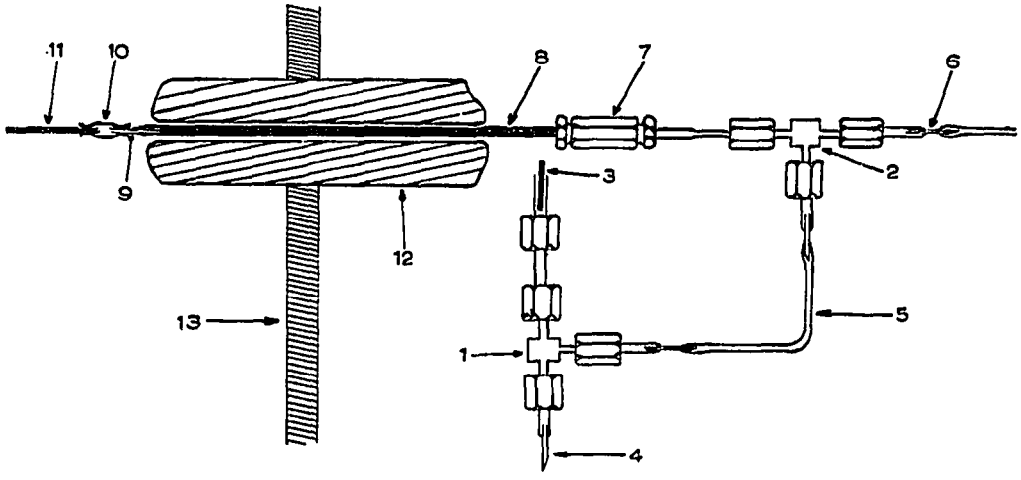

Fig. 1. Schematic representation of a hydrogenolysis reactor system for the detection of nitrogen-containing compounds. 1 and 2 = 1.55 mm O.D. Simplifix three-way connectors (Simplifix, Maidenhead, Berks., Great Britain), providing column effluent split and auxiliary hydrogen purge gas, respectively; 3 = capillary flow restrictor to FID; 4 = 21-gauge hypodermic needle connection to PLOT column; 5 = thin-wall PTFE tubing (0.75 mm I.D.) connected via hypodermic needles; 6 = hypodermic connection to humidified hydrogen purge gas; 7 = 3.1 mm O.D. stainless-steel compression coupling; 8 = 12.5 cm × 3.1 mm O.D. stainless-steel reactor packed with platinum catalyst; 9 = 15 mm × 1.55 mm O.D. stainless-steel capillary tube silver-soldered to reactor tube; 10 = silicone rubber (1 mm I.D.) connecting sleeve; 11 = PLOT capillary coated with test reagent; 12 = reactor furnace; 13 = oven wall of chromatograph.

with a 2% w/v solution of KOH in absolute ethanol, followed by a 2% w/v solution of Carbowax 1540 in methylene chloride. Hydrogen was used as the carrier gas at flow-rates of 3–4 ml/min, while the make-up hydrogen flow to the reactor was at the optimum, 24 ml/min. The preparation of the glass PLOT columns, using Celite 545 and B-37 Pyrex glass as a binding agent, has been described elsewhere[3]. Short lengths (10 mm) of the same tubing, possessing minimal pressure drops were used for the 'on-line' test for nitrogen compounds, while short lengths of PLOT capillaries containing activated alumina, the preparation of which has been described previously[1], were used for the 'off-line' test for ammonia.

Freshly prepared catalyst contains some acidic sites, and required an initial treatment to remove them before it could be used for analysis of nitrogen compounds. Three or four 0.5-μl injections of a 1% solution of aqueous ammonia onto the catalyst produced the desired effect, and no further treatment was then required during the working life of the catalyst. The catalyst was normally operated at a temperature of 250°.

Tests for nitrogen compounds

Off-line (ninhydrin) test. A straight length (usually about 1 m, but depending mainly on the number of traps expected to be required for a given set of experiments) of a PLOT capillary containing activated alumina was coated with a freshly prepared solution of ninhydrin (1% w/v in Analar acetone) by flushing the solution through the tubing from a hypodermic syringe, and drying in a stream of nitrogen.

When the leading edge of a peak to be examined just appeared on the base line, a short length (3–4 cm) of the capillary was connected to the reactor outlet. After trapping over the complete area of the peak, the trap was removed and placed in a labelled position in a holder. The procedure was repeated for other peaks in the chromatogram, and a number of blanks were taken in a similar manner over regions of the chromatogram where no peaks were observed. On completion of the run, the PLOT traps were developed by heating at 110° for 3 min, and examined in relation to the appropriate peaks in the GC trace. A positive test for nitrogen was indicated by a blue-violet coloration over the first few millimetres of a trap, the colour intensity being related to the quantity of ammonia evolved.

On-line (phenolphthalein) test. A length (5–6 cm) of Celite PLOT capillary was moistened by dipping it in a solution of phenolphthalein (0.5% w/v in 50% aqueous ethanol). The tube was connected to the reactor outlet and, upon elution of a nitrogen compound, the indicator changed rapidly from colourless through pink to a sharp bright red, and, as the ammonia ceased to be evolved after passage of the peak, faded through pink back to colourless. The fading resulted from the ammonia's being carried away by the continual purge of hydrogen gas. If the first nitrogen peak was followed soon after (1–2 min) by a second nitrogen compound, the colour change was observed again, but this change occurred slightly further along the tube than the first, since with time the tubing dries out. Dried out tubing no longer responds to ammonia, and it becomes necessary either to redip it in the indicator or to replace it with a new piece. In general, during the course of a chromatographic run, it was found necessary not to leave the tube connected to the reactor for more than 5 min without remoistening it.

RESULTS AND DISCUSSION

The limit of detection for a range of representative nitrogen compounds using both tests, which are given in Table I, was generally 0.2–0.4 μg, and, in favourable cases, as low as 0.1 μg. The dynamic nature of the phenolphthalein test meant that sensitivity depended on the amount of ammonia eluted per second, and peak sharpness was an important factor in determining the detection limit. In fact, provided the GC peaks were sharp, two nitrogen compounds following one another, even when not completely separated, could be easily detected, as long as the first one was not present in very large excess; this was the principal advantage of the test. The sensitivity of the test was also dependent on the pH of the tubing, being most sensitive when the indicator was held slightly below its end point, *i.e.*, just below pH 8.3. PLOT capillaries prepared using acid- and base-washed Celite 545 had a consistent pH of 7–7.5 and were most suitable for this indicator. Other acid–base indicators were examined and proved unsatisfactory for various reasons, but principally because the observed changes were from one colour to another and so were not as easy to observe as the colourless-to-red change for phenolphthalein.

The principal advantage of the ninhydrin test was that it provided a semipermanent record for each nitrogen compound, which could also be made semiquantitative by chromatographing and collecting in the manner described known quantities of a chosen nitrogen compound, and then comparing the intensity of the coloured bands with those of unknown peaks.

TABLE I

LIMIT OF DETECTION OF NITROGEN COMPOUNDS USING COLOUR TESTS

Compound	*Minimum detectable amount (μg)**	
	Phenolphthalein test	*Ninhydrin test*
Pyridine	0.2	0.2
2,6-Lutidine	0.25	—
Aniline	0.1	0.1
Nitrobenzene	0.1	—
Allyl nitrile	0.2	0.2
2,6-Dimethylpyrazine	0.2	—
2-Methoxy-3-isopropylpyrazine	0.2	0.25
Pyrrole	0.1	0.15
Pyrrole-2-carboxaldehyde	0.2	—
Pyrrolidine	0.4	—
Allylamine, Diethylamine, Butylamine, Triethylamine	0.4	0.3
Ethyl isothiocyanate	0.25	—
2-Butyl isothiocyanate, Allyl isothiocyanate	0.2	—
Acrylonitrile	0.1	—

* Limit determined by injecting 0.5-μl quantities of the appropriate concentrations of the compounds in ether.

A catalyst, which would effect total and rapid hydrogenolysis of the eluants followed by rapid elution of the evolved ammonia to produce a colour response coincident with the FID signal, was a prime requirement for the present work. Several batches of palladium and platinum catalysts on both neutral and basic diatomaceous earth supports were evaluated, but none approached the effectiveness of the platinum-on-glass catalyst described above in respect of these criteria. In the absence of sulphur compounds, the catalyst may be expected to perform with unimpaired efficiency for prolonged periods. However, in the presence of even small quantities (20 μg or less) of sulphur compounds, it was quickly poisoned and lost its ability to denitrogenate pyridine, which was used as a test compound to periodically check catalyst performance. For samples suspected to contain sulphur, it was therefore necessary to use a reactor and furnace twice as long as the original one, the first 10 cm of which was packed with a 2% w/w 'basic' palladium catalyst on 100–150 mesh ground Pyrex glass, containing a slight excess (0.2% by wt.) of NaOH and prepared as described by Beroza and Sarmiento[4]. The palladium catalyst, which was separated from the platinum catalyst by a short length of an acid absorber (1 cm of glass wool previously saturated with LiOH or SrOH), effectively removed all sulphur compounds, forming H_2S, which was absorbed, and allowed nitrogen compounds to pass through unchanged to the platinum catalyst.

The combined system was tested with synthetic mixtures containing nitrogen and sulphur compounds, as well as with horse-radish oil which contains isothiocyanates as major constituents, and was found to be totally effective for the detection of nitrogen compounds.

It was not possible to extend the above techniques directly to the detection of sulphur compounds in GC eluates by hydrogenolysis and detection of the resulting H_2S on PLOT traps, *e.g.*, by the sensitive and specific lead acetate test. A variety of platinum and palladium catalysts operated between 200–300° provided 'hold-up' times for H_2S in the reactor which were far in excess of the elution time of the corresponding GC peak. Such broad signals were of little use for pin-pointing sulphur compounds. The literature indicates that nickel wire or nickel-on-quartz wool operated at 850–1000° would be necessary to overcome this catalyst problem, and the present apparatus could not tolerate such high temperatures.

A further use of the techniques described in the present paper is envisaged in the area of 'carbon-skeleton' chromatography, in which the hydrocarbon fragments produced by hydrogenolysis of small amounts of unknown peaks eluted from complex mixtures may be trapped from the reactor in lengths of cooled PLOT capillaries, which are then sealed off. The fragments may then be transferred to another GC column for identification by retention times, with or without mass spectrometry, as recently described for products of hydrogenation and fragments produced by ozonolysis[2].

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