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A multi-component preparative liquid chromatography system

The Pye "moving wire" liquid chromatography (LC) detector in its original version has good sensitivity for lipids[1-3], but it is not suitable for quantitative analyses because the wire coating efficiency and pyrolysis yields are strongly dependent on both the solvent mixture and the solute structure. This detector was constructed primarily for effluent monitoring in preparative chromatography. For work with lipids, other detectors such as UV or RI monitors are often useless owing to lack of UV absorption of the samples and to the great differences in solute polarity, necessitating the use of gradient elution techniques. As the "moving wire" detector, however, is not disturbed by gas bubbles in the effluent stream nor by pulsations,

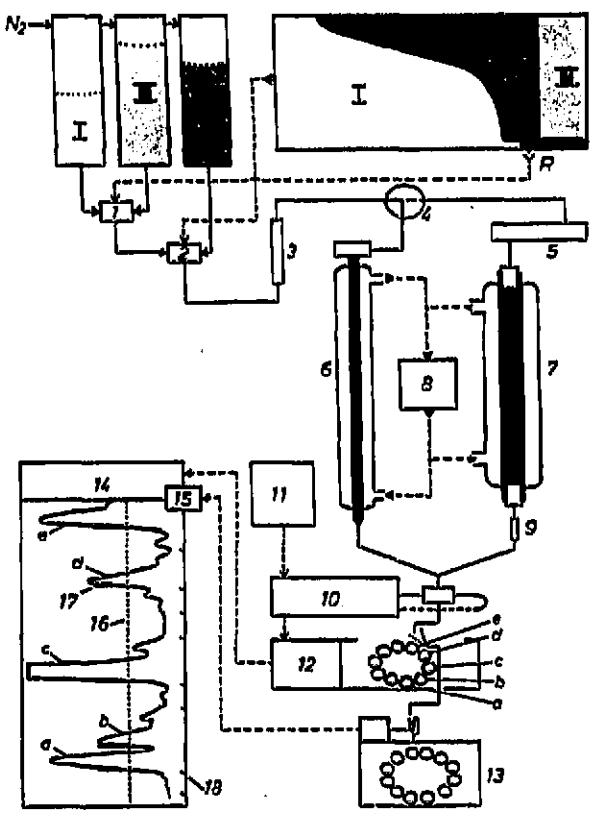

Fig. 1. Scheme of preparative liquid chromatography system. I, II, III = pressurized solvent reservoirs and corresponding Ultrograd chart sections. 1 = LKB flow valve, actuated by rider R; 2 = LKB flow valve, actuated by Ultrograd programme sheet; 3 = mixing device; 4 = Serva PTFE 3-way valve; 5 = Chromatronix sample injection valve with loops; 6 = 4 mm I.D. glass column with Pye injection head and cooling jacket; 7 = preparative column with adapters and cooling jacket; 8 = Haake water bath with pump, for circulating ice-water; 9 = external flow resistance; 10 = Pye moving wire liquid chromatography detector with FID, coating block, and ionization amplifier; 11 = gas flow controls for N_2, H_2 and air; and pyrolyzer detector oven controls; 12 = Pye eluant collector with trap-drain mechanism, producing "Event 1" marks (a, b, c, d, e = trapped peaks); 13 = LKB fraction collector with siphon, producing "Event 2" marks; 14 = 1- or 10-mV recorder (FID analogue signal from 10 via 12-14); 15 = event marker (Event 2 from siphon of No. 13); 16 = chosen trap level index (0-10 mV); 17 = Event 1 mark; 18 = Event 2 mark. Alternatively, de-gassed solvents and a pump between parts 3 and 4 may be used.

simple arrangements for solvent transport can be made. An arrangement such as that in Fig. 1 is useful for the preparative "trapping" of liquid chromatographic peaks from the sub-milligram to the gram scale.

Many substances are not sufficiently volatile for gas–liquid chromatography (GLC) and polar functional groups on long-chain fatty acid methyl esters may often be sufficiently labile to be destroyed or altered by the high temperatures needed for GLC peak trapping[4–6]. Therefore, an LC peak trapping system was devised primarily for the recovery of milligram samples for subsequent analysis, *e.g.*, by mass spectrometry. The system described is also useful for preparative chromatography on a larger scale, as the LKB gradient programming unit used is sufficiently flexible to permit elution times from 15 min to 16 days.

Experimental

Equipment. The following equipment was used:

Glass solvent reservoirs with PTFE stopcocks, glass tubing, and cooling jackets.
Nitrogen cylinder with Dräger Saturn D 14650 fine-pressure regulating valve.
LKB 11300 Ultrograd master unit.
Two LKB 11310 flow valves.
Mixing device LKB 11360 and vessel LKB 11361.
Chromatronix CJ-3031 Tee.
Serva 93001-DDD PTFE three-way valve.
Pye No. 14741 injection head with Hamilton F-138 Microsep septa.
Chromatronix SV-8031 sample injection valve with various loops.
Preparative columns, *e.g.*, Sephadex SR 25/100, Whatman, Serva MCS or Chromatronix.
Narrow-bore columns, self-made from glass tubing, or, *e.g.*, Chromatronix Microbore.
Haake Type NBS circulating water bath.
Polyethylene and PTFE tubing for pressure-tight connections.
Pye liquid chromatograph System 2 (FID) with dual temperature controller, ionization amplifier and Brooks Sho-rate rotameters.
Pye No. 792602 eluant collector.
Philips PM 8000 recorder.
LKB RadiRac fraction collector.
Philips PM 9801 event marker.
Hamilton syringes and 0010 pipette control with Luer tip.

Method. From the pressurized solvent reservoirs (Fig. 1), the solvents I, II and III are delivered according to the pre-selected Ultrograd programme. The Ultrograd master unit will deliver solvents I–III via the magnetic valves 1 and 2 according to a pre-selected valve-switching programme (top-right in Fig. 1). Section II of this programme is cut out of black paper, while sections I and III are white. The operating principles and flexibility of this equipment have been described by KECK[7]. Depending on the size and dead volume of the chosen column and tubing, and depending on the solvent flow-rate, a mixing device may be necessary. The mixing device (No. 3 in Fig. 1) may consist of either the LKB units 11360/11361, a short glass tube filled with coarse silica gel, or a "chain" made of alternating short portions of PTFE and polyethylene tubing of different internal diameters, creating a series

of turbulences between points 2 and 4. A mixing device is unnecessary if low flow-rates with fine-grain analytical columns and syringe injection are chosen. Sufficient solvent mixing will occur in the tubing and the top sorbent layer. The syringe needle should penetrate the top sorbent layer so that the solvent present at the point of sample delivery (needle tip) will already be a homogeneous mixture of the ratio given by the black (II) and white (I, III) sections of the Ultrograd programme sheet[7].

As nitrogen under pressure is in direct contact with the solvents in the reservoirs, some nitrogen will dissolve. At room temperature, bubbles of nitrogen gas will develop at regular intervals in the area of greatest pressure drop. To make sure that this will occur only at the very bottom of the column, or at a point beyond the column, a plug of very fine grain material or a separate flow resistance is used. The column itself may be cooled to below room temperature for the same reason (*e.g.*, by circulating ice-water through a jacket).

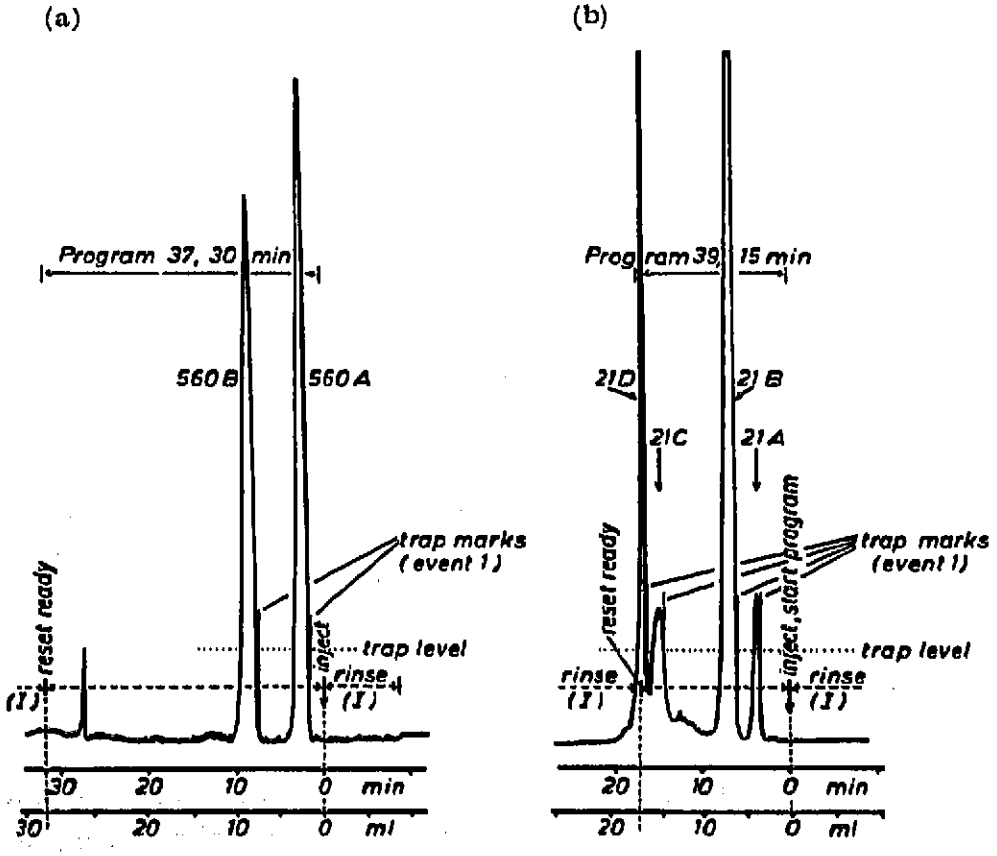

Fig. 2. Preparative liquid chromatography with automatic peak trapping. Pressure: 0.5 atm N_2. Solvents: I = heptane, saturated with water; II = heptane–diisopropyl ether (50:50), saturated with water; III = heptane–diisopropyl ether–ethanol (30:30:40), dry. (a) Liquid chromatography of test mixture. Injected: 100 μl of heptane solution of 5% each of methyl oleate and methyl 9,10-epoxystearate. Programme: No. 37 (see Fig. 1, top-right), 30 min. Column: 430 × 4 mm, bottom 10 mm Merckogel SI 50 of 36–75 μm, main column Merckogel SI 50 of 75–125 μm grain size; rinsed and re-conditioned with solvent I. Trapped peaks: 560 A = methyl oleate; 560 B = methyl 9,10-epoxystearate. (b) Liquid chromatography of reaction mixture. Oxidation of technical methyl ricinoleate with CrO_3. Injected: 20 μl reaction products, diluted 1:1 with heptane. Programme: No. 39 (not shown), 15 min. Column: 300 × 4 mm, Merckogel filling as in (a). Trapped peaks: 21 A = methyl oleate + linoleate; 21 B = methyl 12-keto-9-octadecenoate; 21 C = methyl ricinoleate; 21 D = sum of more polar products.

For preparative columns of larger diameter (1–2.5 cm I.D.), the use of a separate flow resistance in the tubing system between column and coating block is indicated (No. 9 in Fig. 1). Alternatively, a pump may be used between points 3 and 4 in Fig. 1, with de-gassed solvents.

If only the major peaks are to be trapped, the Pye Eluant Collector is operated in the "Auto" position with the trap level index set at some distance from the baseline (*e.g.*, 3 mV of 10 mV f.s.d.), and the smaller peaks are drained into a waste bottle. If the lesser peaks are required for a possible future investigation, the "Drain" position is connected to the second (LKB) fraction collector which is operated at 5-ml intervals by a siphon (No. 13 in Fig. 1).

Alternatively, the Pye eluant collector may be disconnected or kept at the "Drain" position and 5-ml fractions may be taken by the LKB collector while recording the whole chromatogram with "Event 2" marks only. As a preliminary experiment, this procedure should always be carried out with unknown samples in order to obtain an initial idea of the nature of the final chromatogram. This may be important for the correct final choice of attenuation, trap index level and width of Ultrograd programme sections. The "Event 2" marks will normally be found to occur at increasing distances owing to increases in solvent viscosity and column resistance according to the chosen elution programme. This means that, for example, a sheet section of equal width near the end of the Ultrograd programme will lead to a lower solvent volume than at the beginning of the programme, and knowledge of the time–volume relation from the "Event 2" recording of the initial experiment will facilitate the correct adjustment of the Ultrograd programme sections.

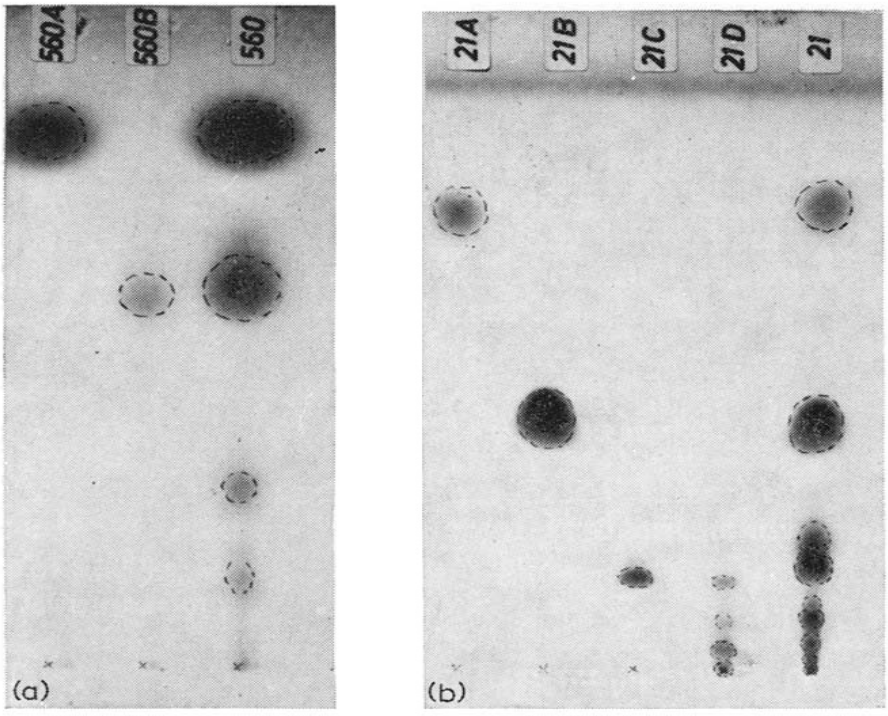

Fig. 3. (a) TLC of test mixture and traps. Silica gel; light petroleum–ether–ethanol (90:10:2). (b) TLC of reaction mixture and traps. Silica gel; light petroleum–ether–ethanol (90:10:2). For spot numbers *cf.* Fig. 2 and text.

Results

Fig. 2 shows examples of chromatograms obtained from both a test mixture and an actual experiment. In Fig. 2a, a test mixture consisting of methyl oleate and methyl 9,10-epoxystearate (containing a small amount of the dihydroxystearate) was separated and two peaks (560 A and 560 B in Figs. 2 and 3) were automatically trapped and then analyzed by TLC (Fig. 3a). The simple Ultrograd programme that was used to produce the chromatogram in Fig. 2a is shown in the top-right section of Fig. 1. In this instance, solvent I was heptane saturated with water, II was heptane–diisopropyl ether (50:50) saturated with water, and III, used for cleaning the column, was heptane–diisopropyl ether–ethanol (30:30:40).

In Fig. 2b, the reaction mixture obtained from a chromic acid oxidation experiment with technical methyl ricinoleate was chromatographed with automatic peak trapping. The purpose of this experiment was to recover the main oxidation product, methyl 12-keto-9-octadecenoate (peak 21 B), in a pure state as a reference compound for other investigations.

The thin-layer chromatogram in Fig. 3b shows that peaks 21 A (non-polar C_{18} methyl ester, mostly oleate), 21 B and 21 C (methyl ricinoleate) were trapped in a fairly pure state, while peak 21 D, which was eluted by the final ethanol front used to purify the column before re-conditioning and re-use, consisted of a mixture of several more polar products.

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Unilever Forschungsgesellschaft mbH, K. AITZETMÜLLER
Behringstrasse 154, D-2000 Hamburg 50 (G.F.R.)

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