

SUBSTANCE RECOVERY IN PREPARATIVE GAS CHROMATOGRAPHY

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A serious problem in preparative gas chromatography is the recovery of the separated substances. Difficulties can arise because of only partial condensation or because the condensation produces an aerosol. In each case some of the material is not held back in the collecting trap and is swept away by the carrier gas. Various methods have been advanced to solve this problem, such as electrical precipitation^{1,2}; centrifugation of the collector bottle during the chromatography³; the use of specially heated or gradient cooled collectors^{4,5} to prevent fogging; filling the collecting bottles with asbestos, glass or cotton wool to trap the aerosols⁶. A 100 % efficient recovery system has not been devised and it seems improbable that this will ever be the case.

In this paper some factors are discussed which have an influence on the recovery percentage and ways of improvement are investigated.

EXPERIMENTAL

The collector trap

Apart from special effects like centrifugation or electrical precipitation, the form of the collector could have some influence. We tried several models and some are depicted schematically in Fig. 1. Of these, model E was most efficient and we did not succeed in improving it by prolonging the tube inlet, drawing the tube inlet to a fine point, or by using thin walled vessels. As reported in the literature, the efficiency of these traps is greatly improved by filling them with cotton wool, asbestos wool or glass wool. In

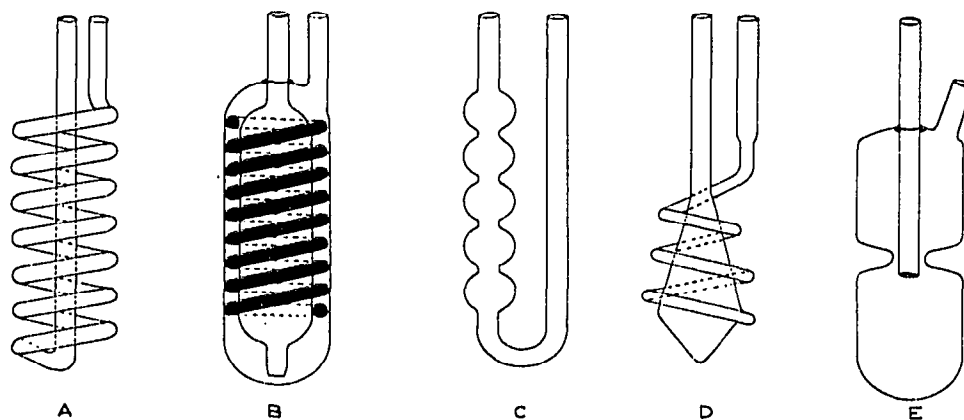


Fig. 1. Collecting bottle types. A = spiral; B = Widmer; C = ball condenser; D = cyclone; E = Aerograph 700.

this case, however, the resistance to carrier gas flow increases and the connection between the column exit end and the trap must be adequate to prevent losses at this point.

Volatility of the substances

Two hundred microlitres of ether, cyclohexane, iso-octane and *trans*-decalin were chromatographed at their respective boiling temperatures on an Aerograph Autoprep 700 (6 m³/₈ in. column, filled with 30 % SE 30 on chromosorb P; 200 ml/min hydrogen) and collected at -20° (ice-salt mixture*). The recoveries were 0, 39, 44.6 and 88 % respectively. This shows clearly the influence of the volatility of the samples.

Concentration of the substances in the gas phase

The concentration of the substances in the gas phase plays an important role in the recovery, which increases with increased concentration. This concentration is determined by column parameters such as the volume of mobile phase in the column (column length and diameter) and the separation efficiency of the column expressible in plate numbers (more plates per metre gives narrower bands and higher concentrations). For a given column, however, these are not very variable. Other factors directly related to the concentration are the amount of material put on the column, and more particularly the temperature of elution**. Large samples chromatographed at a high temperature will show the highest concentration in the eluate. Unfortunately, however, the most interesting are nearly always the rarest and most complicated chemical mixtures which are therefore difficult to separate, so that in this case only small samples and long retention times (low temperatures) can be used. The recovery percentage will be low although it is just with such mixtures that high recovery is most interesting. That the sample size has a direct influence on the concentration of the eluted substances is obvious, but the influence of the temperature of elution is shown by the results given in Table I.

TABLE I
PERCENTAGE RECOVERY OF *trans*-DECALIN AS A FUNCTION OF ELUTION TEMPERATURE
(ISOTHERMAL CHROMATOGRAPHY)

Quantity (μ l)	Column temperature					
	200°	180°	160°	140°	120°	100°
200	93.1	87.8	72.7	71.3	66.7	47.0
100			69.0			

Table I gives the mean value of five determinations. The collecting bottles were tared and the collected sample weight was compared with the weight of the injected sample as delivered by a syringe. Other conditions were: on column injection on Aerograph Autoprep 700; gas rate 200 ml/min H₂; collecting bottles cooled in ice water.

* It is most efficient to use the same salt water solution again and again, by simply freezing it in the Dewar flask by repeated addition of liquid air with vigorous stirring.

** Unpublished work shows that, as stated, the temperature at elution and not the retention time is important in this connection⁷.

High separation effect, long retention times and high temperature at elution can be obtained together by programming the gas chromatographic separation. That programming results in better recovery has been noted before⁸.

An alternative is to run the chromatogram at a lower temperature for the desired time, stop the flow of carrier gas, increase the temperature of the column oven and then restore the gas flow. In this way the substances are chromatographed long enough to be separated and are eluted at a high temperature. That recovery is better by this technique is shown by the figures for 200 μ l ether, cyclohexane, iso-octane and *trans*-decalin which were run in the conditions as described before, but with an additional temperature increase of 70° with the gas flow stopped just before elution. The recovery was respectively 14.4, 42.4, 61.5 and 95.4 %, which is decidedly higher than without temperature increase.

Gas flow rate

High speeds of the gas flow through the collecting trap must be detrimental to the recovery. One way to ascertain this is by diminishing the gas flow rate just before elution of a peak. In a recovery experiment on 200 μ l ether, cyclohexane, iso-octane and *trans*-decalin, as described above, the temperature was increased by programming and the gas flow was reduced to 25–30 ml/min just before elution of the peaks. This was very easily effected without changing any control setting of the instrument, by inserting a suitable bleeding capillary through the injection gasket at the appropriate moment.

The recovery results were now respectively 31.8, 73.4, 84.6 and 97.0 %. While such a drastic gas rate reduction would have a marked influence on baseline stability in normal analyses with high sensitivity, this is not the case in preparative work, since the sensitivity of the instruments is much reduced with regard to the high sample load. Comparative gas chromatograms on decalin are shown in Fig. 2.

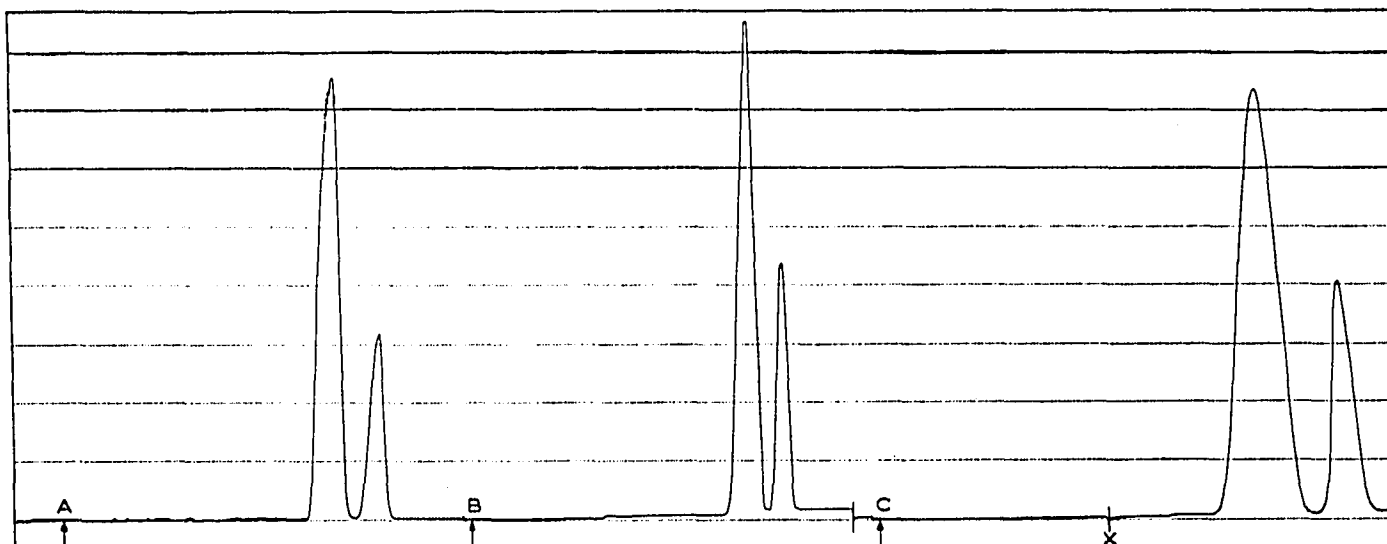


Fig. 2. Chromatograms of decalin on Autoprep 700. Sample load 200 μ l; bridge current 150 mA. Attenuator 32 for A and B, 64 for C. Collecting bottles cooled in ice water. Other conditions: (A) isotherm at 170°—200 ml/min H₂; (B) programmed from 150 to 190° at peak elution; 200 ml/min H₂; (C) programmed from 150 to 200° at peak elution. At point X the gas rate was changed from 200 to 25 ml/min H₂.

A and B have been run in such conditions that the retention times are about the same. The elution temperature for the programmed run is, however, higher and therefore gives narrower and more concentrated peaks (higher signal for identical sample). The recorded band broadening of C is of course due to the lower gas rate while eluting the peaks.

Another possibility seemed to be to collect the eluate of very volatile materials in inflatable toy balloons and then to press the gas very slowly through a collecting bottle. Several attempts in this direction gave no interesting results.

The nature of the carrier gas

Three gases can be considered for general use in preparative gas chromatography, hydrogen, helium and nitrogen. Helium is very expensive, especially in Europe, and therefore often out of the question. Hydrogen is dangerous, although it may be pointed out that this should not be exaggerated since hydrogen-air mixtures become inflammable only above 4 % hydrogen concentration.

Nitrogen, on the other hand, compared with hydrogen, produces a serious efficiency drop in long columns with high resistance to gas flow. From the point of view of recovery discussed in this paper, hydrogen and nitrogen were compared in experiments on 200 μ l ether. The starting temperature was 35° and programming produced a 50° increase to the elution point. The gas rate was 200 ml/min at the start of each run. The instrument was again a Aerograph Autoprep 700 with a 6 m column filled with 30 % SE30 on chromosorb P and the collector bottles were cooled at -15° in an ice-salt mixture.

The recovery was slightly better with nitrogen than with hydrogen. The mean value for five determinations with each gas was 16.5 and 13.0 % respectively. The difference between hydrogen and nitrogen with regard to the recovery percentages is therefore not significant.

Temperature of the cooling bath

In the experiments described above the collector bottles were cooled by immersion in a bath containing ice water (0°) or an ice-salt mixture (-15° to -20°). The effect of further cooling on the recovery of ether was studied. 200 μ l of ether was chromatographed isothermally at 70° on an Aerograph 700 with hydrogen as carrier gas, the other conditions being as described above. The mean of five recovery determinations per temperature are found in Table II. Adequate cooling is therefore very important for the recovery in preparative gas chromatography.

Availability of the recovered substances

Efficient recovery is only possible when using glass wool scrubbers in the collecting

TABLE II
PERCENTAGE RECOVERY OF ETHER AS A FUNCTION OF COOLING BATH TEMPERATURE

	<i>Temperature of the cooling bath</i>					
	0°	-8°	-14°	-25°	-65°	-80°
Recovery %	0	2.3	16.7	24.2	83.3	89.4

bottles. High boiling substances can be rinsed quantitatively from the glass wool with suitable solvents, but low boiling substances cannot be processed in this way and losses through wetting of the glass wool are unavoidable. Centrifuging, however, forces most of the material to the bottom of the collecting bottle, where it can be sucked out with a syringe or a pipette. This problem was investigated with increasing amounts of iso-octane chromatographed at 120° and with cooling of the collecting bottles in an ice-salt mixture, the instrument and other conditions being as described before. For 50, 100, 200, 500 and 1000 μ l of iso-octane the recovery in the collecting bottles increased in the same order from 80 to 97%. The amount which could be drawn out with a syringe was then 31, 37, 74, 78 and 86% respectively. This is rather low for the 50 and 100 μ l samples, and such small samples are indeed necessary with difficult separations. The separation will have to be repeated on a large number of small samples to obtain the desired amount of the substances. With such repeated separations and collection in the same bottle over and over again the recovery increases markedly. Five injections of 100 μ l iso-octane gave a recovery⁷ in the collector of 79% while 67% could be sucked out with a syringe.

CONCLUSION

With higher boiling substances, the recovery in preparative gas chromatography is not a big problem. This is not so with substances boiling below 100° and in this case preparative gas chromatography should be run, with cooling in a dry ice-acetone mixture or even in liquid air. Improvement of the recovery percentage can also be obtained by programming the temperature and diminishing the gas flow rate at the moment of peak elution. This gas rate reduction is something which could easily be controlled automatically by the signal switch on the recorder which actuates the movement of the collecting bottles. As shown, linear programming of the temperature should be better than asymptotic programming. It seems thus that high (> 85%) recovery is possible with all liquids using simple collecting vessels.

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SUMMARY

Ways to improve recovery in preparative gas chromatography were systematically investigated. The results show that good recovery is possible in all cases with simple collecting recipients.

REFERENCES

- ¹ P. KRATZ, M. JACOBS AND B. M. MITZNER, *Analyst*, 84 (1959) 671.
- ² A. E. THOMPSON, *J. Chromatog.*, 6 (1961) 454.
- ³ A. WEHRLI AND E. KOVATS, *J. Chromatog.*, 3 (1960) 313.
- ⁴ R. TERANISHI, J. W. CORSE, J. C. DAY AND W. G. JENNINGS, *J. Chromatog.*, 9 (1962) 244.
- ⁵ R. K. STEVENS AND J. D. MOLD, *J. Chromatog.*, 10 (1963) 398.
- ⁶ K. P. DIMICK AND E. M. TAFT, *J. Gas Chromatog.*, 1, March (1963) 7.
- ⁷ M. VERZELE, F. ALDERWEIRELDT AND J. BOUCHE, unpublished results.
- ⁸ M. VERZELE, *J. Chromatog.*, 9 (1962) 116.