stainless-steel, Teflon or glass. Columns may be either of heavy walled glass [up to 7 MN/m^2 (1000 lbf/in^2)] or of stainless-steel. Injection is generally by $10\mu\text{l}$ micro-syringe for pressures up to 10 MN/m^2 (1500 lbf/in^2), and sample sizes range from 10 ng to $100\mu\text{g}$. Pressure measurement is made by a Bourdon gauge or a pressure transducer. Thermostatic control of the column can be by a water jacket. Electrical thermostatic control can be dangerous if leakage of flammable solvents occurs.

The pump, column packing and detector are the key parts of any high-pressure liquid-chromatographic system.

(1) Pumps. The use of high-pressure N_2 from a cylinder in direct contact with the solvent is the simplest way of generating a pressure up to 21 MN/m^2 (3000 lbf/in^2). The solvent is held in a long narrow tube. The system is cheap but potentially dangerous. Constant pressure may also be generated by a safer but more expensive pressure-intensifier in which a low gas pressure bears on a large-area piston rigidly connected to a small-area piston bearing on the solvent. These systems deliver liquid at a controlled pressure to the column, they are readily adjusted and they deliver $50\text{--}100\text{ cm}^3$ per stroke. Reciprocating plunger pumps with a variable stroke give a controlled flow rate, but the flow pulsates and a damper is required between the pump and column. Piston pumps have low swept volumes, and are particularly useful for gradient elution since the gradient-producing system can be operated at low pressure; with constant-pressure systems the gradient has to be produced on the high-pressure side, and this is expensive. Motor-driven syringes combine constant flow with continuous delivery: the best versions employ a pair of reciprocating small-volume syringe pumps.

(2) Column packings for high-pressure liquid chromatography must be exceptionally well graded and should have particle diameters between 20 and $50\mu\text{m}$. Many are forms of silica gel, but the most satisfactory materials have a thin absorptive layer deposited on an impervious core. Great care has to be taken with column packing to achieve good trans-column uniformity.

(3) Detection methods used are of three types: those that monitor the change in bulk property of the eluent (refractive index for instance); those that monitor a selective property of the eluent (u.v.- or visible-light absorption, fluorescence, electro-reducibility); and those that physically separate solute from solvent and subsequently determining the solute by a g.l.c. method (for example the Pye wire-transport detector). Bulk detectors generally have a sensitivity limit of between 1 in 10^6 and 1 in 10^7 of solute in mobile phase, whereas the selective detectors can detect as little as 1 in 10^9 . The separation-type detectors are potentially very sensitive, but mechanical and other problems have prevented the

attainment of sensitivity limits lower than about 1 in 10^6 .

Aids to Column Chromatography

By ALAN J. THOMAS (*Department of Biochemistry, Imperial College, London SW7 2AY, U.K.*)

Fast liquid-phase column chromatography is potentially a powerful tool for the sensitive measurement of many important biochemicals. Its greatest use may well lie in the separation and determination of the more hydrophilic compounds, which cannot be easily and quantitatively converted into stable volatile derivatives and thus determined by g.l.c. Good examples are amino acids, sugars, phosphate esters and nucleotides.

For optimum separation of a relatively large number of components at high resolution and in a short time, micro-spherical column packings are required, with narrow-bore columns and, inevitably, considerable pressures. Medium pressures, up to 7 MN/m^2 (1000 lbf/in^2), are relatively easy to handle, and the Milton Roy Minipump, at a cost of under £200, is extremely suitable. Medium pressures, combined with gradient elution and low flow rates, can be extremely effective.

Since the introduction of the multichamber Varigrad by Piez & Morris (1960) various devices have become available for producing both multi-exponential gradients and linear gradients. Although these devices are useful and can be comparatively inexpensive, they are difficult to use for repetitive work and are limited in the forms of gradient that can be generated. A completely flexible electronic gradient maker that could be programmed in an analogue form was described by Thomas (1970), and similar devices are now commercially available. Although these instruments are relatively expensive (about £1000), they permit a total control of the elution process with a very high reproducibility and allow the optimum separation of a large number of components in the minimum time. Their high cost can be frequently justified by the speeding up of the chromatographic process and therefore the amount of effective analysis that can be achieved by a single column, high-pressure pump and detection system.

Many of the compounds best separated in the liquid phase require elution with aqueous gradients most frequently composed of low-volatility organic buffers. In such circumstances physical detection systems can present problems. For example, detection methods that use refraction, conductivity or u.v. absorption will show large base-line shifts due to change of eluent composition upon which are superimposed very small changes due to the separated components. Attempts to compensate for these base-line shifts by

differential two-column working are both expensive and difficult. In any case, physical detectors are frequently of comparatively low sensitivity compared with many colorimetric chemical methods. The low specificity of physical detection methods can be advantageous when subsequent radioactivity measurements are required, in that all the components separated by the column can be seen, but at other times the controlled specificity of colorimetric chemistry can simplify the chromatographic method. A carefully designed chemical-analysis system can achieve response times of the order of 3–5 s with total liquid flow rates as low as 0.5 ml/min. This compares favourably with direct physical methods. At the same time it will generally show no response to the eluting solutions and therefore will measure the eluted components against a stable base-line. This makes high-sensitivity working much easier and, as many suitable colour reactions are extremely sensitive, detection limits can be extremely low.

Flexible-gradient elution combined with a fast-response chemical-detection system offers great possibilities for extending chromatographic technique. This is well illustrated by a system developed in this laboratory, where a programmed continuously varying gradient of borate–acetate is used to elute a column (18 cm × 0.2 cm) of Dowex 1 (micro-spherical beads) at 60°C. With a flow rate of 0.4 ml/min ribose, glucose, fructose, glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, ribose 5-phosphate, fructose 1,6-diphosphate, glucose 1,6-diphosphate, UDP-glucose, ADP, ATP and UTP are successively eluted in under 4 h. The flowing-stream chemical-detection system, based on the orcinol reaction, gives a detection limit of about 50 nmol for each of the components.

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Thin-Layer-Chromatographic Equipment

By J. P. LEPPARD (*Wolfson Bioanalytical Centre, University of Surrey, Guildford, Surrey, U.K.*)

T.l.c. is a widely used technique for qualitative analysis of reaction mixtures, quality control, screening and the like. In many cases routine t.l.c. of a large number of samples is required, which leads to practical problems with regard to efficient use of laboratory personnel and to their morale. Also, although t.l.c. is a very useful technique for qualitative analysis, it has been found difficult to use it to obtain quantitative results in a reasonably straightforward manner. A number of research workers and manufacturers

have therefore designed equipment to mitigate either or both of these handicaps. Since the solvent development of the plates is generally straightforward, and a wide range of commercially prepared plates is now available, most of the problems of quantitative t.l.c. arise either in the application of the sample to the plate or in evaluation of the separated spots of material after development. Innovations have therefore been made particularly in the production of sample-applicators and of plate-scanners (since direct quantitative determination *in situ* presents the greatest attractions in terms of simplicity and speed).

Sample-applicators range from relatively inexpensive controlled-bore capillary tubes, through various modified syringes, to 'sophisticated' (and expensive) semi-automatic machines. The latter, which make use of several methods of sample transfer such as electrostatic precipitation or 'firing' on to the plate, may work well, but are often useful only for preparative t.l.c. or will not cope with more than one or two different samples at a time; moreover, they may be tedious to clean. In our laboratory a machine of unorthodox type has been designed, initially intended mainly to relieve the tedium of repetitive application of an array of different samples (as would be required, for example, in laboratories concerned with quality control or screening programmes). It consists essentially of a reciprocating drive that moves mounted pick-up arms into a row of sample cups and then on to the t.l.c. plate. Up to 12 samples can be 'spotted' simultaneously, and up to 99 aliquots can be 'spotted' without intervention of the operator. Timers allow for drainage of the sample on to the plate and for drying of the spots, and the machine has exceeded expectations by giving a precision of 2–3% even with solvents as volatile as chloroform.

Many densitometric plate-scanners are now available, mostly at prices well above £1000; some are self-contained and some are for attachment to a spectrophotometer. Many of the instruments depend on a simple and well-established design principle, involving linear traverse of the lanes on the t.l.c. plate by a slit of light, usually with reflectance measurement; others involve techniques such as 'flying spot', double-beam and even double-wavelength scanning. Most of the more expensive scanners measure in various modes (transmission, reflectance, fluorescence, fluorescence quenching), all with visible-light illumination and some with u.v. light also. A common feature of the more refined instruments is that they measure the intensity of the reflected, transmitted or emitted light over the whole area of the spot and subsequently present an integrated value for the spot. In our laboratory a scanner has been produced that makes use of a rather different principle of quantitative determination that is well founded but has been rather neglected: it depends on the measure-