COLLECTION OF GAS-CHROMATOGRAPHIC EFFLUENTS FOR INFRARED SPECTRAL ANALYSIS

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INTRODUCTION

In recent years, high-temperature gas–liquid chromatography has become the method of choice in the analysis of high-boiling liquids, particularly in the field of lipid chemistry. This is to be expected, since the method is rapid, reproducible, and nearly quantitative. However, the results obtained must be interpreted with caution. Particularly in the case of unknown mixtures, the analyst must have some means of qualitatively identifying the components he has separated and quantified. In many cases the amount of the pure components available is so small that classical methods of analysis are impractical, but valuable information may be obtained from an infrared spectrum.

For this purpose, the vaporized sample in the effluent from the chromatographic column is generally condensed in a chilled glass tube and later transferred to a suitable cell. The methods presently in common use for non-volatile liquids include smearing the material on a salt plate (AgCl, KBr, or NaCl), or transferring it with solvent to a standard micro-cell[1–4]. A technique has recently been developed which eliminates the necessity of transferring the sample, by condensing it on the same medium that is used to support the sample in the infrared spectrophotometer. For this purpose, a material is required which can efficiently remove the sample from the exit stream of a gas-chromatographic column without interrupting the flow of gas; furthermore, the material must be somewhat transparent in the infrared region of the spectrum.

To meet these requirements, what is needed is essentially a filter or thin, porous membrane. SLOANE has previously shown that specially made, thin (25 μ) cellulosic membranes, of the type manufactured by Millipore, can be used in a differential technique for infrared analysis[5]. While the cellulose esters, of which the filter is composed, have four strong absorption bands in the infrared (see Fig. 1a), these can be effectively nullified in a double-beam instrument (Fig. 1b), allowing the material to be used as a sample support. This fact, combined with the high porosity and uniformity of the Millipore® membrane, makes it ideally suited to the purpose. Tests on this membrane indicate that the fine, uniform pores (0.45 $\pm$ 0.02 μ in diameter) constitute up to 70% of the membrane volume. Thus it is fine enough to trap effectively small droplets, and porous enough to permit a very high flow rate per unit area. This is supported by the

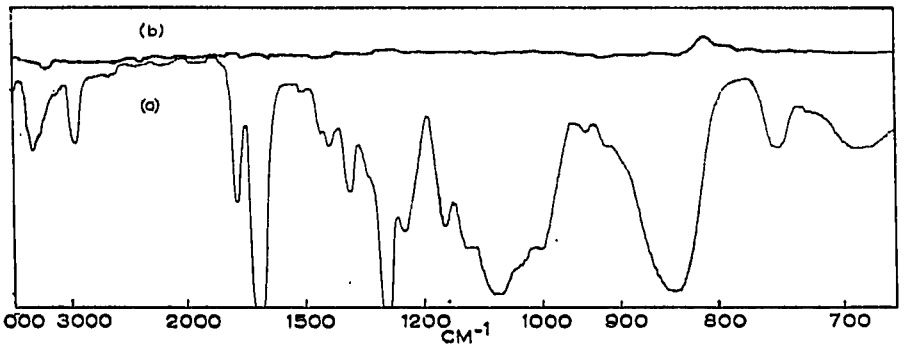

Fig. 1. (a) Infrared spectrum of thin Millipore membrane filter uncompensated. (b) Differential spectrum of compensating filters.

fact that standard thickness Millipore filters have previously been used as a supplementary collection device in gas–liquid chromatography[6].

EXPERIMENTAL

To collect samples, a $^1/_2$ in. filter disk in a Millipore "Swinny" Adapter was tried initially. The exit of a gas-chromatographic column (F & M Scientific Co., Avondale, Pa., Model 500) was fitted with a male luer-taper adapter which provided a leak-proof seal to the filter holder while permitting quick changes. Studies with methyl 1-^{14}C-stearate indicated, however, that only 10% of the radioactive material injected into the column was collected on the filter. Cooling the filter produced no improvement. For efficient collection, the filter and its support screen must be kept cold, but the passages upstream from the filter must be hot to prevent condensation of the sample before reaching the filter. This apparent dilemma was solved by making the front half of the holder from Teflon® (see Fig. 2), which prevented heat transfer from the heated

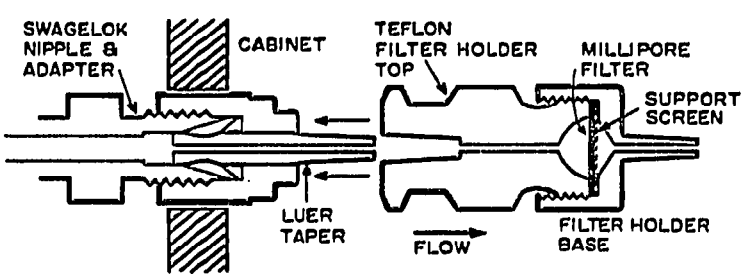

Fig. 2. Holder for collecting chromatographic effluents on $^1/_2$-in. filter disk, showing adapter on column exit.

exit port to the filter. The base of the holder was then cooled by wrapping it with a strip of cloth which dipped into an acetone–dry ice bath in a Dewar flask. With this arrangement, collection efficiency was increased significantly. To determine this collection efficiency 1 μl of methyl 1-^{14}C-stearate was injected into the chromatographic column and an equal quantity was placed in a counting vial, No. 1. The effluent from the column was collected on a filter as described and the filter was then placed in vial No. 2. A second filter was used to collect effluent following emergence of the stearate; this filter was placed in vial No. 3. Fifteen ml of polyether scintillation fluid was added

to each, and to a blank vial No. 4, and all were counted in a Packard liquid scintillation counter. The results are shown in Table I.

To record spectra of samples after collection, the filters were mounted in the spectrophotometer using a simple holder designed for the $^1/_2$ in. KBr disc. A blank filter of the same size and thickness was mounted in the reference beam, and the spectra were recorded in the usual manner on a Perkin-Elmer Infracord®.

In experiments using minimal amounts of sample, greater sensitivity was attained by reducing the effective area of the filter, to conform with the effective beam area of the spectrophotometer. This was done most simply by pressing a mask of gummed paper over the filter; a more elaborate holder using a specially-designed Teflon® insert was also used (Fig. 3).

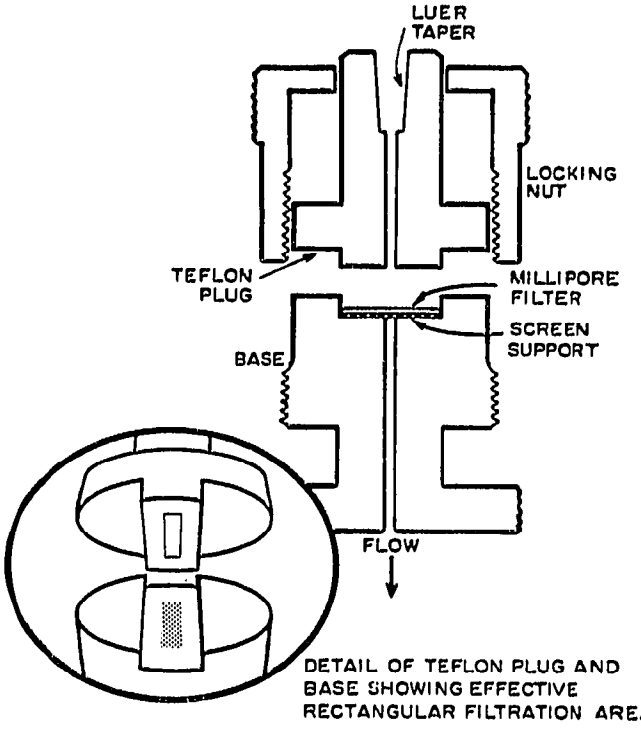

Fig. 3. Improved holder which concentrates sample on 2 mm by 10 mm area, for greater efficiency.

RESULTS AND DISCUSSION

The data from the experiment to determine collection efficiency are shown in Table I. They indicate that about 45% of the injected material was collected on this filter. Since recovery from chromatographic columns rarely exceeds 80% these were results considered quite favorable.

TABLE I

No.		*c.p.m.*	*% of standard corrected*
1	Standard	3400	100
2	Collected on filter	1620	44
3	Column background	130	—
4	Counter background	30	—

Fig. 4a shows the spectrum of diethyl malonate collected on a filter. The sample of the pure compound was separated from 1 μl of a 30% solution in benzene, on a silicone rubber column at 120°. Fig. 4b is the spectrum of an equivalent amount of pure

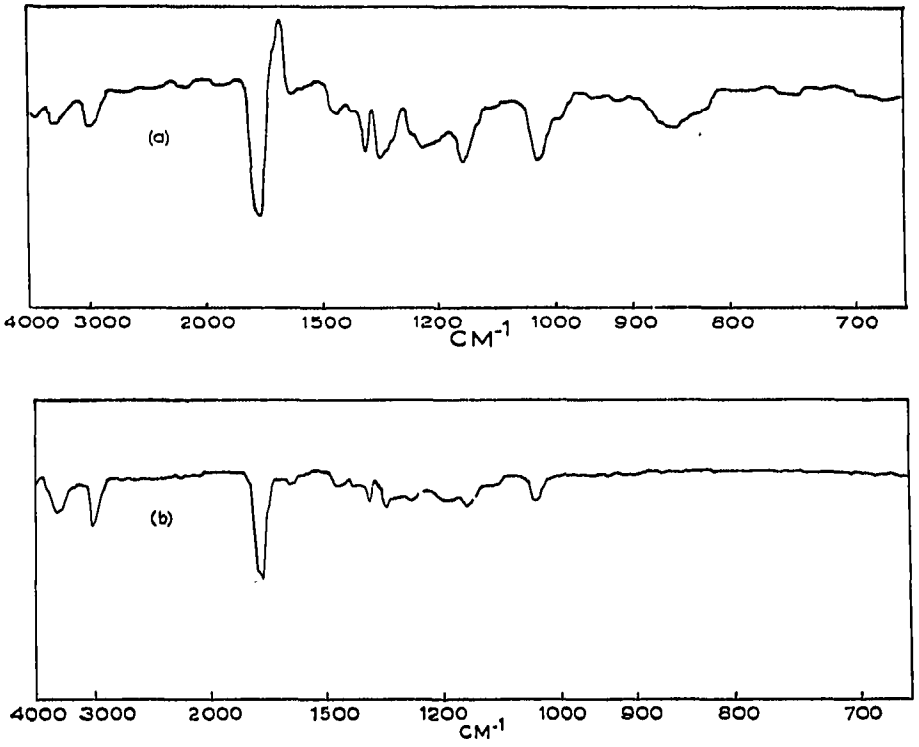

Fig. 4. Infrared spectra of diethyl malonate, (a) on membrane filter and (b) on salt plate.

diethyl malonate smeared on a salt plate. The absorption is weaker because not all of the sample is concentrated in the beam area, and because some evaporation occurred. The main differences between the two spectra occur at 1650 cm^{-1} and 850 cm^{-1}, frequencies at which the filter is very strongly absorbing (see Fig. 1). This is an example of imperfect compensation.

Fig. 5a shows the spectrum obtained from 2-hydroxydodecanoic acid (methyl ester), a component of the fatty acids in the bound lipids of *Azotobacter vinelandii*, collected on a filter from a silicone rubber column at 190°. Although these fatty acids are normally separated on a polyester column, we found new absorption bands in samples collected from a polyester column at 190°; these were shown to be due to substrate bleeding from the column. Readers are cautioned to be aware of this possibility when collecting from columns at high temperatures.

Fig. 5b shows the spectrum of synthetic 2-hydroxydodecanoic acid (methyl ester) smeared on a salt plate. The spectrum obtained on the filter in this case differs in two respects: (1) the additional band at 850 cm^{-1}, due to strong filter absorption and (2) the gradual lowering of the base line at higher frequencies. The most probable explanation for the latter result is the formation of tiny crystals on the filter, which disperse the incident radiation of short wavelength.

From the results of these and other tests which have been run it appears that the membrane filter technique is a useful supplement to the standard methods of collecting gas-chromatographic effluents for infrared spectral analysis. The technique is best

applied to relatively non-volatile liquids. Good spectra cannot be obtained from crystalline solids owing to the dispersion of the incident beam. Because of the energy of the infrared beam, however, some low-melting solids may become liquid, and provide characteristic spectra.

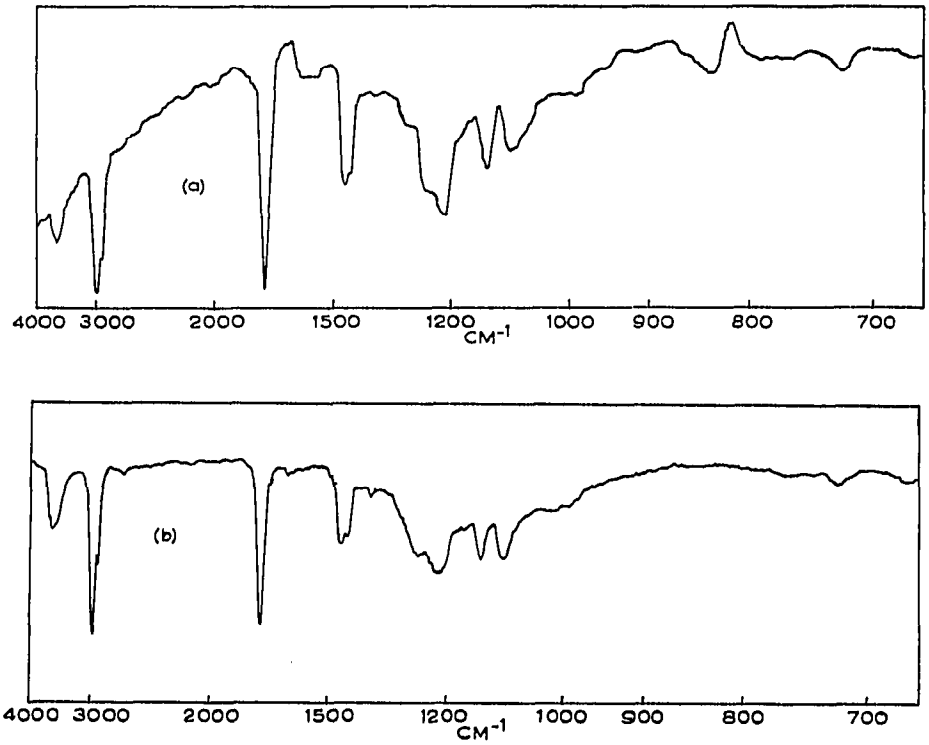

Fig. 5. Infrared spectra of 2-hydroxydodecanoic acid (methyl ester), (a) on membrane filter and (b) on salt plate.

As with any differential technique, matching of the two beams is important. In this case, the blank filter must have the same thickness as the sample filter, within 1%. Since the tolerance cannot be maintained within this range in the manufacturing, it is necessary to cut the two filters from adjacent areas of the same piece. Attempts are being made to improve the variation in manufacturing, but for the best results at present it is advisable to match the filters. If they are cut with a die having a constant area, they can be matched with respect to thickness simply by weighing them on a micro-balance.

The spectra shown in Fig. 4 and 5 were obtained from samples collected with the specially designed holder, but the results obtained with the Teflon Swinny adapter are equally good. These holders and the filters are available from Millipore. Adapters to fit most chromatographs can be furnished on request if the details of the fitting are supplied.

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SUMMARY

Thin, porous, cellulosic membrane filters were used to collect components of the effluent from a high temperature gas-chromatographic column. These filters were then mounted directly in the sample beam of an infrared spectrophotometer, while a blank filter of the same thickness was mounted in the reference beam. Characteristic spectra were obtained from the samples.

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