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A TECHNIQUE FOR THE HYDROGENATION OR HYDROGENOLYSIS OF SUB-MICROGRAM AMOUNTS OF MATERIAL COLLECTED FROM A GAS CHROMATOGRAPH

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SUMMARY

A short stainless-steel tube containing segments of gas chromatographic column packing and catalyst forms a trap-reactor. Gas chromatographic fractions may be collected in the trap-reactor, stored until required, reacted with hydrogen at elevated temperatures and re-injected from it into the gas chromatograph. The technique is applicable to both hydrogenation and hydrogenolysis in the microgram and sub-microgram range.

INTRODUCTION

The examination of complex mixtures such as food flavour volatiles is commonly made by combined gas chromatography–mass spectrometry (GC–MS) using capillary columns. For fully resolved components, the mass spectrum and the retention data can usually provide sufficient evidence for their positive identification, but in doubtful cases and certainly with unknown compounds, additional evidence of structure is essential. With the limited amounts of material recoverable from single peaks on capillary columns and especially with trace components, the effective use of other micro-spectrometric techniques (UV, IR, NMR) is rarely feasible. Chemical micro-techniques are available and perhaps the most useful of these is the catalytic reaction with hydrogen to give hydrogenation or hydrogenolysis products, these being examined by GC or preferably by GC–MS.

The simplest means of hydrogenating material is by placing a catalytic reactor between the injection point and the gas chromatographic column as described by BEROZA AND SARMIENTO[1], MOUNTS AND DUTTON[2] and KOMAN[3]. This technique, however, is applicable only to pure compounds or very simple mixtures. TEETER *et al.*[4] and ISSENBERG *et al.*[5] placed the reactor between the column and the mass spectrometer inlet in GC–MS combinations. This makes hydrogenation applicable to complex mixtures which show "hydrogenated" peaks at the same retention times as the original material but giving the mass spectra of the hydrogenated product. A serious limitation of this approach is that if hydrogenation is incomplete, the interpretation of

superimposed spectra of product and unchanged material presents difficulties. With the variation in reactivity of unsaturated compounds occurring in a flavour mixture, for example, the realization of a single set of conditions to fully hydrogenate all compounds could be impossible.

In examining flavour volatiles in this laboratory, we have, for the above reasons, avoided "on-line" hydrogenation and preferred to collect resolved components from the stream splitter at a GC–MS interface[6] concurrent with the recording of their mass spectra. These compounds are reacted with hydrogen and are subsequently re-examined by GC–MS. Some excellent reactor systems have been described for hydrogenation externally or "off-line" to the gas chromatograph[7–10]. Our requirement, however, was for a simple technique by which fractions could be trapped, reacted with an external source of hydrogen, and re-injected into the same GC–MS combination. The ability to store material either before or after reaction is essential to permit one operator to handle the entire process, and to allow time for the gas chromatograph to be cleared of the previous sample.

EXPERIMENTAL

Apparatus

The trap-reactor (A) (Fig. 1) is a thin-walled stainless-steel tube (82 mm × 3.175 mm O.D.) cut to a 60° face at one end and threaded internally for 6 mm at the other. The tube is packed in three sections, the centre section consisting of the catalyst bed (*e.g.* Pd on Chromosorb W[1]) and the outer sections of GC packing such as silanized Chromosorb A (20–30 mesh) coated with 10% silicone SF 96-100. The packed sections are separated and retained by 4-mm plugs of rolled-up fine stainless-steel mesh (B). A regulated hydrogen supply (not shown) is delivered by a flexible capillary line and attached to the trap-reactor by a threaded adaptor (C). Other items

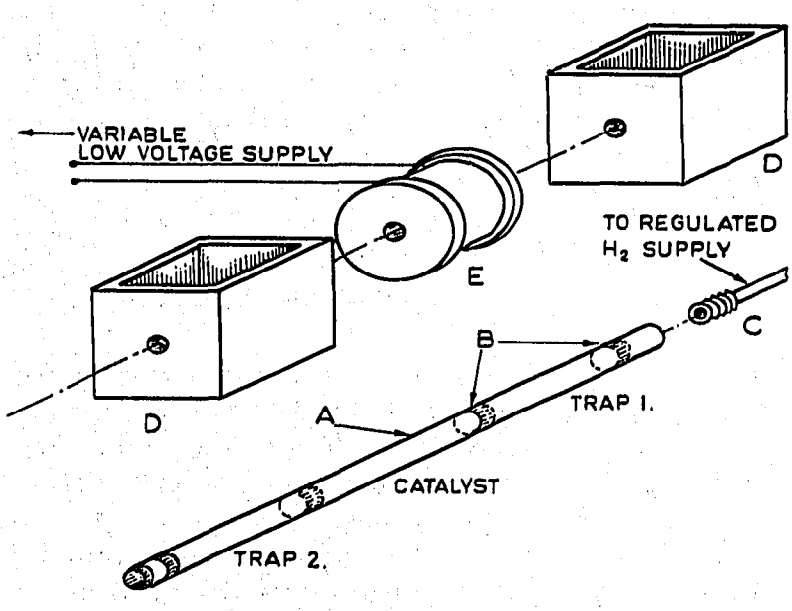

Fig. 1. (A) Stainless-steel trap-reactor tube, (B) stainless-steel gauze plugs, (C) hydrogen supply line and threaded connector, (D) aluminum boats for solid CO_2, (E) electric heater.

include two aluminum boats (D) for solid CO_2, a low voltage electric heater (E) and a heated brass block (F) (Fig. 3) which is grooved and spring loaded to clip onto the trap-reactor.

Operation

The trap-reactor is conditioned for use by heating to 180° in a stream of hydrogen until the background due to contamination is no longer significant, this conditioning period being determined by a blank run. Material to be hydrogenated

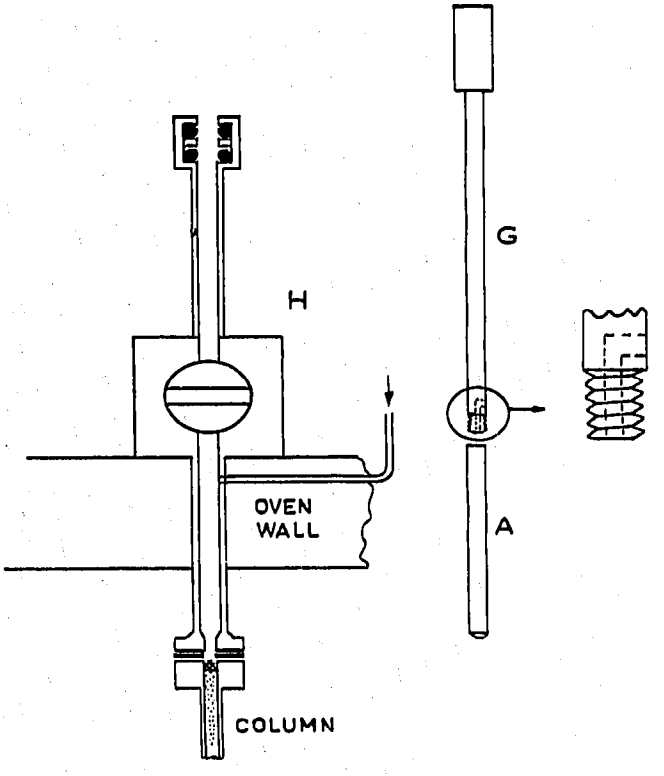

Fig. 2. (A) Trap-reactor, (G) threaded holder, (H) ball-valve introducer.

is collected in trap 1 (threaded end) by enclosing it in a boat of solid CO_2 and attaching the trap-reactor to the exit port of the GC–MS interface. After collection the ends of the trap-reactor are sealed with PTFE caps and the material may then be stored under solid CO_2 while subsequent collections are made in additional trap-reactors.

With the cooling boat still on trap 1, the electric heater (E) is slipped over the catalyst section and the other solid CO_2 boat over trap 2. The hydrogen supply, preset to about 5 ml/min, is connected. A voltage to the heater is selected to give the appropriate catalyst temperature, voltage and temperature having previously been calibrated against one another using a dummy reactor tube with a thermocouple inserted. When the temperature is steady (4 min for 150°) the boat on trap 1 is slid off along the hydrogen supply line and the metal block (F), preheated to 150°, is clipped on the tube in its place. The trapped material is liberated and swept over the catalyst bed by the hydrogen stream, the reaction products being condensed in

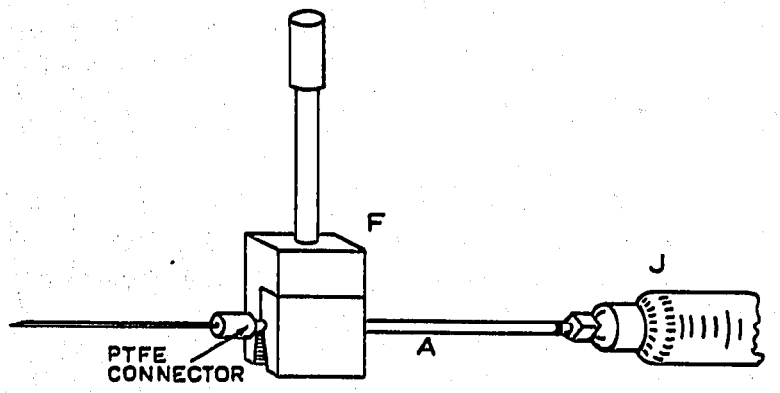

Fig. 3. (A) Trap-reactor, (J) 2-ml syringe, (F) heated brass block.

trap 2 cooled in solid CO_2. The hydrogenation is carried out for 2 min. If necessary, the reaction products may be stored in the trap-reactor at the temperature of solid CO_2.

The injection of the reaction products into the gas chromatograph is most conveniently made by a ball-valve type of introducer (Fig. 2). The trap-reactor is simply attached to the threaded holder (G), inserted through the O-rings and ball-valve (H) until it seals against the 60° face just above the column. The preheated carrier gas now passes through the hole in the holder and the trap-reactor, thus sweeping the reaction products into the column.

Injection through a septum is made by attaching a 2-ml syringe (J) (Fig. 3) filled with carrier gas to the threaded end of the trap-reactor and a needle to the other. After insertion through the septum, the hot metal block (F) is applied to trap 2 and the sample is injected with the 2 ml of carrier gas. Although somewhat cumbersome, this technique gives a satisfactory injection.

TABLE I

REACTION CONDITIONS AND PRODUCTS

Catalysts: (1) 5% Pt/$CaCO_3$ (Light & Co.) mixed with 44–52 mesh Chromosorb G to 2% Pt. (2) 2% Pt on 44–52 mesh Chromosorb G. (3) 1% Pd on Chromosorb W, trap 0.5%, SF 96-100 on silanized glass beads.

Compounds	*Amount (μg)*	*Catalyst*		*Products*
		No.	*Temp. (°C)*	
cis-Hex-3-enol	10	1	125	Hexane + pentane
cis-Hex-2-enol	2	2	125	Hexanol
Hexanol	2	2	125	Hexanol
Octanol	1	1	125	Octane + heptane, 3:1
Nonanol		3	150	Octane + nonane (trace)
Nona-2,6-dienol	1	3	150	Octane + nonane, 8:1
Octanal		1	125	Heptane + octane, 4:1
Octa-2,4-dienal	5	1	125	Heptane + octane, 4:1
Nonanal		1	125	Octane + nonane, 6:1
cis-Non-2-enal	0.1	3	150	Octane + nonane, 6:1
trans-Non-2-enal	0.1	3	150	Octane + nonane, 6:1
Geranial	0.1	3	150	2,6-Dimethylheptane
	0.01	3	150	2,6-Dimethylheptane

RESULTS AND DISCUSSION

Some preliminary results with the above technique using pure saturated and unsaturated alcohols and aldehydes are summarised in Table I. It is evident that with two of the catalysts, hydrogenolysis has occurred with considerable ease and at much lower temperatures than those reported by BEROZA AND SARMIENTO[1,11] who obtained no hydrogenolysis of decanal at 150°. With a similarly prepared Pd catalyst (No. 3) we have, by comparison, observed extents of reaction ranging from 14% at 70° to 100% at 175°. These discrepancies are probably due to differences in catalytic activity, although other factors such as the sample–catalyst ratio and reaction time could be significant. It is noteworthy that with the third catalyst (No. 2) at 125°, no hydrogenolysis of hexanol took place whereas *cis*-hex-2-enol was cleanly hydrogenated to hexanol.

With the present design of the trap-reactor, the catalyst temperature is limited to about 200°, above which a background on the gas chromatograph becomes troublesome when examining the hydrogenolysis products of sub-microgram samples. This background is apparently derived from the heating of the ends of the packed traps adjacent to the catalyst bed resulting in a bleed of phase which is decomposed on the hot catalyst. Separation of the packed sections by a gap of 10 mm reduced this background, which suggests that much higher catalyst temperatures could be tolerated if heat conduction to the traps could be eliminated. The operation at higher temperatures could be further improved by the use in the traps of liquid phases of higher stability, *e.g.* silicone OV-101.

The main feature of the present technique is the low handling losses. Octane (1 μg), for example, was "hydrogenated" and recovered without loss when injected on the gas chromatograph. Hexane was easily recoverable as a product. However, losses with butane were high unless liquid nitrogen was used as a trap coolant, but even under these conditions propane and ethane were not fully recoverable as hydrogenolysis products. The smallest size of sample to give a significant result was 0.01 μg of geranial, the hydrogenolysis product, 2,6-dimethylheptane, being identified by its mass spectrum and retention time. Samples of 0.1 μg were easily handled. However, for samples of this size the precaution should be taken to check the cleanliness of the trap-reactor beforehand in a "blank" run.

CONCLUSION

The present technique is well suited to the examination of food flavour constituents at the sub-microgram level, especially when the products can be examined by GC–MS. The results to date suggest that by selection of catalysts for hydrogenolysis and hydrogenation the reaction can be conducted at relatively low temperatures, a decided advantage with heat-labile substances.

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