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# Laboratoriums- und Meßtechnik in der Chemie

## *Trennverfahren**

## Quantitative Aspects of Gas Chromatography**

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### Introduction

During the last few years the developments in gas chromatography have been largely confined to improving the efficiency or resolving power of column systems, developing more sensitive detectors and extending the fields of application. Relatively little work however has been published dealing with the accuracy and precision of analyses carried out with gas chromatographic equipment. There is a general feeling amongst workers using the technique that the precision of quantitative results obtained from the gas chromatograph leaves a lot to be desired and it is not surprising that considerable attention is now being paid to the accuracy that can be obtained from gas chromatographic apparatus. This paper will consider the various factors which may produce sources of error in quantitative analysis, and will attempt to suggest techniques for overcoming them. Methods for examining the different parts of the equipment will be described with a view to assessing their effect on quantitative accuracy and recommendations will be given as to the best method of operation of the apparatus and the best method of assessing the result. As the technique develops, the design of gas chromatographic equipment will change and some of the recommendations given in this paper may need modification or eventually may even become unnecessary. However, it is hoped that the ideas put forward will aid present day workers in obtaining improved accuracy and precision from their apparatus.

### Factors Affecting Quantitative Accuracy

In any gas chromatographic analysis there are five main sources of error that can affect the accuracy of the result:

1. The sample obtained must be representative and it must be stored in such a manner that its composition remains unaltered throughout a series of duplicate or replicate tests.
2. The injection system must be capable of placing a representative sample on the column and the injection device must not permit preferential loss of low boiling materials. Its absolute precision must be sufficient to permit a charge to be placed on the column to produce peaks of the required size.
3. The column system must be capable of giving adequate separation of the substances of interest, and the adsorption effects of the support or column wall must be reduced to the limit where the accuracy of the quantitative analysis is not affected. If this is not possible, the effect of the residual adsorption must be taken into account in assessing the results.
4. The detecting system (which includes both the detector itself and any ancillary amplifier and recorder) must have a linear or known response.
5. The results obtained, whether as a chromatogram drawn upon a chart or as a series of numbers from a digital integrator, must be correctly interpreted.

The effect of each of these aspects will now be considered in detail.

* Aus urheberrechtlichen Gründen können folgende drei an der ILMAC-Fachtagung 1962 des Schweizerischen Chemiker-Verbandes über Trennverfahren gehaltenen Vorträge nicht abgedruckt werden:

E. Stahl: Entwicklung und Anwendung der Dünnschichtchromatographie.

M. Brenner: Dünnschichtchromatographie, $R_f$-Wert und chemische Struktur.

H.R. Bolliger: Die Dünnschichtchromatographie der Vitamine.

Der stoffliche Inhalt dieser Vorträge findet sich, von denselben Autoren behandelt, in dem 1962 vom Springer-Verlag, Berlin/Göttingen/Heidelberg, herausgebrachten Buch: *Dünnschichtchromatographie.* Ein Laboratoriumshandbuch. Herausgegeben von Egon Stahl.

Der Vortrag von E. Bayer (Präparative Gas-Chromatographie) erscheint erst im Juni-Heft

** Lecture presented at the Technical Congress of the 2nd International Exhibition of Laboratory Measurement and Automation Techniques in Chemistry (ILMAC), October 15th to 20th 1962, in Basel (Switzerland).

### Sample

Gas chromatography is inherently a method for analysing volatile substances; if these substances are highly volatile (e.g. low boiling esters, alcohols or hydrocarbons) then the method of obtaining the sample and the method of storing it can be a considerable source of error if carelessly carried out. If a sample containing a small percentage of a very volatile substance is contained in a stoppered vessel half filled and if $k$ is the partition coefficient of that substance with respect to the bulk of the liquid, then already $\frac{1}{k}$ of the sample is lost in the air space above the liquid. Each time the vessel is opened to take a sample, due to the high rate of diffusion of the sample in the gas phase, $\frac{1}{k}$ of the substance contained in the bottle is lost. If the vessel, half filled, contains a 1 % solution of a volatile substance and $k$ for that substance is 20 with respect to the bulk of the liquid, and if at the same time, it is assumed that there is no error associated with the gas chromatograph, then the first analysis will give a result for the component of 0·95 % instead of 1 %. The second analysis will give 0·90 % and the third 0·86 %. The difference between each result is solely due to the method of storing the sample. In the practice of gas chromatography, it is very common to store the sample in a small phial, often only about a quarter filled, and under such circumstances the errors quoted may be increased by a factor of three. It follows, therefore, that samples of volatile mixtures for analysis should be stored in vessels that are completely filled, the aperture being as narrow as possible, and the sample taken for analysis as rapidly as possible. If the sample is to be stored for any length of time it is essential to seal it in a glass tube. It should be pointed out, however, that as the partition coefficient $k$ increases, this effect is reduced, particularly if the component becomes a major constituent of the mixture.

### Injection Systems

There are two main types of injection device for liquids, one employing the hypodermic syringe and the other employing the micropipette.

Prior to the introduction of the microlitre syringe the pipette system was usually employed with the high sensitivity detectors. This was because, with the aid of the glass capillary, pipettes could be made that held a few micrograms of charge. The pipette system can be used in two ways, either by discharge onto an open column as described by MARTIN and JAMES[1] or with a closed system as described by SCOTT[2]. The former necessitates the interruption of the column flow, removal of the column closure and the discharge of the pipette onto the top of the column packing. The column closure is then replaced and column flow continued. The closed pipette system incorporates a sealed chamber exterior to the column in which the pipette is placed prior to injection. Connection is then made to the column by way of a suitable tap system and the pipette allowed to fall onto the top of the column and discharge onto the packing. The pipette is then withdrawn into the chamber by means of a magnet and the tap system closed. The pipette system tends to allow a certain loss of the more volatile constituents of the mixture into the gas phase above the packing of the column. This results in a low value being obtained for the more volatile constituents, and, if normalisation of the chromatogram has been carried out, a corresponding increase in the apparent proportion of the high boiling materials. Due to the fact that the pipette normally has a large exterior surface, this also tends to hold some of the charge from which the more volatile substances evaporate during transfer to the column. When the pipette is placed on the packing at the column temperature the higher boiling materials from the exterior surface of the pipette evaporate onto the column and again produce an apparent increase in the quantity of these components. Until recently the closed pipette system was the only method for introducing the charge onto a column operating at high inlet pressure. HURRELL[3] however, has devised a serum cap system that allows injection of samples onto a column operating at high inlet pressures, using the Hamilton microlitre syringe. With the device of HURRELL available, the microlitre syringe system of injection is likely to become the preferred method. The advantage of the syringe system of injection lies in the fact that the needle can be plunged into the column packing and thus when discharged, vapour is prevented from diffusing back into the air space above the column packing. The G.L.C. Working Group of the Standard Tar Products Tests Committee has recently carried out a series of repetitive tests in Britain to assess, amongst other factors, the representability of charges made by the two types of injection system. It is hoped that this work will be published in due course in *The Analyst*. In the meantime it can be stated that there appears to be strong evidence that the hypodermic syringe method of injection gives a more representative charge onto the column than does the pipette system. It should be pointed out, however, that if the sample concerned is relatively involatile, such as the higher fatty acid esters, high boiling hydrocarbons etc. then the relative advantage of the hypodermic syringe over the pipette system is not nearly so significant.

### Column System

It is obvious that in order to obtain accurate quantitative results the substances concerned must be completely resolved. Many papers have been published on

[1] A.J.P.MARTIN and A.T.JAMES, *Biochem. J. 63* (1956) 138–43.
[2] R.P.W.SCOTT, *Gas Chromatography* (Ed. D.H.DESTY), 1958, p. 189.
[3] R.AMOS and R.A.HURRELL, *Gas Chromatography* (Ed. M. VAN SWAAY), 1962. To be published.

column efficiency and column resolution; others have described the preparation of long columns. The problem of resolution will therefore not be considered in this paper. The second problem concerned with the column is that of adsorption of the surfaces and support material. Due to the fact that adsorption isotherms are non-linear, any adsorption on the support or column walls results in asymmetric peaks. An example of a chromatogram obtained from a column that has adsorptive properties is shown in Figure 1, peaks being due to non-polar and polar materials respectively. It may be seen that the peak for the polar material exhibits a tail and that this tail can extend a long way along the chromatogram, hardly discernable from the base line, but constituting a significant area of the peak. The area of such a peak measured either geometrically or by digital integration will not include the tail and thus an analysis using such a chromatogram will show a deficiency in this particular substance. The effect of adsorption on quantitative accuracy is at present being studied by the Research Department at W. G. Pye & Co. Ltd. and it may be of interest to describe some of the results obtained so far, to illustrate the effect of the phenomena on quantitative accuracy.

The significance of the quantity of solute adsorbed on the column on the resultant analysis will be dependent upon the size of the charge. The smaller the charge the greater the amount of solute existing in the tail of the peak relative to that contained within the peak. A 120 cm column packed with brick dust, a relatively adsorptive support, carrying 10 % of squalane was used to separate mixtures of decane, toluene and methyl *n*-hexoate. The polarity of these substances increases from decane to methyl *n*-hexoate. The column was used in conjunction with a macro argon ionisation detector whose linearity

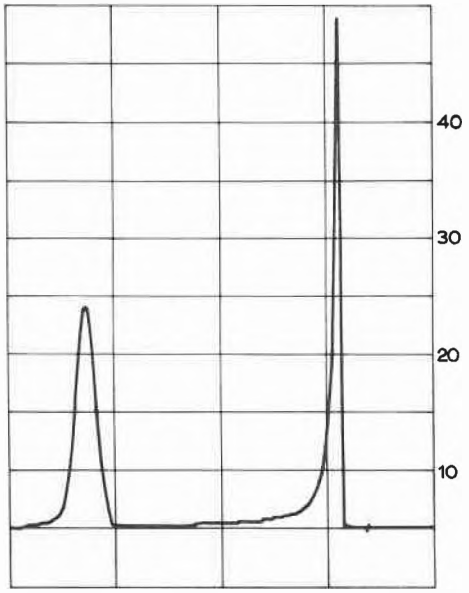
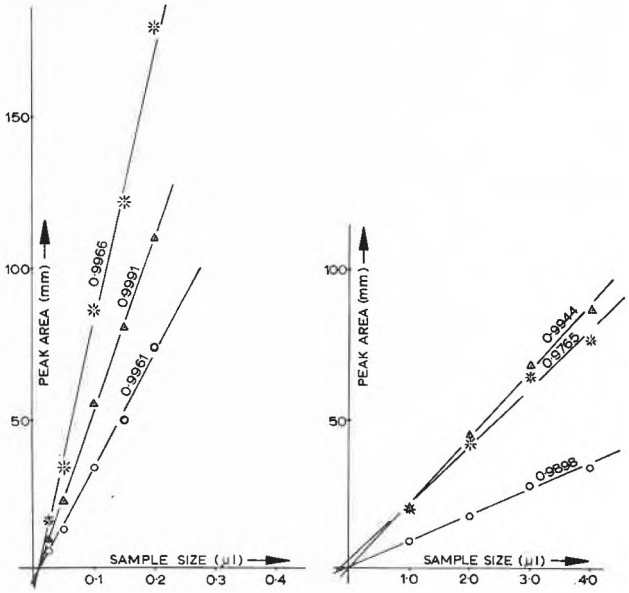



*Fig. 1.* Chromatogram of Polar and Non-Polar Compounds Obtained from a Column with Significant Adsorption Properties




Graphic I: Sensitivity × 10. Column Flow Rate 54·5 ml/min

Graphic II: Sensitivity × 10. Column Flow Rate 54·5 ml/min Detector Flow 300 ml/min

*Fig. 2.* Effect of Charge Size on Quantitative Accuracy. A column 5% Squalane on Brickdust (see note [3])

had been proved by the method of FOWLIS and SCOTT[4] to be described later. The Hamilton micro syringe was used for injecting the sample onto the column. A series of samples of increasing size was placed onto the column and the peak areas for each component measured by means of an electronic integrator. Two series of experiments were carried out using two detector sensitivities. Detector voltage and amplifier gain were identical in each case, the only difference in the second series of experiments being the provision of a secondary flow of argon through the detector of 300 ml/min to dilute the sample as it was eluted. The curves obtained by plotting charge size against each peak area are shown in Figure 2. It should be emphasised that the linear mass/peak area curves shown were obtained with a complete chromatographic system, and include errors associated with sample injection as well as column, detector and peak area measurement effects. The correlation coefficients of these curves are shown, together with the intercepts of the curves on the area axis, in Table 1.

It is seen that the intercept due to the adsorption is less at the lower sensitivity due to the effect of adsorp-

Table 1

| Sample | Low Sensitivity | | High Sensitivity | |
|---|---|---|---|---|
| | Correlation Coefficient | Intercept | Correlation Coefficient | Intercept |
| *n*-Decane | 0·976 | 2·7 | 0·996 | −5·9 |
| Toluene | 0·989 | 0·7 | 0·999 | −5·0 |
| Methyl *n*-Hexoate | 0·994 | −0·3 | 0·996 | −3·4 |

[4] I. E. FOWLIS and R. P. W. SCOTT, *Journal of Chromatography*. To be published.

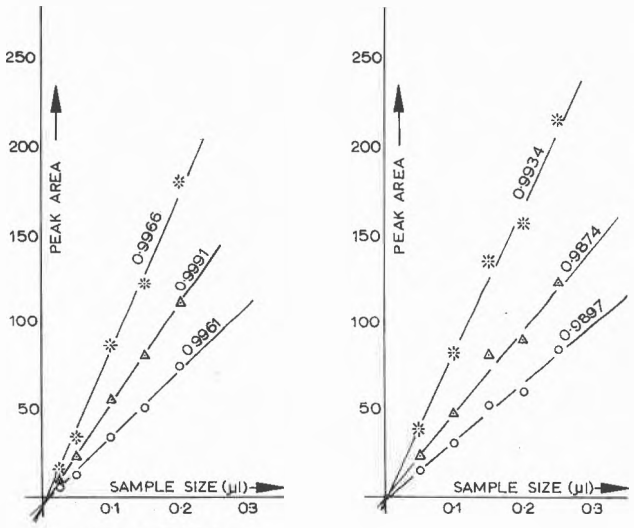



*Fig. 3.* Effect of Different Supports on Quantitative Accuracy (see Note [5])

tion being less significant with the larger charge. An intercept on the peak area axis could be due to the syringe not having zero charge when reading zero. This possibility was eliminated by ensuring that the same volume of charge was used in both sets of experiments, the concentration of the test substance in the solvent being adjusted to suit the overall sensitivity of the system. The change of intercept with sensitivity confirmed the significance of the adsorption effect at higher sensitivities.

Table 2

| Sample | Fire Brick Support | | Celite Support | |
|---|---|---|---|---|
| | Correlation Coefficient | Intercept | Correlation Coefficient | Intercept |
| *n*-Decane | 0·996 | −5·9 | 0·993 | −3·42 |
| Toluene | 0·999 | −5·0 | 0·990 | −0·28 |
| Methyl *n*-Hexoate | 0·996 | −3·4 | 0·987 | −2·95 |

The effect of the relative adsorptive capacities of different supports is shown in Figure 3, taken from a similar set of experiments which compared brick dust and celite as the supports. In Table 2 the correlation coefficient for the substances on each column is shown, together with the area axis intercepts. It is seen that the adsorption of brick dust is significantly greater than that of celite, and that celite is preferable for quantitative analysis. It is also seen that the effect of adsorption of the support would be increased at higher detector sensitivities, and therefore supports with the smallest adsorptive capacity should be employed and the detector should be adjusted to the minimum sensitivity commensurate with other column requirements so that a maximum charge size can be used. The chromatography of very high boiling substances, such as sterols, or the determination of trace components in a mixture, may call for maximum detector sensitivity; under these circumstances a mass/peak area curve should be determined prior to the analysis in order to correct for the

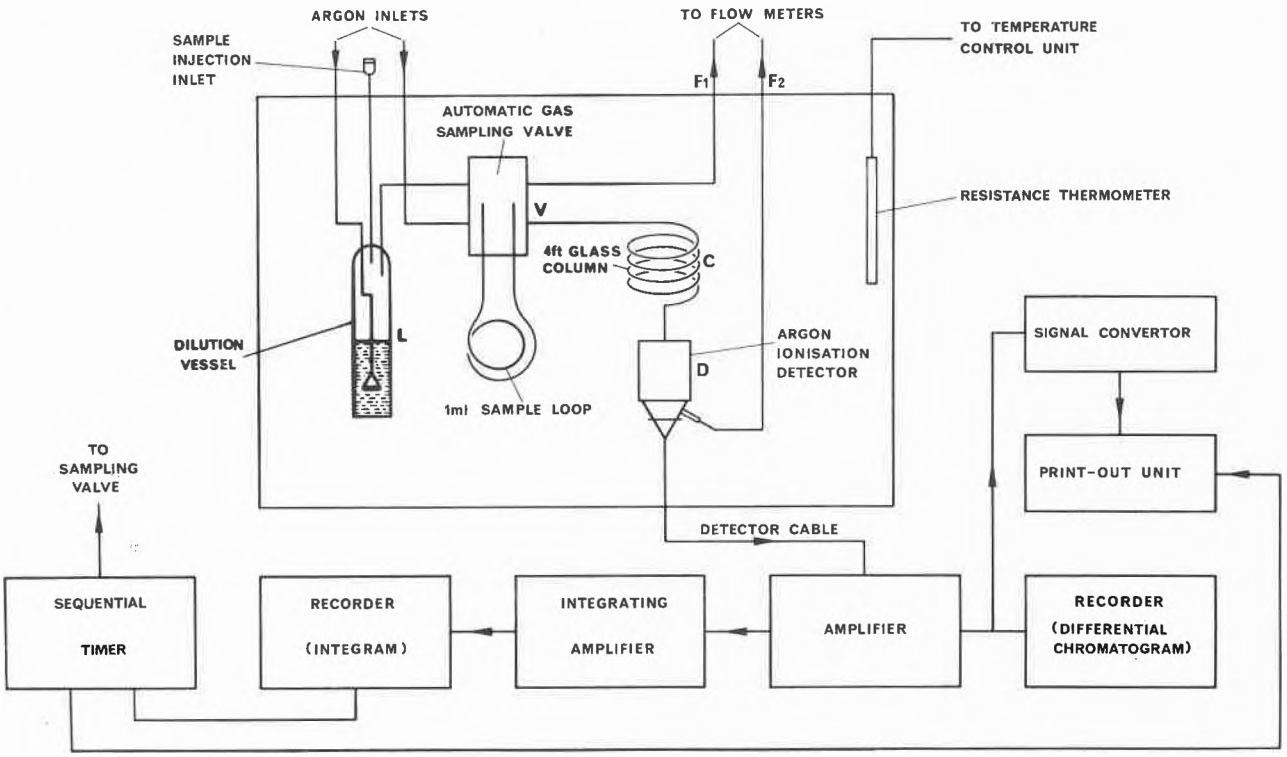



*Fig. 4.* Detector Calibration Apparatus

proportion of the substance which is adsorbed and is not included under the peak. It is also obvious that the method of measurement of the peak area becomes critical when measuring asymmetric peaks due to adsorption. This problem however will be dealt with under the assessment of chromatographic results.

### Detector Systems

The detecting system consists of the detector itself together with ancillary amplifier or bridge circuits and recorder. Due to the development of amplifiers for other fields of research over a number of years, this part of the gas chromatographic equipment is probably the most precise. Amplifiers and recording devices are manufactured with linearity deviations of less than 0·25 %. This section will only consider the performance of the detector. Two methods for examining detector performance have so far been published. One described by DESTY[5] utilises the diffusion of a solute from the open end of a glass capillary into a gas stream which is subsequently led to the detector. By adjustment of the quantity of solute in the capillary various concentrations of solute vapour can be fed to the detector, and its linearity examined. This method however, can only calibrate the detector for one substance at a time. The second method described by LOVELOCK[6] depends on the continual dilution of a solute contained in a vessel by a gas stream which then passes through the detector. The concentration of solute in the gas stream passing from the dilution vessel decays exponentially with time, and thus if a detector is linear, a plot of the logarithm of the detector signal against time will give a straight line. This method has the disadvantage that at a low concentration the rate of change of solute concentration is no longer solely dependent upon the diluent gas stream, but on the rate of desorption of the solute from the surface of the dilution vessel. This system, however, has been modified by FOWLIS and SCOTT[4], who introduced

[5] D.H. DESTY, C.J. GEACH and A. GOLDUP, *Gas Chromatography* (Ed. R.P.W. SCOTT), 1960, page 46.

[6] J.E. LOVELOCK, *Gas Chromatography* (Ed. R.P.W. SCOTT), 1960, page 26.

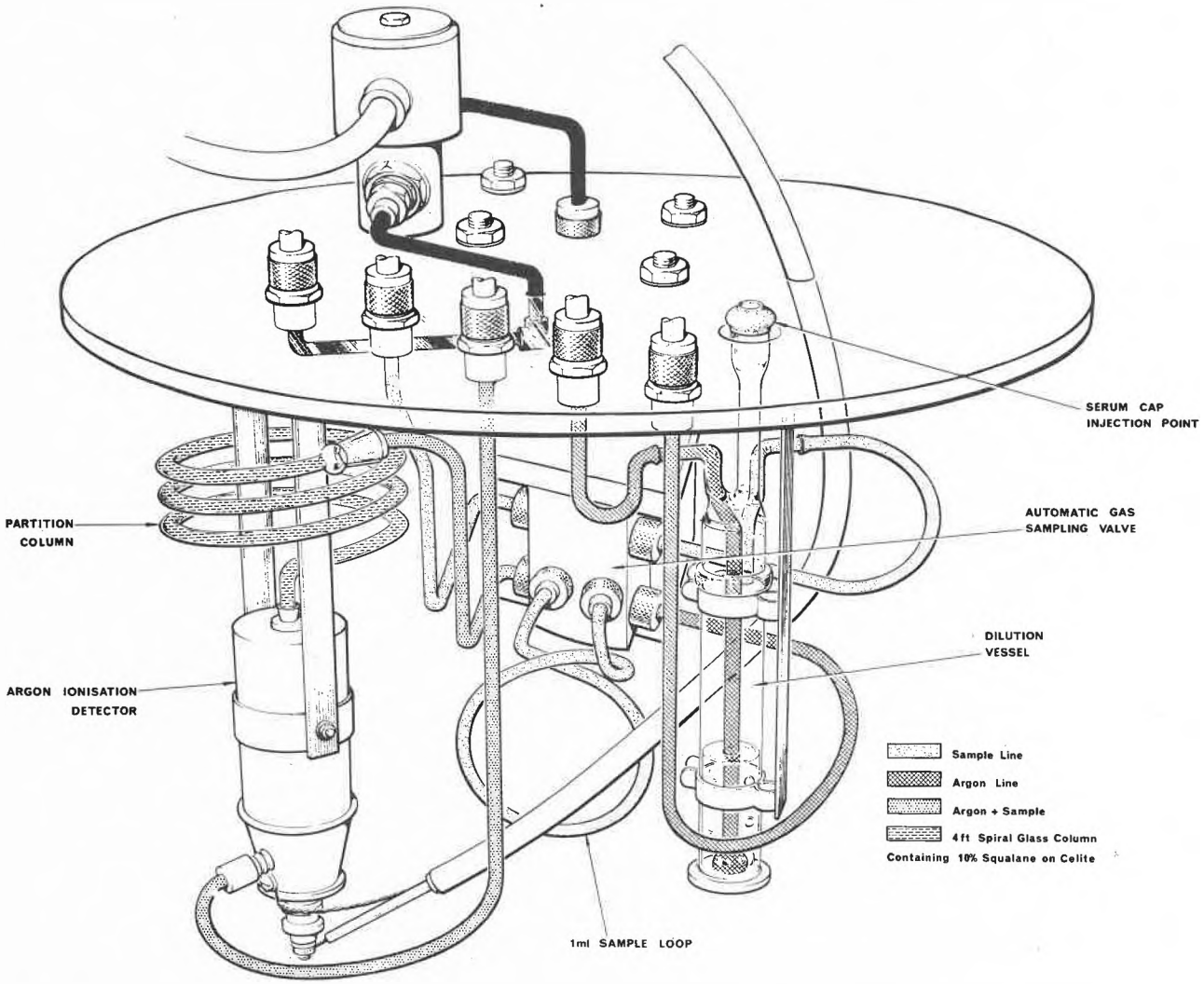



*Fig. 5.* Detector Calibration Apparatus. Detail of Dilution Vessel and Column System

a high boiling liquid into the dilution vessel producing what was effectively a high capacity one plate chromatographic column. The concentration of solute in the eluent gas again decays logarithmically with time but due to the bulk of solute contained in the liquid at all times the effect of the adsorption on the surface of the system is rendered insignificant. Figure 4 illustrates schematically the arrangement employed, Argon, carefully dried by passage through activated Linde Molecular Sieve 5A, is supplied to the glass dilution vessel and to the chromatographic column via an automatic gas sampling valve. The dilution vessel is charged at the beginning of a run by hypodermic syringe injection through a serum cap. An automatic timing system operating the sampling valve at given intervals of time places a constant volume sample of the exit gas from the dilution vessel in line with the argon supply to the chromatographic column and detector. The detector produces a conventional chromatogram after each injection, peak area integration being provided by the use of an electronic integrating amplifier. Automatic zero reset of the integral record is carried out by the timing control unit. Facilities are provided to include digital area print-out if required. Figure 5 shows in greater detail the construction of those parts of the apparatus enclosed in a carefully temperature controlled oven.

Providing the resolution of the column is adequate any number of solutes can be introduced into the dilution vessel at one time, the plot of the logarithm of their respective peak areas against time being a straight line providing the response of the detector is linear. It can also be shown that the system can simultaneously be used to provide relative response factors. An example of the results obtained from examination of the macro argon detector, using this system, is shown in Figure 6. The curves shown have correlation coefficients of better than 0·99 and were obtained for the mixture of chlorobenzene, toluene, chloroform and di-iso-propyl ether; the results are tabulated in Table 3.

Investigation of the Gow-Mac density bridge using the same dilution system is shown in Figure 7. A critical investigation of all types of detector using this apparatus is being undertaken, but of necessity this project must be of a somewhat lenthy nature. The examination of the

Table 3

| Compound | Correlation Coefficient | Number of Observations | Confidence Limit % | Concentration Range Studied |
|---|---|---|---|---|
| Chloroform | 1·0 | 4 | > 99·0 | $10^3$ |
| Di-isopropyl Ether | > 0·999 < 1·0 | 4 | > 99·0 | $10^3$ |
| Toluene | > 0·999 < 1·0 | 8 | > 99·0 | $10^1$ |
| Chlorobenzene | > 0·999 < 1·0 | 8 | > 99·0 | $10^1$ |

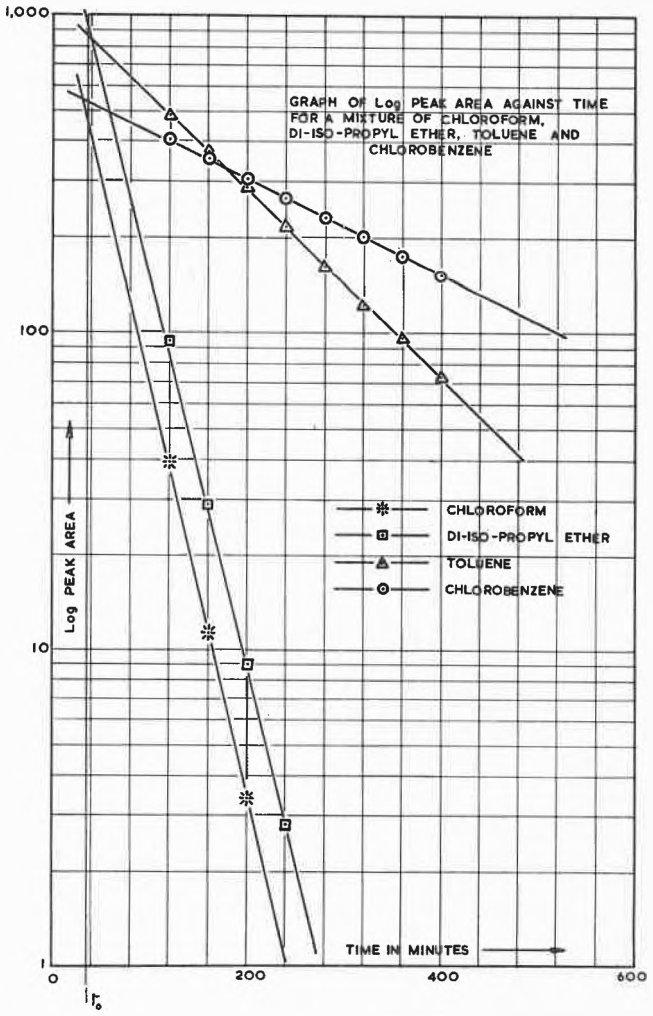



*Fig. 6.* Macro Argon Ionisation Detector Investigation (section of a typical linearity graph)

detector of each chromatographic equipment by the user, using this method is hardly practical; it should not in fact be necessary. Equipment manufacturers should be responsible for defining the range of any detector they

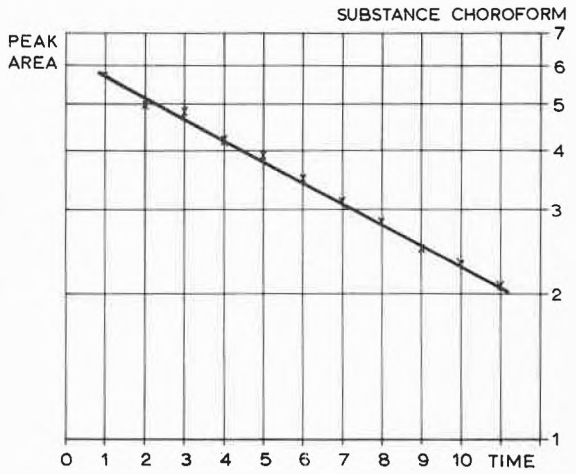



*Fig. 7.* Calibration Curve for Gow-Mac Gas Density Balance Using Sample Dilution System

make. In our experience it has been generally found that errors in quantitative analysis are not due to non-linearity of detectors but to the other factors described in this paper. Because adsorption in the column system has its greatest effect when using very small charges and thus very sensitive detectors, the effect of the adsorption has often been misinterpreted as non-linearity of the high sensitivity detectors.

### Quantitative Interpretation of Gas Chromatographic Results

The quantitative assessment of a chromatogram is obtained from the measurement of peak heights or peak areas. Peak heights can be taken as a direct measurement from the differential record, peak areas can be obtained by geometrical measurements or by the use of an automatic integrator. The use of peak height measurements, although probably the simplest method of obtaining quantitative results, has a disadvantage in that peak heights are very dependent on constant column operating conditions. Furthermore, the precision of the measurement is not merely dependent on the mass of substance present, but on its retention volume. Peaks representing equal masses of substances with different retention ratios may have widely different peak heights. The accuracy of any result obtained by peak height measurements also depend upon the peak being symmetrical or gaussian. For relatively non-polar materials chromatographed with low sensitivity detectors, peak height measurements could give satisfactory results. However, when polar materials are being chromatographed on columns that have adsorption effects, and particularly when high sensitivity detectors are employed, the peak height measurement technique can give serious errors in quantitative analysis.

There are several methods that have been described for determining areas from differential records. Peak areas can be mesured with a planimeter, by calculating the product of peak height and peak width at half the peak height, by measuring the area of the triangle formed by the tangents to the points of inflection of the gaussian curve with the peak base and also by cutting the peak out and weighing it. The last method has the disadvantage of being tedious and furthermore destroys the record of results. It does, however, take into account asymmetric peaks and if carefully carried out by an experienced operator, can give relatively precise results. The other three methods of peak area assessment have been investigated by the Gas Chromatography Working Group of the Standard Tar Products Test Committee. These results are due for publication shortly in *The Analyst*. The repeatability of each of the three methods of area measurement was determined for the values obtained from a number of different operators from a number of different laboratories. The results obtained indicate that the method of measuring peak area by calculation of the product of peak height and peak width at half the peak height gives the most repeatable results. If, however, the peak is asymmetric due to adsorption, or there is incomplete resolution of the peaks, then the best method will be either to take the average of a large number of readings determined with the aid of a planimeter or by cutting the peak out and weighing it.

Providing the peaks are resolved, a better method of area measurement is by use of one of the automatic integrators. There are two main types of integrator used in gas chromatographic equipment, the digital integrator and the electronic integrating amplifier. For satisfactory operation of both methods of integration a very stable base line is required. Unless a very expensive form of digital integrator is employed, this type of instrument requires peaks to be completely resolved. The digital integrator must initiate at a particular signal value. That is to say counting will not begin until a

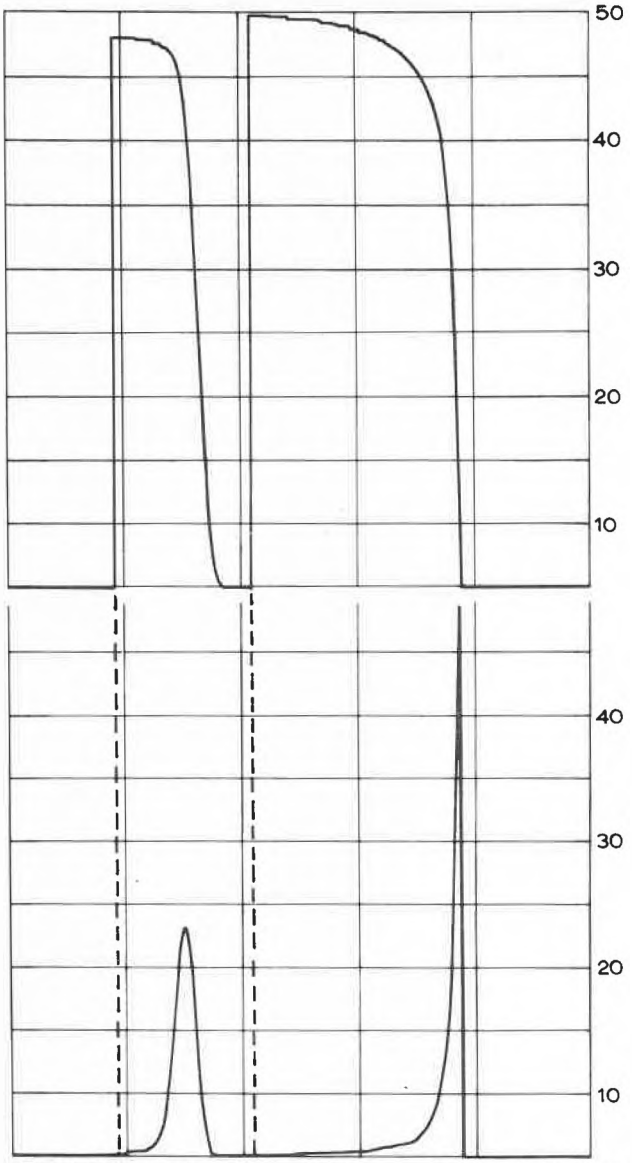



*Fig. 8.* Differential and Integral Curves for Polar and Non-Polar Compounds Obtained from a Column with Significant Adsorptive Properties

finite deflection of the recorder has taken place. This means that the digital integrator can not take into account the long tail of an asymmetric peak, when its tail is below the initial deflection required for counting to begin. The results from the digital integrator are, however, much easier to interpret as they are merely a series of figures that can be easily handled arithmetically. The electronic integrator produces an integram as opposed to a differential chromatogram: this takes the form of steps on a chart. Results from the electronic integrator are therefore still dependent on the measurement of lengths, and the use of the electronic integrator is more tedious and time-consuming that the digital integrator. However, the electronic integrator can take into account the long tail of an adsorption peak, and it will contue to integrate this tail until either it has become insignificant or another peak appears for integration. Figure 8 shows the integral and differential curves obtained for two peaks, one affected by the adsorptive capacity of the column and the other a pure gaussian peak. It is seen that the electronic integrator takes into account the thin tail of the asymmetric peak. An example of the advantages of the use of the electronic integrator over the geometric method of measuring peaks is shown in Figure 9, which shows a plot of peak area against charge size that has been obtained in experiments investigating adsorption effects of column supports.

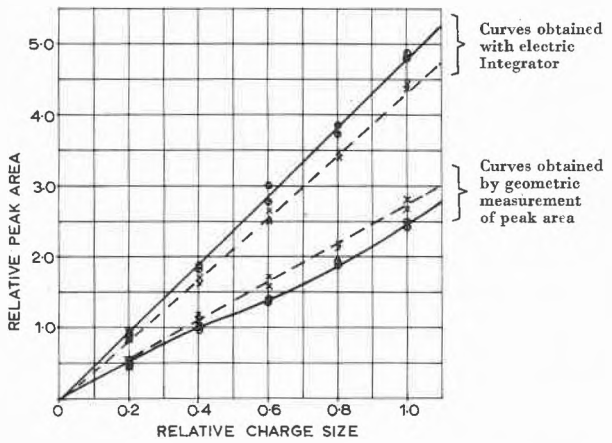



*Fig. 9.* Graph of Peak Area Against Charge Size Using Different Methods of Area Assesment. – – – *n*-Nonane, —— Methyl Alcohol

The linear curve is that obtained from the electronic integrator, and includes the tail of the peak. The other curve is obtained from the same peaks but the area is measured by means of the product of the peak height times the peak width at half the peak height. It is clear that the method of measuring peak areas can seriously affect quantitative analysis. It is apparent that the best method of measuring peak areas will vary from one set of operating conditions to another. At one extreme, chromatographing non-polar materials with a celite support using low sensitivity detectors and therefore large charges, accurate quantitative results could be obtained by the use of peak height measurements only. At the other extreme, separating polar materials on a column system which exhibits support adsorption and using a high sensitivity detector (and therefore small charges) quantitative accuracy can only be obtained with the use of tedious area measurements or the electronic integrator.

One final aspect in the assessment of quantitative results is the method of obtaining the mass percentage analysis from the peak areas on the chromatogram. There are two alternatives: either by normalisation or by the use of an internal standard. Normalisation is a method by which each peak area is corrected for the respective response factors of the detector and it is expressed as a percentage of the total area of all the corrected peaks. This method is satisfactory providing it is certain that the total charge has been eluted from the column and all the peaks are taken into account. The more common method is to add a known quantity of a standard substance, the mass percentage of any other substance present being obtained by direct comparison with the peak area of the standard.

## Conclusions

Many parts of the gas chromatographic apparatus can contribute errors to quantitative analysis. Generally the following conditions should be adhered to wherever possible.

1. The sample should be stored correctly.
2. A hypodermic syringe method of injection should be employed.
3. The adsorption of the support should be reduced to a minimum.
4. The detector should be operated at the minimum sensitivity commensurate with the other column requirements. When small charges and very high sensitivities have to be employed, a calibration should be carried out to account for the effects of adsorption or other factors affecting peak symmetry.
5. The detector used should have been proved to have a linear response over the sensitivity range of operation. For detectors with significant non-linearity, mass/peak area calibration *must* be carried out for each substance under the column conditions to be used for analysis. Any change in substance or column conditions will necessitate recalibration.
6. With large charges and low sensitivity detectors chromatographing substances of a non polar nature, quantitative analysis can be obtained with peak height measurements. If adsorption effects are present, it is essential to use peak area measurements that must be assessed either by planimeter, by cutting the peak out and weighing it or by the use of an electronic integrator. The digital integrator gives a more easily interpreted result, the electronic integrator can assess the area of asymmetric peaks more accurately.