

GAS-LIQUID CHROMATOGRAPHY

A METHOD OF SEPARATION AND IDENTIFICATION OF VOLATILE MATERIALS

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MOST workers concerned with the separation of closely related compounds are familiar with chromatographic techniques involving the use of a stationary solid or liquid and a moving liquid. Less widely known however are chromatographic techniques utilising a stationary solid or liquid and a moving gas. The absorption chromatography of gases (gas-solid system) has been studied by many workers, in particular Claesson¹ and Janak² but until recently has not come into general use owing to the frequent occurrence of non-linear distribution isotherms with consequent impairment of separations. Gas-liquid chromatography is a recent innovation, suggested originally by Martin and Synge³ and developed by James and Martin⁴ for the separation of volatile fatty acids.

Since the mobile phase of the chromatogram is a gas, it is possible to use long thin columns and to obtain high rates of flow of mobile phase impossible with liquid-liquid chromatograms of similar shape. In all chromatograms the main factor limiting the efficiency is the rate of diffusion in the two phases concerned. In the gas-liquid system, diffusion in the gas phase is virtually instantaneous and the elevated temperatures at which the columns are generally run increases the rate of diffusion in the stationary liquid phase. For these reasons gas-liquid columns have very high efficiencies even at high rates of flow so that refined separations may be carried out more rapidly than with any other chromatographic technique. In addition it is generally much easier to detect low concentrations of vapours in gas streams than it is to detect low concentrations of solutes in solvents.

The columns are constructed of lengths of glass or metal tube into which is packed by vibration a suitably inert solid, usually kieselguhr, over whose surface is distributed a thin layer of the stationary liquid, a thermally stable substance of high boiling point. The column is held in a heated jacket at the desired temperature. The substances to be separated are applied to the top of the column packing with a micropipette and are then blown down the column by a stream of permanent gas, usually nitrogen, from a constant pressure source. The substances separate according to their relative volatility in the stationary liquid chosen and are detected as they leave the column in vapour form in the stream of permanent gas.

There are many theoretically possible methods of detecting vapours in gases but so far only three have been widely used. The first method is limited to those substances capable of ionising in solution or which can be caused to produce ions by rapid chemical reaction in solution. The gas stream from the column is led into the base of a small cell

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containing water or other solvent. The acids or bases are extracted as the nitrogen bubbles through the solvent and can be continuously titrated. James and Martin⁴ devised a simple automatic recording burette to carry out the titration continuously so that after loading the column and turning on the gas stream the whole process was automatic, the final result being recorded on a moving drum (see Fig. 1). The method was

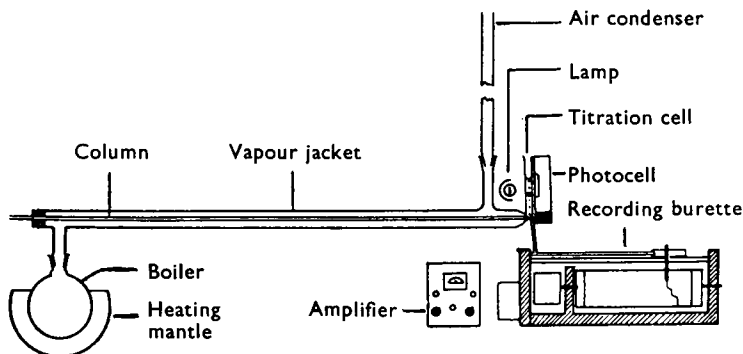


FIG. 1. Schematic lay-out of apparatus for separating volatile acids or bases, using titration as the method of detection and estimation.

sufficiently sensitive to allow the detection of 0.02 mg. quantities of acids such as acetic acid or of 0.002 to 0.01 mg. quantities of volatile amines such as ethylamine.

The records produced by the automatic burette show a series of steps (see Fig. 2, lower curve) rather reminiscent of the types of diagrams

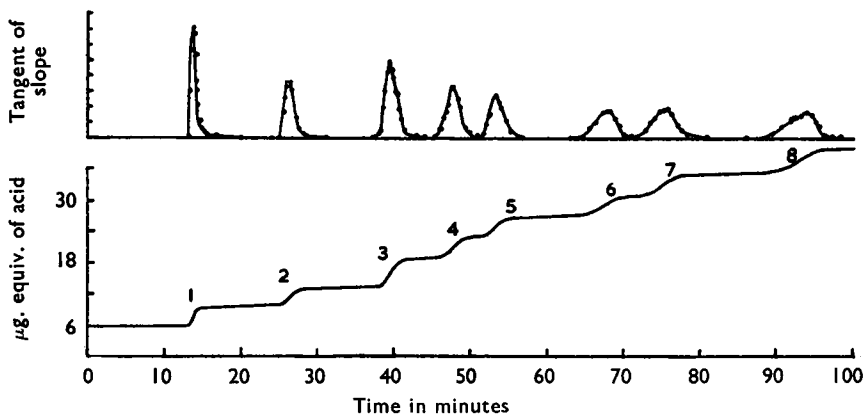


FIG. 2. The separation of acetic (1), propionic (2), *isobutyric* (3), *n*-butyric (4), trimethylacetic (5), *isovaleric* (6), methylethylacetic (7) and *n*-valeric (8) acids, showing complete resolution of all acids.

Upper curve: Differential of experimental curve.

Lower curve: Experimental results.

Column length, 11 ft.; stationary liquid phase, DC550 silicone fluid containing 10 per cent. (w/w) stearic acid; temperature, 137° C.; rate of flow of nitrogen, 18.2 ml./min.; pressure of nitrogen, 74 cm. Hg.

used to follow fractional distillations. When only carrier gas is leaving the column a horizontal line is drawn by the automatic burette and the emergence of titratable material is denoted by a step in the curve, the height of the step being a direct measure of the amount of material titrated. The machine is recording in effect a measure of the total amount of

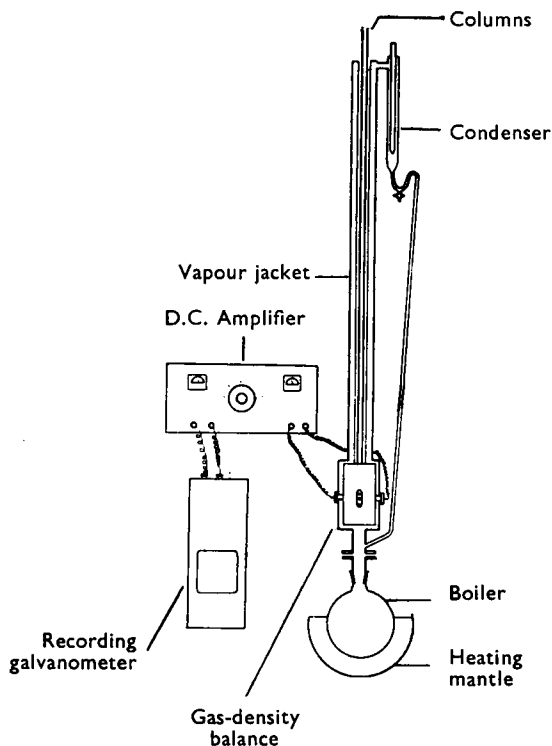


FIG. 3. Schematic layout of gas-liquid chromatograph using measurement of gas density as the detecting technique. Applicable to all volatile compounds.

this purpose by Ray⁵, and by Phillips and his colleagues⁶. The apparatus (the catharometer) consists of a platinum wire heated electrically and fixed along the axis of a tube through which is passed the gas stream. The composition of the gas stream determines the rate of loss of heat of the wire and hence its electrical resistance. By using two cells, one for the gas stream leaving the column and the other for the gas stream entering the column, and connecting the two wires in a bridge circuit, any change in resistance of the wire in the chromatogram gas can with the aid of a suitable amplifier and recorder be plotted automatically. Whilst having the advantages of simplicity and cheapness the method suffers from two main defects: (a) not very high sensitivity and (b) a high sensitivity of its zero to changes in rate of flow of gas through the cells.

The third method utilises two columns, one being the chromatogram,

material that has emerged from the column. By plotting the differential of the experimental curve there result the peaks so familiar to those who have used chromatographic techniques (see upper curve, Fig. 2).

Wider application of the gas-liquid chromatogram necessitates a detection technique that does not depend on the presence of particular types of chemical grouping in the molecules to be detected, so physical methods are indicated.

The second method used is to measure the thermal conductivity of the gas leaving the column and has been chiefly exploited for

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the other acting as a reference source of carrier gas, any volatility of stationary phase being thus compensated for. The densities of the two gas streams are continuously compared in an instrument known as the gas density meter developed by Martin⁷. In general the emergence of a substance from the chromatogram increases the density of the carrier gas. The instrument consists of a series of tubes bored in a copper block, maintained at the same temperature as the columns in order to prevent any condensation of the vapours, and connected in a manner analogous to a Wheatstone bridge. Adjustable throttles are placed in some of the channels so that when the instrument is first set up, resistance to flow in the channels can be adjusted until no pressure difference is generated across the network when the rate of flow of either gas stream changes. Two sets of channels, one for each gas stream, are connected by a cross channel in such a way that any difference in density between the two gas streams causes a small flow of gas through the cross channel. This channel contains a flow detector consisting of a small filament, heated electrically, arranged below and equidistant from two connected thermojunctions; any cross-flow of gas causes the stream of hot convected gas to be diverted to one or other of the thermojunctions, heating one and cooling the other. The resultant thermo e.m.f. is fed to a D.C. amplifier whose output is led to a recording galvanometer. The galvanometer deflection is linearly related to the density difference of the two gas streams making quantitative measurement relatively simple. The

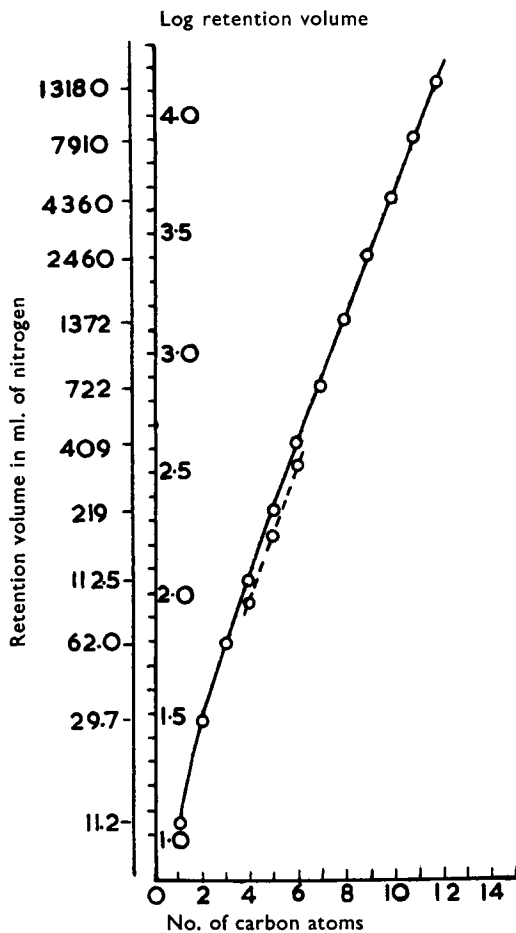


FIG. 4. The relationship between the retention volume at zero pressure difference, V_r^0 , and the number of carbon atoms in the lower fatty acids. Data are for a 4-ft. silicone-stearic acid column at 137°C. ○—○. *n*-acids; ○---○. *iso*-acids.

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instrument is highly sensitive, density differences such as those caused by the presence of one molecule of amyl alcohol in 50,000 to 100,000 molecules of nitrogen being easily detectable. Its zero is relatively insensitive to changes in rate of gas flow and its response is determined only by the molecular weight of the substance being detected. As with the other two methods described, the whole process is automatic once the column has been loaded and the carrier gas stream started. A schematic diagram of the whole apparatus is shown in Figure 3.

SOME APPLICATIONS OF THE TECHNIQUE

The separation of fatty acids and their esters

In Figure 2 is shown a separation of acetic, propionic, *isobutyric*, *n*-butyric, trimethylacetic, *isovaleric*, methylethylacetic, and *n*-valeric acids using a column 11 ft. long, arranged in a hairpin shape so that its overall length is only 4 ft., at 137°C. The total time for the separation is only 100 minutes. All the acids are well separated as is demonstrated in the upper curve, the differential of the lower experimental curve. The four isomeric valeric acids are difficult if not impossible to separate in such small amounts by any other technique. This result was obtained by using the automatic burette to detect the zones leaving the column.

The columns give highly reproducible results and can be used some hundreds of times without exhaustion. The time at which a given substance emerges from a column depends on the temperature, the flow rate of nitrogen and on the nature of the stationary phase in the column. At a given temperature and with a particular stationary phase it is found that there exists a simple relationship between the retention volume (the volume of nitrogen passing through the column before the centre of the peak emerges) and the number of carbon atoms in the molecule for the members of any homologous series. In Figure 4 is shown the result obtained with fatty acids when log. retention volume is plotted against the number of carbon atoms in the molecule. The straight line produced demonstrates that the free energy of solution of the CH₂-group added to the molecule in ascending the series is constant and independent of its position in the chain. This regularity of chromatographic behaviour enables one to identify an acid from its time of appearance relative to a standard acid; this relative retention volume can be considered as a physical constant as useful as an R_f value or melting point or boiling point for identification purposes.

The range of acids capable of being detected is limited by the titration technique because it is not possible to raise the column temperature to a value high enough for a reasonable rate of movement of the longer chain fatty acids. Recently the range has been extended to include the C₁₈ acids, using methyl esters instead of the free acids, with the gas density meter⁸ as the detector. In Figure 5 is shown the separation of a variety of saturated and unsaturated methyl esters carried out with a 4 ft. column at 200° C. The range covered here is from methyl-*n*-heptanoate to methyl stearate in a time of 100 minutes. With this type

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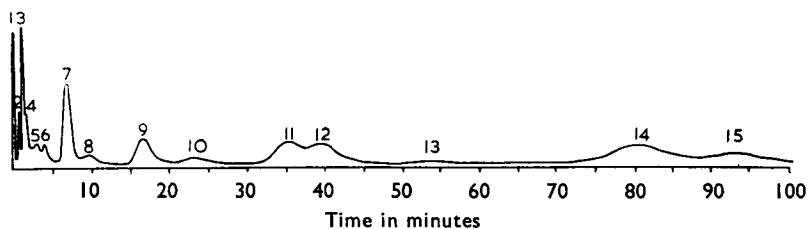


FIG. 5. The separation of methyl esters of some branched- and straight-chain saturated acids C_8 to C_{18} and also palmitoleic and oleic acids on a 4 ft. column with Apiezon M vacuum grease as stationary phase at 197°C . Nitrogen pressure 76.5 cm. mercury, nitrogen flow rate 98 ml./min.

Peaks in order of appearance:—

- | | |
|----------------------------------|-------------------------------------|
| 1. Air | 9. Methyl- <i>n</i> -tetradecanoate |
| 2. Methyl- <i>n</i> -heptanoate | 10. Methyl-10-methyltetradecanoate |
| 3. Methyl- <i>n</i> -octanoate | 11. Methyl- <i>cis</i> -palmitoleic |
| 4. Methyl- <i>n</i> -nonanoate | 12. Methyl- <i>n</i> -hexadecanoate |
| 5. Methyl- <i>n</i> -decanoate | 13. Methyl-14-methylhexadecanoate |
| 6. Methyl-8-methyldecanoate | 14. Methyl oleate |
| 7. Methyl- <i>n</i> -dodecanoate | 15. Methyl- <i>n</i> -octadecanoate |
| 8. Methyl 10-methyldodecanoate | |

of column acids differing in chain length by one carbon atom can be separated, (cf. an earlier attempt by Cropper and Heywood⁹, an *iso* or anti-*iso* acid can be separated from its straight chain homologue, and mono- and di-unsaturated acids can be separated from the corresponding straight-chain saturated acids.

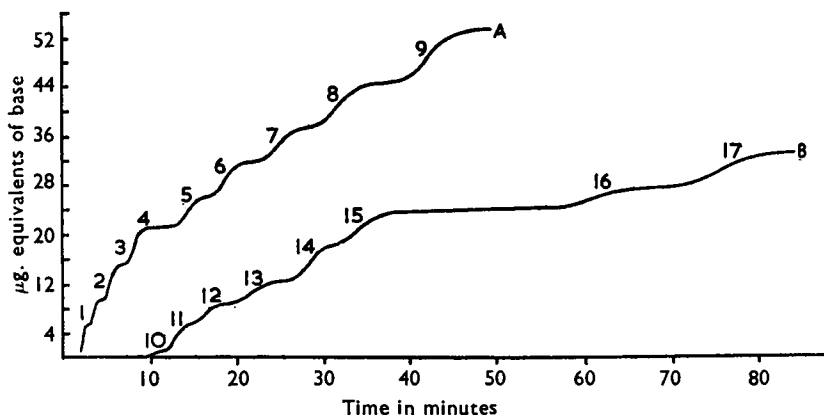


FIG. 6. The separation of 15 aliphatic amines on a 4 ft. column, with liquid paraffin as the stationary phase, at 100°C .

Curve A: Rate of flow of nitrogen, 5.7 ml./min.; pressure of nitrogen, 7.5 cm. Hg.

Curve B: Rate of flow of nitrogen 18.7 ml./min.; pressure of nitrogen, 22.5 cm. Hg.

- | | | |
|---------------------------|-------------------------------|---------------------------------|
| 1. Methylamine | 7. Diisopropylamine | 13. <i>iso</i> Hexylamine |
| 2. Ethylamine | 8. <i>iso</i> Amylamine | 14. <i>n</i> -Hexylamine |
| 3. <i>iso</i> Propylamine | 9. <i>n</i> -Amylamine | 15. Di- <i>sec.</i> -butylamine |
| 4. <i>n</i> -Propylamine | 10. <i>iso</i> Amylamine | 16. <i>n</i> -Heptylamine |
| 5. <i>iso</i> Butylamine | 11. <i>n</i> -Amylamine | 17. Di- <i>n</i> -butylamine |
| 6. <i>n</i> -Butylamine | 12. Di- <i>n</i> -propylamine | |

The separation of volatile bases

The bases dealt with so far by this technique have been ammonia and the three methylamines¹⁰, higher aliphatic amines and substituted pyridines¹¹ and aromatic amines¹². In all cases titration was used for detection

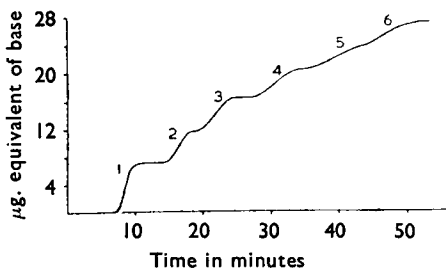


FIG. 7. The separation of aromatic bases. Column length, 4 ft.; stationary phase, liquid paraffin; temperature, 137°C.; rate of flow of nitrogen, 75 ml./min.; pressure of nitrogen, 47.2 cm. Hg; titration carried out in glacial acetic acid with perchloric acid.

- | | |
|--------------------|----------------------------------|
| 1. Aniline | 4. <i>m</i> -4-Xylidine |
| 2. Methylaniline | 5. Dimethyl- <i>o</i> -toluidine |
| 3. Dimethylaniline | 6. Diethylaniline |

and estimation. The aromatic amines, because of their weak basicity, were titrated in glacial acetic acid solution with perchloric acid. An example of a separation of aliphatic amines is shown in Figure 6, and an example of a separation of aromatic bases in Figure 7.

The separation of other types of compounds

Two further examples of some typical separations are shown in Figures 8 and 9. Figure 8 shows a range of alcohols from methanol to *n*-pentanol, the separation being completed in only 32 minutes. The separation of ethanol and *isopropanol* would be improved if the column were run more slowly. Figure 9 shows the separation of five ketones carried out in less than thirty minutes.

FACTORS INFLUENCING THE SEPARATIONS

At a constant temperature and a constant flow rate of mobile phase the relative times of emergence of a mixture of compounds are dependent on the differences in their free energies of solution in the stationary phase. In solvents such as paraffin hydrocarbons the forces involved in solution are Van der Waals forces, which vary with molecular weight, configuration

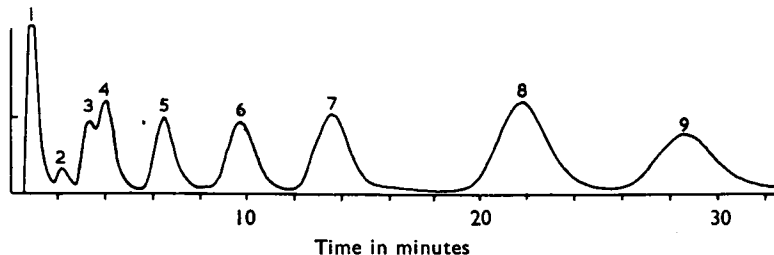


FIG. 8. The separation of alcohols. Column length, 4 ft.; stationary phase, benzylidiphenyl; temperature, 100°C.; rate of flow of nitrogen, 12 ml./min.; pressure of nitrogen, 13 cm. Hg.

- | | | |
|-------------|------------------------|----------------------------|
| 1. Air | 4. <i>iso</i> Propanol | 7. <i>n</i> -Butanol |
| 2. Methanol | 5. <i>n</i> -Propanol | 8. <i>iso</i> Amyl alcohol |
| 3. Ethanol | 6. <i>iso</i> Butanol | 9. <i>n</i> -Amyl alcohol. |

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etc., and not with the polarity of the solute molecule. In such paraffinic stationary phases therefore separations are based on differences in molecular weight, etc.

In more polar stationary phases such as polyethers, long-chain alcohols, aromatic hydrocarbons etc., polar forces such as hydrogen bonding are also involved. Separations in these phases can therefore take advantage of differences in polarity of the solute molecules. Aliphatic amines for

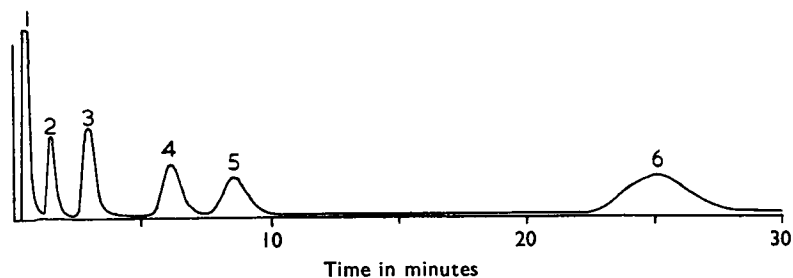


FIG. 9. The Separation of ketones. Column length, 4 ft.; stationary phase, paraffin wax; temperature, 100°C.; rate of flow of nitrogen, 24.8 ml./min.; pressure of nitrogen, 17.8 cm. Hg.

- | | |
|------------------------|----------------------------------|
| 1. Air | 4. Diethyl ketone |
| 2. Dimethyl ketone | 5. Methylisobutyl ketone |
| 3. Methyl ethyl ketone | 6. Methyl- <i>n</i> -amyl ketone |

example fall into three types, primary, secondary and tertiary. Primary and secondary amines possess an -NH group and are therefore capable of hydrogen bonding with a suitable acceptor molecule such as an alcohol or an ether. Tertiary amines possess no -NH group and cannot hydrogen bond in the same way. Thus methylamine, dimethylamine and trimethylamine emerge in order of molecular weight from paraffin columns and in order of hydrogen bonding power (i.e. the reverse order) from columns with substances such as glycerol as stationary phase. A study of the behaviour of different types of amine on two types of column, one with a paraffin hydrocarbon stationary phase and the other a polyether stationary phase showed that plotting relative retention volumes, relative that is to a standard substance, in one stationary phase against the corresponding values obtained in the other gave rise to a family of straight lines of different slope. Each line corresponded to a different type of amine, i.e. primary, secondary, tertiary, diamino, hydroxyamine, so that an unknown amine could be classified by running it on both types of column and comparing the values obtained with the standard values from the graph. The different slopes refer to different ratios of Van der Waals solution forces to Van der Waals and hydrogen bonding solution forces¹¹.

A similar study carried out with a wider range of substances, comparing behaviour in the paraffin hydrocarbon stationary phase with that in an aromatic hydrocarbon stationary phase gave rise to the result shown in Figure 10. Again substances of similar chemical type fall along one of a family of straight lines. In practice it was found that aliphatic

hydrocarbons, alkyl chlorides, bromides, iodides, amines, alcohols, methyl ketones, nitriles and nitro compounds could be readily differentiated. In this manner a great deal of information can be obtained as to the

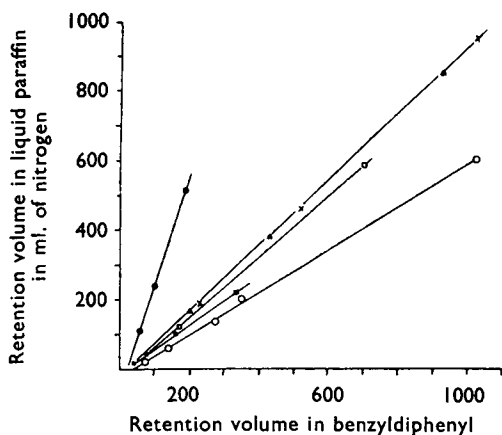


FIG. 10. Graphical representation of relative interaction forces in two types of stationary phase, liquid paraffin and benzyldiphenyl.

- *n*-Aliphatic hydrocarbons
- ▲—▲ Aromatic hydrocarbons
- ×—× *iso*Alkyl iodides
- *n*-Alkyl iodides
- Alcohols
- Ketones.

structure of an unknown compound.

To conclude, the advantages of gas-liquid chromatography can be seen to be: (1) highly refined separations can be carried out very rapidly; (2) the technique can be used with quantities of material ranging from micrograms to grams; (3) a study of the chromatographic behaviour of an unknown substance can give valuable information as to its structure; (4) it is applicable to all substances capable of being distilled, and (5) suitable commercial apparatus is already available in the United Kingdom and the United States. Indeed

the advantages of the technique over analytical distillation in terms of efficiency, time and convenience are so great that in time it will replace the older technique.

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