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COMPUTER AND INSTRUMENTATION GROUP COLLOQUIUM ON 'CHROMATOGRAPHIC EQUIPMENT'

Trends in Chromatographic Equipment

By E. REID (*Wolfson Bioanalytical Centre, University of Surrey, Guildford, Surrey, U.K.*)

An outlay of £1000 or more on g.l.c. (vapour-phase chromatography) equipment is a good investment for any laboratory that has to analyse vaporizable substances, the technique being notable for resolving power if not speed. The following contribution, by G. Guiochon, outlines present-day trends in g.l.c. equipment. Although the usefulness of g.l.c. apparatus is enormously enhanced by coupling it to a mass spectrometer, this entails expense that, even if it becomes less formidable during the next few years, will remain prohibitive to most laboratories. Another approach, still with few practitioners, is to interface g.l.c. with t.l.c.; this may not facilitate actual measurement, but it does help with identification or with resolving a mixed peak into its components (Kaiser, 1969).

Already g.l.c. has proved useful for preparative as well as for analytical work. This may also be the case in future for high-pressure chromatography of liquids. For the latter up-and-coming technique a satisfactory name is badly needed in place of the unpardonable term 'liquid chromatography' that has found its way into sales literature. To the uninitiated this term might signify conventional column chromatography such as has been practised for decades. Even where literature does emphasize the high-pressure aspect, and the superior resolution and speed achievable, seldom is it pointed out that the key feature of the technique is the use of micro-granular column materials, through which the flow of liquid would be hopelessly slow if merely under gravity. For high performance a daunting outlay of more than

£5000 has to be made. Fair performance may, however, be achievable with medium-pressure apparatus such as is now appearing on the market, sometimes glibly termed 'low-cost' (actual cost £1000-2000); components for do-it-yourself assemblies are also becoming available.

Gravity-flow column chromatography will continue to be the method of choice for many workers, on the grounds of cheapness and/or convenience; indeed, high pressure must be avoided if present-day molecular-sieve materials are to be used. The fraction collector represents the only significant outlay, and need cost no more than £100 if of turntable type rather than modular-rack type; manufacturers are still introducing new models into what seems to be a saturated market. With overnight running, and possibly use of an automatic solvent-changing device, the slowness of gravity flow is often no handicap.

Speed of operation may likewise, in some contexts, be a minor factor when it comes to choosing between paper and thin-layer materials. One advantage of paper is that it stays intact when elution of separated spots is performed. Yet t.l.c. is nowadays the method of choice except where paper chromatography has an over-riding advantage for a particular reason. Centrifugal or other methods to speed up t.l.c. seem to be 'gilding the lily'. The tedious stages in layer chromatography are commonly the processes of applying the material (particularly where multiple spotting has to be done) and of measuring the amounts of the separated components quantitatively. For the latter purpose scanning devices that obviate elution are deservedly becoming popular, although users should beware of unsuspected errors in the readings obtained.

'On-line' quantitative measurement may come to occupy a limited but useful place in the t.l.c. analysis

of solutes that, passing in succession along a rod or wire, are finally pyrolysed or volatilized for measurement. In connexion with column chromatography of liquids a variety of equipment for on-line quantitative determination is already available—at a price. Refractometric devices are deservedly, if somewhat belatedly, becoming popular, but will not supersede u.v. devices. Although the outlay (of the order of £500) for a u.v. device will not guarantee that meaningful values will be obtained for separated constituents, such a device at least has a valuable function as a monitor for detecting peaks, which may subsequently be examined off-line.

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Gas-Liquid Chromatography in Biochemical Analysis: a Review of Recent Trends in Equipment

By GEORGES GUIOCHON (*Laboratoire de Chimie Analytique Physique, École Polytechnique, 17 Rue Descartes, Paris 5, France*)

Although g.l.c. was invented by two biochemists, most people in this field have, until relatively recently, regarded this technique more as a snare than as a tool. The main reason is that, in spite of many papers by leading experts working with equipment of their own make, in spite of many advertisements for the 'new gas chromatograph X for biochemical analysis', most commercial equipment has been designed, often successfully, for the analysis of petroleum fractions. This situation is slowly changing, however, and analysis of many compounds of biochemical interest may now be carried out successfully on a routine basis.

It must be taken for granted that the conventional systems for pressure, flow-rate and temperature controls, signal recording and data acquisition and handling are working properly, since they are common to all kinds of chromatographs. Problems associated with sampling, column design and detectors will be discussed.

Many compounds of biochemical interest cannot be analysed as such because they are too polar and not volatile enough. The formation of suitable derivatives is often a good solution, and the various reagents and methods that can be used will be reviewed. Another solution, though much less frequently used, is pyrolysis g.l.c. Some examples of this will be given. The carbon-skeleton determinator will also be exemplified.

Column injection is not too difficult with conventional packed columns. It is much more of a problem with capillary columns, and various ways of solving this will be discussed, with emphasis on the direct injection of very diluted solutions.

Column packings have changed little during the last 10 years, although a few stationary phases that can be used in the 300–400°C range have been developed. A truly inert support is still to be obtained, but the art of chemical modification of solid surfaces by grafting is now fairly well advanced and can offer satisfactory solutions.

New detectors have been developed that have very high sensitivity and selectivity. The flame-photometric detector can determine separately compounds containing nitrogen, sulphur and phosphorus. The electron-capture detector is highly sensitive to halogens, though it is somewhat less predictable. The mass spectrometer is a very sensitive detector, and simultaneously offers a solution to a problem that is usually not solvable by g.l.c. alone, namely the identification of a truly unknown compound.

Finally, some recent examples of difficult analyses will be discussed, as well as the scope of the method. A few problems will be discussed in detail, this seeming to be more useful than a superficial review of the very abundant scientific and technical literature.

Equipment for High-Pressure Liquid Chromatography

By JOHN H. KNOX (*Department of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ, U.K.*)

High-pressure liquid chromatography has developed within the last 5 years and has brought to liquid chromatography the speed, resolution, sensitivity and ease of operation previously associated with g.l.c. The technique promises to be of immense importance for clinical, forensic and biochemical analysis.

The essential features of a modern liquid chromatograph are: (a) a pressurization or pump system capable of delivering degassed dust-free solvent against back pressures up to 21 MN/m² (3000 lbf/in²) and at flow rates up to 10 cm³/min; (b) an injection system for placing samples of volume a few microlitres on the column head; (c) a column, generally 25–100 cm long × 2–4 mm diam., that is packed with 20–50 μm-diam. partitioning material and preferably thermostatically controlled; (d) a highly sensitive detector, of not more than 10 μl volume attached directly to the column outlet, that produces an electrical signal that can be processed by a recorder or other data-handling system.

Commercial equipment costs between £3000 and £10 000, depending on the 'sophistication' and versatility desired. Home-made equipment is cheaper but has its drawbacks. The expensive components in home-made equipment are the pressurization system or pump (£600–1000) and the detector (£600–1200). Couplings should be of the compression type, and all parts coming in contact with the eluent must be of