THE PROBLEM OF DETECTION

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ABSTRACT

A number of untried ideas, or ideas not properly worked out, which might improve or supplement present chromatographic techniques are suggested. For high sensitivity the active part of a detector should be small to lessen troubles due to adsorbtion and to maintain highest possible concentrations of substances to be measured with a short time constant. Further, the problems of attaining highly quantitative results demand that measurement be made on simple decomposition products, e.g. water, CO_2, CH_4, etc., rather than the parent substances, which have to be individually calibrated. By splitting the chromatographic stream into several fractions and performing simple reactions on each, a quantitative elementary analysis of each chromatographic peak should be possible.

One of the most attractive methods of accurate measurement would be conversion first to water. Three methods are discussed in outline: electrolysis, chemical amplification, and exchange of the hydrogen in the water for tritium followed by absorption in a photographic film and counting developed grains. This latter method is probably the most sensitive that could be devised.

A number of other miscellaneous suggestions are made.

INTRODUCTION

To speak at an occasion such as this, celebrating the centenary of Tswett's birth and the twentieth and thirtieth anniversaries of the publication of the papers on gas and partition chromatography, is a great honour. But it also puts me in a great difficulty. I have done little work on chromatography for very many years past. I am no historian, and have not read the literature thoroughly enough to be able to give a fair review of others' work. I am therefore reduced, if I wish to avoid telling you what you already know, to giving an essentially speculative account of the various things that I think could be more or less easily done to improve or supplement present techniques, and I should warn you that many of the suggestions are quite untried. I must also ask your indulgence in that I have extended my consideration to detectors for liquid chromatography as well. Many facets are common to both, but it may, in general, be said that while gas chromatographic detectors excel in sensitivity, some of the methods used in liquid chromatography are selective for groups of substances in a way that is of great value in examining complicated mixtures, particularly those of biological origin.

THE PROBLEM OF DETECTION

Though gas–liquid chromatography is now some twenty years old, a surprising number of problems of technique have not yet been adequately solved. Thus it is still common practice to include a standard reference substance in any mixture to reduce errors in the amount of material put on the column, or to allow for a change in the performance of the column or the detector.

But in some respects, perhaps, the performance of the detectors shows the biggest gap between what is provided and the ideal. The very first method of detection that James and I used, titration, had a virtue of giving a response that did not require calibration. There are few detectors at present in use of which this can be said. Even the second we used, the gas-density balance, gave the mass if the molecular weight were known, and this could be determined if a second run were made in another carrier gas. A variant of this technique is now being advertised as a great forward step!

There have, indeed, been enormous increases in sensitivity, and the hydrogen flame, Lovelock's various ionization detectors, and the various spectroscopic detectors are prime examples. So far, however, the sensitivity of some biological detectors has not nearly been reached. Thus man's nose and the bee's perception of a sexual pheromone† are respectively suggested to be 10^8 and 10^5 times more sensitive than Lovelock's recently described electron-capture detector.

This is not the place to discuss the mechanism of the biological detectors, but perhaps two aspects of them may be relevant. I believe that they involve a chemical multiplication, and that the small size of the essential detector element permits the concentration at the essential part to be relatively high, in spite of the extreme dilution.

No one has yet succeeded in making detectors very small, but the success of the capillary column is due to its small diameter, and its small volume per theoretical plate. As a result of the latter, though the column is very long, the concentration of each peak is relatively high, and providing a detector with a relatively small dead volume can be employed high sensitivity results.

But no detector, depending upon the same properties in the carrier gas as in the substance to be measured, e.g. thermal conductivity, viscosity or gas density, can ever be very sensitive. With any such general property, the constancy of pressure, temperature and rate of flow, absence of vibration and low levels of electrical noise in the measuring part, impose impossible demands for very high sensitivity. By disrupting the substance and using the ionization of the whole molecule, or its conversion products, while the carrier gas remains un-ionized, or by changing the nature of the ions, high sensitivities can be attained. High sensitivities can also be reached by using radiation, excited or adsorbed by simple atoms or molecules derived from the substance to be measured, but which cannot arise from the carrier gas. In this case several different atoms may be separately, but simultaneously, measured.

A number of detectors have utilized chemical reactions to produce

† L. Marton, National Bureau of Standards.

products more easily measured than the parent substances, for instance, S, P, N or halogen. Except for C, H and O, they have not gained very wide acceptance, perhaps because it has been difficult to match the small reaction volume of a capillary column.

There have been few attempts to apply simple quantitative chemical reactions to detectors. There are several, perhaps many, directions in which these could go, and I believe that they will in future play an important role. Let me reiterate the advantages of converting a substance to be measured to a number of simple compounds. Thus, if the substance be oxidized to water, CO_2 and N_2, detectors can be made that respond to only one such compound and a single calibration with a known substance then suffices for all other substances. The value of the measurement made, gives the total mass of a given element in the sample before oxidation; and if several elements are measured simultaneously in separate detectors, an elementary analysis can be obtained.

One of the most attractive substances to convert to, and measure, is water. It can be measured in several ways. It can be electrolysed and the quantity of electricity required measured. This is potentially a very sensitive method. Thus, a comparison may be made with a hydrogen flame detector. Here, perhaps, one in 10^5 atoms are ionized. After conversion to water and electrolysis, each hydrogen atom is ionized.

Further, the size of the apparatus required is very small indeed, and it should be very resistant to disturbance by change of external conditions.

A possible form would consist of two combs of noble metal (evaporated, or otherwise deposited on a quartz, alumina, or other ceramic plane) whose teeth interdigitated, and the teeth of the combs would be nearly covered with phosphoric acid. An electrical potential applied between the combs electrolyses the water and phosphoric acid, probably to P_2O_5. The reaction appears to be quantitative. The time for completion of the electrolysis is probably dependent upon the time taken for water or phosphoric acid to diffuse to places where the potential gradient is high enough to speed it to the electrode. In an existing apparatus, used for measuring water content of dry gases, made of 25 μm Pt wires wound within a 1 mm glass tube, the time for equilibration is a few minutes. Hence, for a chromatographic detector the width of the comb teeth should probably be no more than a few microns. It would then be reasonable to expect a response in seconds or tenths of seconds. The useful sensitivity of such a detector would depend upon the amount of electrical noise in the detector and the associated amplifier. I do not at present know the semi-conductor properties of P_2O_5, which will, presumably, be the chief contributor to noise. Clearly, there will be an optimum temperature to work at, and since the concentration of water is so low, a wide choice may be available.

Simple chemistry should suffice to exchange CO_2 for H_2O with a column of alkali metal or alkaline earth hydroxide. Similarly, N_2 could be exchanged for H_2 in a suitably chosen metal hydride column and the H_2 produced could be combusted to water. Thus hydrogen, carbon and nitrogen should all be determinable.

Combined oxygen would require conversion to CO by passing over hot carbon. Oxidation to CO_2 should be possible, without oxidizing the hydro-

carbons accompanying it to water. Exchange to water would be done as before.

It should normally be possible to determine an empirical formula from such results, even without great accuracy of measurement, since with a figure for each element, stoichiometric ratios should be easy to guess. Even when the empirical formula was a submultiple of the molecular weight, the position occupied on the chromatographic column should enable the molecular weight to be inferred.

There is another very attractive method of estimating water, which involves replacing hydrogen in water by tritium. This cannot be done by exchange in a column of, say, a metallic hydroxide. To maintain a constant exchange ratio a large excess of tritium is necessary. However, disintegration would lead to vacant sites on the column which would pick up hydrogen from the sample. But a device recently invented by Lovelock should give a true exchange. This consists of a tube made of an alloy of palladium and silver, which is the cathode of a cell with an electrolyte of fused alkali metal hydroxide. The electrolyte surrounds the tube. With an appropriate potential applied to the cell, a partial pressure of hydrogen of any desired value may be maintained within the tube.

If instead of KOH, KOH* be used as the electrolyte, then water passing through the lumen of the tube will be converted to H_2^*O with a controllable partial pressure of H_2^* in the carrier gas. If this gas mixture be led to a narrow slit a few microns away from a cold, dry photographic film, which passes the slit at a constant or programmed rate, then the film will pick up the water quantitatively, while allowing almost all of the H_2^* to escape.

If the film consists mostly of fine grains of suitable silver bromide, a yield of one developable grain for each disintegrated tritium atom is to be expected. Note that the sensitivity is almost proportional to the time that elapses between collecting the H_2^*O and the time at which the film is developed. Individual grains would be counted (making a very thin layer of emulsion desirable) and the background measured on either side of the area of H_2^*O uptake.

It is worth pausing to see how great is the gain of sensitivity over any method employing a flow-through counter. Coupling to any high efficiency column a mean residence time of only a few seconds is desirable. Thus, if the film be kept only one hour before development (i.e. note that the film should be developed at the same rate as it runs through the detector), an increase in sensitivity of the order of 10^3 is to be expected. If kept for four days, a 10^5 increase in sensitivity is to be expected, and if for a year, about 10^7 increase. After keeping for a year, there should be one black grain for about every ten atoms of tritium adsorbed.

This primitive calculation illustrates how very poorly the measurement of most radioactive substances from chromatograms is normally carried out. Unless the analyst must have an immediate answer, combustion of an aliquot of the stream and absorption in film is almost certainly the best method.

There is also another method which is potentially very powerful if development work be done upon it. This is the method of chemical amplification. Scott and I made a first attempt to utilize the reaction sequence, substance $\overset{Cu}{\rightleftharpoons} CO_2 \overset{C}{\rightarrow} 2CO \overset{Cu}{\rightleftharpoons} 2CO_2$, etc., for chromatographic purposes. With twenty

such sequences a 10^6 increase in amount is theoretically possible. We were limited by our apparatus to about 1200°C for the carbon reduction step, and the results were unsatisfactory. At 1000°C the reduction was not complete and up to 1200°C chemisorption of the CO made its release too slow to match a chromatogram. The behaviour might be better at high temperature, but even if still unsatisfactory, there are many other reactions that should be tried. One method that is attractive is the use of a metal surface, perhaps Al or Zn, loaded with alkyl groups. Water from the combustion should liberate alkanes. These, when combusted, would yield many molecules of water for each one entering the exchange column, and the cycle could be repeated as desired.

The alkanes, from the last step, could be measured in any suitable detector, and a final conversion to halogen-containing substances should be feasible, permitting the use of the currently most sensitive detectors, the electron-capture ones.

The CO_2 multiplier has a real advantage, if it can be made to work quantitatively. It can be readily regenerated to its original state a large number of times. If the reactive carbon is in the form of a thin filament, which has the advantage that it can readily be made very hot indeed by an electric current, the filament will, of course, lose carbon in the course of the reaction. It can, however, readily pick up carbon if at the end of the measurements a hydrocarbon gas be circulated while it is suitably hot. Further, since the thinnest parts will have the highest electrical resistance, these will be hottest and deposit most carbon, a method of making uniform the diameter invented by Edison of electric lamp fame.

It is interesting to consider what noise is to be associated with a chemical amplification method. The original intake to the apparatus is a statistical sample of $N \pm (N)^{\frac{1}{2}}$. But if the energetics of the reactions employed are such as to drive the reactions almost to completion, then negligible error is introduced. There will, of course, be errors due to other causes, essentially instrumental ones, such as molecules which pass through without meeting the reagents, or are adsorbed or reacted with parts of the apparatus. Nevertheless, it could well be possible to make all the errors small, compared to the original sampling error, if only the best reactions can be chosen.

If, however, reactions have to be used which do not go nearly to completion, then not only statistical errors arise at each step, but also constancy of instrumental conditions plays an important part, and large errors could accumulate. It is for this type of reason that I do not see much future for an essentially continuous chemical amplifier, e.g. a tube with carbon on one side and CuO on the other, in which the degree of amplification would be a function of temperature and rate of flow. I think the accuracy attainable in such a system would be insufficient for most purposes. Perhaps for something such as an alarm, detecting organic vapours (or, indeed, many other chemicals), it could be a useful method.

I would like to return for a moment to the subject of the size of the active volume of the detector. It will be noticed that the three methods I have discussed all involve very small volumes of gas. In the case of the electrolytic water detector, the active volume is a circle a few millimeters in diameter and a few microns thick. The radioactive detector is in one sense a single

grain of silver bromide, with a volume rivalling a biological cell. Even the chemical multipliers are essentially small volume devices.

I believe that it is highly desirable that sensitive devices should be small. The higher the concentrations can be kept, the less the risk of contamination and error.

In one respect, the present day detectors fall woefully short of biological detectors. Not only is the sensitivity of the latter astonishingly high, but biological detectors are also able to distinguish an astonishing number of different chemical substances even when large numbers are mixed together. This power of distinguishing substances is not due to separation such as we must perform on our chromatographic columns. It is due, I believe, to a very large number of different detectors, many probably specific to single substances. At present we could rival this specificity only by employing enzymes of biological origin, whose function in the living systems from which they are extracted are preparative, not analytic as in the organs of smell. The clinical chemist has, of course, made use of enzymes for very many years, usually finishing up with colour reactions to measure quantity. The sensitivities of the methods used by clinical chemists cover the range roughly from milligrammes to nanogrammes, many being in the microgramme region.

We would, indeed, make use of many of these enzyme reagents in gas chromatography, if the effluent gas were split into many streams and fed into flowing streams of enzymes and detecting substances or apparatuses. Enzymes of varying degrees of specificity could be useful. Oxidases, reductases and hydrolases could give useful information in many cases. But a hunt for enzymes for specific substances could well yield, particularly from microorganisms, an astonishing variety of useful reagents.

There do, indeed, exist well-tried methods of identifying particular groups in molecules by chromatographic methods. To mention a single name, Beroza has used short columns of reactive materials to retain chemicals with particular groups. These short columns are placed at the beginning or end of the separating column and a particular peak from a previous run can be inferred to possess a particular group by its disappearance.

I am attracted by the idea of a battery of such reagent columns, each with its own general detector at the end of the analytical column. How many such different types of reactor can be devised I cannot estimate, but I believe that they could be very useful in problems of flavour analysis and in identifying the volatile materials from human or animal body fluids and tissues.

The current general methods of identification of unknown substances, all, one or another form of spectroscopy, are of course essential tools in any complicated analysis. But when one is faced with the hundreds of substances such as are present in coffee or urine, the need for very sensitive methods becomes apparent. The amounts of materials obtainable from capillary columns often do not suffice for examination by u.v., i.r., or n.m.r. The lower resolving power of packed columns makes accumulating enough material for these examinations difficult.

The mass spectroscope is, of course, the great stand-by in this dilemma. It is entertaining to recollect how ridiculous the suggestion seemed to be to most mass spectroscopists, when I first proposed to dedicate such an

instrument to a gas chromatograph. But the analysis of the flood of data that these machines pour out is a task that seems now to call for computer assistance, and there are still many substances that can be shown as peaks, not present in large enough amounts to be properly examined.

Another spectroscopic method shows promise of being of value. E.S.C.A. seems able to give useful information, particularly when quantities are very small.

In liquid chromatography the limitations of any general property detectors are the same as in gas chromatography detectors. Thus those depending upon refractive index, dielectric constant, density, or vapour pressure cannot be very sensitive unless the essential volume can be made extremely small and concentration of substance to be detected kept high. Electrical conductivity or surface tension detectors could be more specific and eliminate to some extent the effects of the sensitivity of solvent properties to external changes.

In the current renaissance of liquid chromatography, new detectors are urgently needed to catch up with advances in column technique, which can now effect resolution in a few minutes, but only with small amounts of material. The high sensitivity of gas chromatographic detectors is now essential.

As in the case of gas chromatography, if the detector is sensitive enough, it does not greatly matter if it destroys the substance measured.

In principle, it is not difficult to use a mobile phase that is so different in volatility that recovery of the wanted substance is almost quantitative after evaporation of either the solvent or the peak. However, it does demand that the solvents used be very pure, and the degree of purity needed is not easily obtained. I believe that it will be found that the method of zone refining is the only one that can easily yield the required purity. Of course, to use it is a counsel of perfection, which cannot always be followed.

Before gas chromatographic detectors can be used, the solvents must, of course, be removed. So far, the wire detectors, introduced by James and Scott, (or the variants of it) are the only practicable methods available. The replacement of pyrolysis by combustion, followed by reduction to methane looks like being the most valuable recent advance. Any of the methods discussed earlier are potentially available if the solvents can be removed.

At present no convenient method of evaporating the whole of the effluent from the column exists, but many ways in which this can be done are readily available. A wide tape of suitable material seems the most obvious method.

The use of a microscope to examine the dried effluent of the column can be rather surprisingly sensitive. It is possible to resolve particles optically which are of the order of 1 μm diameter. A cube of this dimension of an organic material will weigh about 10^{-12} g.

By using dark-field illumination, much smaller particles can be detected. In general, a pure substance that crystallizes well will give numerous separate crystals under good conditions.

If on evaporating the solvent, the residue left will not crystallize, interference colours will show very thin films of liquid or solid material. It should be possible to devise a photodetective apparatus that does not necessitate

examination by eye. Lenses of liquids will form if the thin films are unstable. It seems probable that counting crystals or lenses of liquid by a flying spot technique could yield reasonably quantitative results, at least for well-separated substances.

Another possible method, though this would involve inspection by eye, could depend upon the alteration of the crystal habit of an indicator substance added for that purpose. The normal shape or habit of the crystals of a given substance is determined by the rate of growth of the individual crystal faces, those faces that grow most slowly being the largest in the final crystal. A foreign substance, present in the solution from which the crystals are growing, will reduce the rate of growth of any face upon which it is adsorbed. In general, it will not be equally adsorbed on different faces since it requires a near identity of spacing of the crystal molecules and the adsorbed layer. A triclinic crystal should probably be chosen in that it will present the greatest variety of faces.

The potential sensitivity of this method should be great. The thickness of the adsorbed layer should be of the order of a few tens of ångstroms, and adsorbed on to a 1 μm cube this represents 1×10^{-14} g. This method should be quite selective, but suffers from the difficulty of knowing, at any rate in the absence of a lot of experience with it, which substances to select as crystal and solvent.

If this method could be made to work at all, it would also be available for gas chromatography. Depositing the substance to be detected is essentially the same problem in gas or liquid chromatography. Provided temperatures are kept low, no great loss need be anticipated.

The clinical chemist has for a long time used colour reactions for analyses of particular substances, and many of the methods are highly specific and sensitive. Attempts have seldom been made to keep the volumes very low. By doing liquid chromatography followed by evaporation and the same colour reactions observed in small volumes, great increases in sensitivity would result. If fluorometric rather than colorimetric methods can be developed, further great increases in sensitivity are possible.

Further, using detection methods of high specificity and sensitivity, the possibility again opens of using a battery of tests of differing sensitivities, and thus simultaneously scanning on different or divided tapes for, say, amino acids and peptides, and carbohydrate-containing substances.

It is perhaps worth pointing out that in examining animal body fluids for amino acids, a general detector is of little value, in that the amino acid peaks, though resolved without too much trouble from each other, cannot easily be resolved from all the other varieties of substances present, and that a specific detector is almost essential.

So far as using colorimetric methods direct on the effluent of a liquid chromatographic column is concerned, I venture the opinion that few serious attempts have been made to push the sensitivity to the limit. It is possible to trade sensitivity and light output for each other. Thus, all highly sensitive colorimeters should use powerful light sources, and photomultipliers as light detectors with long small-diameter cells. Perhaps laser technology will soon provide the first of these requirements.

Polarography or perhaps better microcoulometry can also be readily

adapted. If applied after a reaction the sensitivity and selectivity could again be very great.

Colorimetric reactions are, in general, insensitive to buffer salts and small changes of solvents. By addition of swamping concentrations before the detector, even gradient elution methods can be used in the column. Buffer salts are, of course, a major difficulty where evaporation is desired.

The clinical chemist makes great use of enzymes. They can provide a specificity on occasion limited to a single substance, but can also be much broader, e.g. as in various oxidases.

You will see that I feel that many years of development lie ahead, and that great increases in sensitivity and specificity are possible. Probably in future most difficult analyses will be conducted in rather elaborate composite pieces of apparatus, of which perhaps the combination of chromatogram and mass spectrometer is a typical example.