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Quantitative Gas Chromatography

Quantitative Recovery and Re-injection of a Sample

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Dedicated to Professor *Holger Erdtman* on his 60th birthday

Samples (0.05 μl and 1 μl) of organic compounds (boiling points < 150°C) have been collected at the exhaust tube of a gas chromatograph and then re-injected, using the gas sampling valve of the chromatograph (Perkin-Elmer 116). Two methods have been used to collect the samples, sorption on gel and a freezing-out technique.

The hydrocarbons and chloro compounds, listed in Table 2, have been collected quantitatively on silica gel in U-shaped or F-shaped tubes at room temperature, and then re-injected by rapid heating of the tube to 150°C. The loss of material was found to be very small; samples should be recycled several times in order to permit a determination of the average loss of a collection — re-injection cycle. An average loss of less than 1.5 % was achieved using 1 μl samples, whereas with a sample size of 0.05 μl the loss was found to be somewhat larger.

Interference of water was overcome by the choice of stationary phase or in some cases by applying a CaH_2 pre-column. The importance of ensuring a constant flow-rate of carrier gas for the quantitative estimation of recorded graphs is demonstrated.

Utilizing F-shaped tubes, samples were recycled by a freezing out technique to give a fairly good quantitative yield, in most cases with a loss less than 3 %. This method can be applied more universally since gel was found to interfere with some compounds, *e.g.* 2-pinene decomposed totally, and a loss of 25 % was recorded for butanol.

The presence of an inactive filling (Chromosorb W or glass micro beads) in the F-tube is essential, and with the exception of the low boiling compounds, such as chloroethane and ethyl ether, CO_2-ice gives the same good result as liquid air.

Quantitative gas chromatography may be regarded from at least three different viewpoints. The passage of the sample through the chromatographic instrumentation must proceed without loss, a conception which also includes quantitative injection and recovery of the sample. The instrumentation, including detecting and recording devices, should convert the output

signal to a corresponding quantitative answer, and finally, the physical-chemical background of the response given by the individual chemical compound is to be considered. The publications given in the literature concern the latter two aspects, whereas the first one being the main consideration of this investigation has not, to the knowledge of the authors, been treated earlier.

The technical evolution of commercial chromatographs has advanced considerably during the last years, and there are nowadays no difficulties in running the instruments under such conditions that the important variables are constant enough for a reproducible and quantitative estimation of the graphs. However, an important factor of our investigations, later described, was to keep the flow of carrier gas under control, and the present authors wish to draw the reader's attention to the necessity of checking the flow-rate intermittently during quantitative analysis, *cf.* Fig. 1.

The common errors incurred on estimation of the recorded graphs for quantitative analysis have been thoroughly eluciated in the literature. Janak *et al.*[1,2] have critically compared most methods in use. For this investigation the geometric method has been used when treating sharp and regular peaks, giving the same good result as reported by Janak. However, since changes of flow-rate were sometimes unavoidable, giving an irregular baseline, the planimetric method has been used frequently. For the most part, lower mean errors than those reported by Janak have been obtained for the different peak sizes.

The third viewpoint of quantitative analysis mentioned, *i.e.* the relative signal response of individual compounds using thermal conductivity detectors, has mainly been studied by Rosie and his colleagues [3,4]. We have investigated some of the compounds used in the present investigation, which are also given in the reports of Rosie. As demonstrated by Table 1, fairly good agreement has been obtained both when injecting mixtures of the compounds in benzene (50/50 % by volume), and when injecting equal volumes of the compound sample and benzene separately. However, there are some obvious discrepancies, *e.g.* with 2,2,4-trimethyl pentane, and it is intended to investigate the case further.

Table 1. Relative signal response per mole on thermal conductivity detection (benzene = 100; benzene-compound 50/50 % by volume, average mean error ± 1.5 %

Compounds investigated	Consecutive injection of compound and benzene	Injection of compound-benzene mixtures	
		present authors	Rosie [3,4]
Cyclohexane	103	105	114
Methylcyclohexane	115	113	120
2,2,4-Trimethylpentane	131	134	147
Toluene	113	116	116
Ethanol	67	65	72
Propanol-1	85	83	83
Butanol-1	97	100	95
Ethyl acetate	108	108	111

COLLECTION AND RE-INJECTION OF A SAMPLE

There are several methods in use for collecting a fraction which issues from a gas chromatograph, the purpose of this collection being mostly for further identification by additive analysis. The carrier gas, together with the vapours of the compound of interest, can be carefully drawn by suction into an evacuated container or a syringe. Mostly, the vapours are condensed by cooling, dissolved in a solvent or sorbed on an active surface, and thus more or less effectively freed from the carrier gas. The last three operations are very similar to those for a long time used for sampling vapours in air, but for gas chromatography the problem is not so delicate, since water vapour is not present in large amounts. The quantitative yield of the collection operations mentioned are difficult or impossible to measure by the aid of additive methods, such as UV or IR. It is very obvious that the most convenient way to determine the yield would be gas chromatographic. However, this requires a quantitative re-injection of all the sample collected. *To make this possible, a quantitative circle of gas chromatography is closed, and this makes the gas chromatographic method ready for an enlarged field of analytical work.* The demand for a quantitative re-injection of the collected sample practically eliminates those collecting methods where solvents are used or where the sample is collected together with the carrier gas. To fit the gas chromatographic method the re-injection must proceed rapidly, and thus the only methods which were likely to be applicable were heating techniques of a sorbed or condensed fraction. In this investigation these two methods have been found to give the desired result.

COLLECTION OF A FRACTION ON SILICA GEL

This investigation was begun because of medical interest in benzene vapour, and since the gel technique had been used earlier to collect benzene vapour in air for UV-spectroscopic determination after desorbing with ethanol, attempts were made first to enrich on gel the benzene vapours issuing from the chromatograph. To enable a re-injection into the chromatographs available in these laboratories (Perkin-Elmer 116) the collection tube holding the gel was made U-shaped in order to fit into the gas sampling valve of the apparatus. The U-tube (total length 200 mm pyrex, i.d. 1.5, o.d. 3.0 mm) was filled with 200 mg of activated silica gel, 60—70 mesh, kept in position by plugs of glass yarn.

Since methods of air sampling were simultaneously investigated, and since any disturbance of the base-line height was undesired during collection of the benzene fraction leaving the exhaust tube of the chromatograph, the following method was first adopted. The issuing vapour was drawn by suction into the gel of the U-tube by the aid of a large syringe fastened to one arm of the U-tube by a piece of rubber tubing. The exhaust needle was loosely inserted into the other arm. The rate of suction was kept slightly higher than the flow-rate of carrier gas of the chromatograph to ensure a complete uptake. Due to difficulties in achieving a constant speed when moving the piston of the syringe, an evacuated container was used instead. A constant suction was achieved

during the period of time when benzene was issuing from the chromatograph, by means of attaching a restriction between the U-tube and a vacuum tank of 500 ml capacity.

Later, a third method of collecting the fractions was used. Two U-tubes were carefully calibrated to give exactly the same resistance to gas flow by adjusting the amounts of gel added and the degree of packing. Short teflon tubings were used to connect tightly the two U-tubes to the two way valve of the chromatograph exhaust tube. This arrangement increased the gas pressure at the detector and lifted the baseline. However, on switching the valve from one position to the other, no change of the base-line of the recorder was detected. Thus, one tube could be removed for re-injection without interfering with the recorded graph.

Finally, since the samples were usually recycled several times for determination of the yield of recovery, two uncalibrated gel tubes were interchanged between collection and re-injection positions according to a timed schedule, and the recorded peaks were estimated in two series. This was found to be the most convenient way of collecting the fractions and the procedure rendered it possible to use the more handy F-shaped tubes described later.

RE-INJECTION OF A FRACTION COLLECTED ON SILICA GEL

On re-injection of the collected sample, the U-tube was fastened into the O-fittings of the gas sampling valve of the Perkin-Elmer chromatograph. When opening the valve and flushing the carrier gas through the U-tube, it was found that the gel retained the benzene very effectively at room temperature, and no peak of benzene was detected within 30 min. However, the resistance of the gel layer caused an elevation of the base line. Applying electrical heating to the U-tube (a spiral thread over the tube), the benzene was rapidly desorbed and flushed into the chromatograph, to give a well-defined peak. Heating of the gel layer increased its resistance to the flow of carrier gas, and if heating was continued throughout the analysis, temperature stability was not usually achieved by the time that the sample of benzene entered the detector; thus, the peak of benzene was recorded on a drifting base-line.

It was subsequently demonstrated that the sample of benzene was totally desorbed within 30 sec on heating with the oven used. Thus, the stream of carrier gas through the U-tube or F-tube could be switched off after this time (one minute was used). The peak of benzene appeared usually after 5 min, by which time a good and reproducible baseline had been restored. This made it easier to compare quantitatively the peak areas of repeated re-injections, and thus the yield of each collection — re-injection cycle.

As always on gas analysis, the presence of water vapour interfered. On re-cycling the sample of benzene, the amount of water was increased, giving greater interference on further re-cycling. Furthermore, the water sorbed on the gel was not totally desorbed within 1 min, and sometimes water peaks appeared during subsequent analyses.

The interference of water was successfully overcome by the choice of stationary phase or by the use of a short pre-column containing calcium hydride

Table 2. Average percentage loss of sample at a collecting — re-injection cycle. (Number of cycles minimum 6 and maximum 15).

Substance *a* 0.05 μl *b* 1 μl	Gel method	Freezing-out method Chromosorb-W		Micro glass beads	
		CO_2-ice	liquid air	CO_2-ice	liquid air
Benzene *a*	1.3	4.4	4.4	2.3	
» *b*	0.4	2.7	3.5	2.2	
Toluene *a*	1.1	2.7		2.6	
» *b*	1.0	2.5		2.4	
Ethylbenzene *b*	3.1	3.2		2.6	
Styrene *b*	6.5	3.6			
p-Xylene *b*	2.3			2.7	
Cyclohexane *a*	2.3			15	3.4
» *b*	3.3	2.7	1.5	4.5	
Hexane *b*	3.5	1.3	1.6	29	2.0
Heptane *b*	2.3			2.2	2.0
Pinene *b*	total decomp.	0.5		1.4	
Methanol *a*	6.4 (decomp.)	5.7		9.2	
» *b*	10.3 (decomp.)	6.9	4.5	2.8	
Ethanol *b*	12.7 (decomp.)			2.8	0.6
Propanol-1 *b*	18 (decomp.)			4.1	2.8
Butanol-1 *b*	25 (decomp.)	1.7		2.4	
Ethyl acetat *a*	(decomp.)	10.1	3.3	10.7	5.8
» » *b*				2.1	
Acetone *b*	(decomp.)			9.8	1.4
Methylethyl ketone *b*	(decomp.)	2.3		4.1	
Ethyl ether *b*	(decomp.)	27	4.1	30	2.8
Ethyl chloride *a*	0.3	70	3.9		4.3
» » *b*		34	0.6		0.9
Chloroform *a*	0.6				
Perchloroethylene *a*	2.5				
Dichloroethane *b*	2.9	1.3		2.4	
Trichloroethylene *b*	0.9				

(30 % mixture with Chromosorb-W). The hydride converted the small amounts of water to hydrogen, which did not affect the base-line at these small concentrations, since the thermal conductivity of hydrogen and the carrier gas used were similar.

QUANTITATIVE RESULTS USING SILICA GEL TECHNIQUE

Re-cycling samples of benzene, toluene, chloroform, and trichloro ethylene, and even such a low-boiling compound as ethyl chloride, the loss at each cycle was found to be very small, of the same magnitude as errors made on the estimation of the graphs. Thus, an accurate determination of the mean yield had to be made by re-cycling the sample several times. Up to fourteen cycles have been performed, with a total loss of 10 %. An average loss of 0.4 to 1.0 % for each cycle was achieved for the compounds mentioned when applying a sample size of 1 to 5 μl, the latter being the maximum amount tolerated by our recording device. The loss was found to be somewhat larger

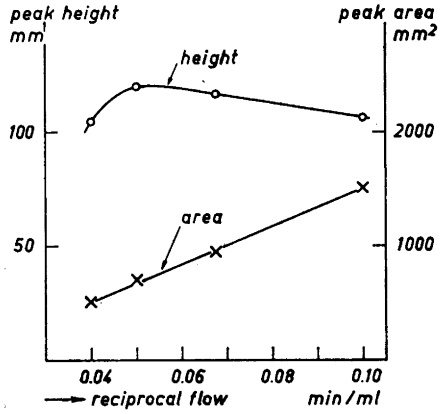

Fig. 1. Effect of flow-rate of carrier gas (helium) on peak height and peak area. Column: 2 m., inner diameter 4 mm., octylphthalate (30 %) on Chromosorb-W; temp. 101°C; sample: 2,2,4-trimethyl pentane, 2 µl; sensitivity 32; chart speed 30 cm/h.

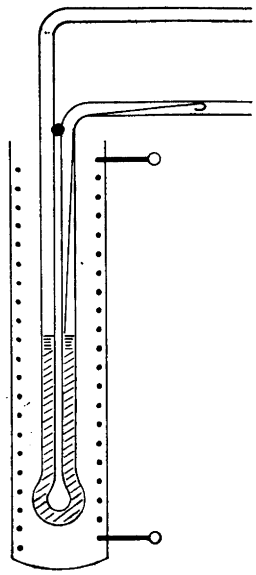

Fig. 2. F- shaped tube and oven. F-shaped tubing of Pyrex glass, o.d. 3 mm, total length about 35 cm, of which the lower 8 cm contained the filling and two small plugs of glass yarn. Since the carrier gas enters *via* the upper arm, the filling is held in position in the lower arm by a stainless steel wire. — The electrical oven consists of a quartz tube holding an inner spiral of resistance wire (4 Ω). With 13 V, the temperature of the F-tube was brought to 170°C within 1 min when the oven was raised over the F-tube.

when 0.05 µl samples were used and the reason for this is now under investigation (*cf.* Table 2).

For the higher boiling aromatic hydrocarbons, a desorbing time of 3 min was found necessary. Slightly distorted peaks were obtained, very likely due to insufficiently high temperatures upon re-injection. For saturated hydrocarbons such as cyclohexane, which were not sorbed as firmly as aromatic hydrocarbons by the gel, the time of uptake on the gel was found to be critical, and should be chosen to be as short as possible and to give no more than the small loss mentioned earlier. Those hydrocarbons isomerized totally by the gel have not been investigated further, *e.g.* 2-pinene.

Certain obstacles were encountered on application of this gel method to lower alcohols, esters, ethers and ketones. These compounds were more or less decomposed on heating the gel upon re-injection. Butanol, for example, gave a loss of 25 %, even more when the CaH_2-pre-column was connected. These findings led to an investigation of the collection method described in the next section.

FREEZING-OUT OF FRACTIONS

Except for work with high vacuum, the freezing-out technique is generally considered not to be quantitative. However, using the F-shaped tubes, shown in Fig. 1, which similarly to the U-tubes could be fitted into the gas sampling valve, fairly quantitative yields were soon obtained. This was achieved when the lower part of the F-tube was filled with packing material to a height of 4 cm; it was demonstrated that the nature of the packing material was of importance. It became apparent that sterchamol and celite-C-22 contained active groups which even at a moderate temperature retained the alcohols, causing tailing. Chromosorb-W (60—80 mesh) or micro glass beads (20—40 mesh) did not give this effect and gave a much lower resistance to the gas than gel. However, on heating at the re-injection of methyl cyclohexanol, an effect was observed which might indicate a decomposition caused by active groups on the filling material.

Cooling of the F-tube was achieved by CO_2-ice; no advantage was found with the use of liquid air when re-cycling benzene or butanol. Naturally, low boiling compounds such as ethyl ether, cyclohexane and ethyl chloride gave better yields by using liquid air. Minimizing the time of collection also increased the yield. Fig. 3 shows chromatograms obtained on re-cycling ethyl ether.

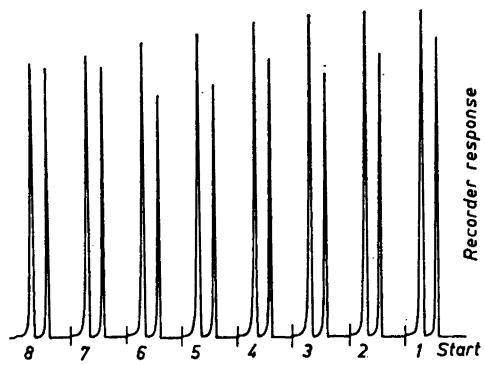

Fig. 3. Chromatograms from recycling of ethyl ether. Chromatogram 1 was given by the injection of 1 μl ethyl ether, and consists of peaks of air and ethyl ether. On repeated re-injection and collection, chromatograms 2—8 were formed. Two F-shaped tubes, filled with micro glass beads, were employed alternatively, and were cooled with liquid air. The first peak of water appeared after the eighth recovery. — Analytical conditions: Reoplex-400 (15 %) on Celite-22; temp. 65°C; pressure 1 kg/cm² (He); sensitivity 128; chart speed 60 cm/h; gas chromatograph Perkin-Elmer 116 e.

Some water vapour was condensed inside the tube, but lessened by protecting the free exit arm of the F-tube with a drying tube containing CaH_2. However, chromatograph columns were chosen where the small peak of water did not interfere, and remaining water was flushed off before a new collection. The use of a pre-column (CaH_2) was omitted since butanol and many other compounds were affected by this pre-column.

On injection, the F-tube was connected rapidly to the gas sampling valve and the hot oven (mostly 150°C) — *cf.* Fig. 2 — was raised over the cooled part of the F-tube. Simultaneously, the valve was opened and the F-tube flushed by the carrier gas. All the samples investigated were vaporized within 45 sec., and after a further 45 sec the valve was turned back to the normal position and base-line stability was rapidly obtained.

The results obtained using the freezing-out technique elaborated up to now are demonstrated by Table 2. For some compounds the yields were quite satisfactory, but mostly the loss was greater than when silica gel was used. At the present stage of the investigation, it is too early to distinguish whether chromosorb or micro glass beads are to be preferred as filling for the collection tubes or to state when cooling by liquid air should be used. With regard to the low resistance to the stream of carrier gas, the micro glass beads seem to be preferable. According to our opinion, the freezing-out technique can be improved further to give a lower loss at each analytical cycle and to allow the treatment of higher boiling compounds.

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