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LIQUID ADSORPTION CHROMATOGRAPHY IN COLUMNS AND ON THIN LAYERS

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SUMMARY

The work described in this paper provides basic practical detail for the construction of liquid chromatographic systems superior in performance to existing column and thin-layer techniques.

Practical detail, both of components and materials, is given and the performance of high efficiency columns compared with existing techniques. Whilst the new column systems are shown to be capable of much better performance, nevertheless because of its simplicity and cheapness thin-layer chromatography will remain the method of choice for multi-sample routine analysis not requiring the highest resolving power.

INTRODUCTION

Liquid chromatography in columns (LCC) has been applied to the analysis of petroleum products for over 70 years. One of the first to make use of it was DAVID DAY who, in 1897 said, "I believe that we will be able to obtain complete resolutions... Imagine how valuable it would be to characterise a lubricant oil by the correct percentages of the component hydrocarbons instead of densities, inflammation points and viscosities"[1]. This prediction of complete resolution remains unfulfilled for the high-boiling fractions of petroleum although gas chromatography (GC) has been used to characterise the more volatile fractions by complete resolution of the components present. The analysis of the high-boiling petroleum fractions and of additive concentrates and finished lubricants, new and used, their decomposition products, and related materials remains a major problem.

Thin-layer chromatography (TLC) has proved of greatest value in separating the high-boiling mixtures not amenable to GC. TLC has been shown to be capable of resolving mixtures of great complexity with speed and convenience. Used on a preparative scale, chromatography on layers (PLC) has been used to prepare pure samples of components of complex blends for further examination by spectroscopic and related methods.

LCC has been used in these laboratories to separate larger samples (1–10 g) where more material was required or where PLC failed to separate a trace component in sufficient quantity. As used in the immediate past, however, LCC has been a crude

and inefficient process. The classical technology has changed very little over 50 years of accumulated experience. A number of guidelines have emerged for the choice of column configuration, desirable sample size, adsorbent properties and solvent elution strengths.

It has recently been realised that LCC is capable of a performance comparable to that routinely expected of GC and that the tedium associated with LCC because of the slow flow rates of solvents is not a necessary limitation of the process. Theoretical[2,3] and practical studies[4–6] have begun to appear in the literature and suitable instrumentation for the re-creation of LCC as a major separations tool has been invented and described[7–9]. The studies so far made have indicated the criteria for increased speed of analysis, optimum separation efficiency and the more important problems and weaknesses that remain.

In order to take advantage of this work it has been necessary to assemble a suitable apparatus capable of meeting the requirements of the revised approach to LCC. It has also been necessary to supplement, and in many instances anticipate, published work of a fundamental nature in order to establish the best conditions for column performance. The interim conclusions we have reached on the criteria for high efficiency LCC have also been subjected to a limited comparison with the established techniques of TLC and PLC. An attempt has been made to indicate the future role of LC in columns and on layers and the strengths and weaknesses of the methods have been critically examined.

EXPERIMENTAL

Equipment and the preparation of stationary phase

Columns. Conventional LCC requires a column, usually of glass, with average dimensions of 1–3 cm i.d. and 30–100 cm length, fitted with a stop-cock and packed with a suitable adsorbent, *e.g.* alumina or silica gel. Experience has shown that narrow-bore columns give better separations than wide ones. Studies in GC have established the same principle. Columns were constructed for preliminary experiments from medium wall glass tubing (1.5–2.0 mm wall) with 2, 4 and 6.5 mm i.d. Contrary to one report in the literature[10] that glass columns can only be used to ~ 10 p.s.i.g. we found these able to withstand solvent pressures up to 300 p.s.i.g. For more advanced work at higher pressures stainless steel columns were used. These were 0.25 in. o.d., 0.18 in. i.d. (4.65 mm). It was found inconvenient to pack columns in lengths greater than 1 m. Columns were therefore made up in 75 cm lengths when made of glass and 1 m lengths when of stainless steel.

Column connections. In order to make up columns in lengths greater than 1 m it was necessary to connect a number of metre lengths in series. Remixing of solutes in connections between column lengths is to be avoided and connectors having a very low volume were constructed from 0.020 in. i.d. × 1/16 in. o.d. stainless steel tubing and ¼ in. × 1/16 in. "Swagelok" couplings specially constructed to minimise dead volume. The adsorbent packing was retained in the column by means of a disc of 0.4 μ pore size "Gelman" filter pressed into the Swagelok coupling. Extra-column connections (*e.g.* to the detector or fraction collector) were made with 0.020 in. i.d. × 1/16 in. o.d. tubing of minimum length.

Introduction of solute. Recently, reports of solute introduction into LC columns

by means of valves[6] and injection devices[4] have appeared. The primary objective of this investigation was a small-scale efficient separation of a variety of petroleum products. Sample sizes not greater than 100 mg (100 μl) were envisaged. Solvent switching valves capable of handling samples smaller than 100 μl are not readily available and the further requirement of operational efficiency at pressures up to 2000 p.s.i.g. was an additional discouragement. It was decided to attempt the construction of an injection device. Experiments with coloured solutes injected into glass columns showed that disturbance of the upper surface of the column packing during solute injection produced very broad bands of irregular distribution across the column cross-section. SCOTT *et al.*[4] have described an injection port giving greatly reduced band widths on injection. This design incorporates a needle guide which ensures axial injection into 8–10 cm of ballotini glass beads (150–170 mesh) at the top of the column. The combined effect of the axial injection and glass beads is to give even distribution of solute across the cross-section of the column, at least for samples of small enough volume (< 100 μl).

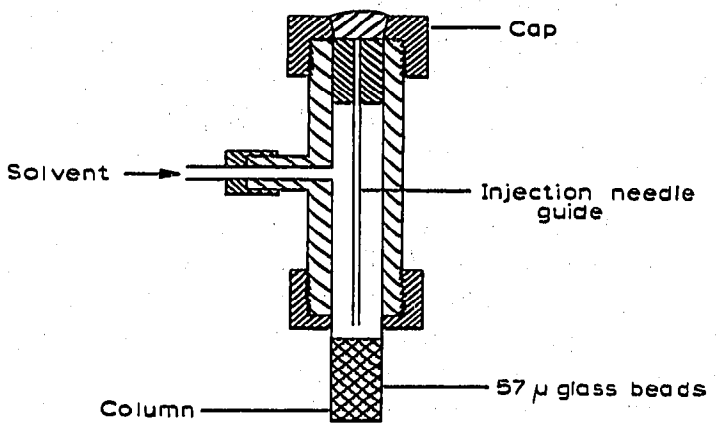

Fig. 1. Injection port constructed from "Swagelok Tee" based on SCOTT's design.

An injection port based on design by SCOTT *et al.* was constructed from a "Swagelok Tee", as illustrated in Fig. 1. With a 4 mm i.d. column, packed with silica gel and with 7 cm of glass beads (60 μ, Perkin-Elmer Co. Ltd., Beaconsfield) injection through this port gave band widths of 0.2–0.5 cm. These were measured with a coloured solute (azulene) at the top of the silica gel. It was observed that the solute band was sharpened up on passing from the beads to the silica gel giving a further improvement in band width at this point. Injection was made by removing the metal cap from the injection port with the solvent pump switched off. The cap was replaced while care was taken to exclude air and the solvent pump restarted. Silicone caps were found to contaminate the solvents with silicone oil, so that injection through a silicone septum, as in GC, was not possible. It was also observed by SCOTT *et al.* that the septa normally used in GC are extruded through even 1 mm orifices at high pressure. This was confirmed in our work.

Solvent pump. Solvents for LCC using the conventional, inefficient systems in common use are either allowed to percolate the column under gravity or are placed under slight (0–10 p.s.i.g.) pressure of nitrogen. With coarse particles of adsorbent (*e.g.* 100–200 mesh) and wide-bore columns this gives satisfactory flow judged by convenience and simplicity.

With the introduction of adsorbents of optimum particle diameter (see below) in tightly packed beds and narrow-bore columns a solvent pump has become necessary. Pumps based on displacement from a solvent reservoir have been widely used[7]. When gas pressure is used to displace solvent into the column a diaphragm or interfacial liquid is necessary to prevent the solvent becoming saturated with the gas which appears as bubbles in the column and ruins its efficiency. More satisfactory is a pump of the plunger type. This suffers from the disadvantage of giving rise to pulsating flow but this can be fairly effectively smoothed out[5]. For preliminary experiments, a DCL Micro-Pump (F. A. Hughes Ltd., Epsom) with a stainless steel plunger head assembly was found to be fairly satisfactory at pressures up to 200 p.s.i.g. Random pressure fluctuations were observed, probably due to poor seating of the non-return valves. This pump is capable of delivering a nominal flow of up to 12 ml/min, continuously adjustable from zero, and was useful for determining the effect of solvent velocity on column performance. For more advanced experiments and subsequent analytical evaluation a more sophisticated pump capable of delivering against higher pressure was required. An Orlita Diaphragm Dosing Pump Type DMP-1515 (Orlita KG, Giessen, Lahn) capable of delivering up to 10.5 ml/min against up to 4000 p.s.i.g. has been used. This pump is completely adjustable by micrometer variation of plunger stroke length over the range from maximum to zero flow. After the replacement of faulty ball valves this pump has given excellent performance.

Pressure protection switch. In the event of a blockage or restriction upstream of the Orlita pump a catastrophic rise in solvent pressure between the blockage and pump could occur. To prevent this happening a pressure switch (K.D.G. Instruments Ltd., Crawley) has been incorporated in the solvent flow line immediately after the pump. This switch is adjustable over the range 0–3000 p.s.i.g. and is set to trip the power supply to the pump in the event of a pressure rise in excess of safe operation.

Pressure gauge. Although the actual solvent pressure is of minimal interest in LCC, the solvent velocity being the important related parameter, it is nevertheless necessary to have an indication of the pressure for safe operation. For low pressures up to 300 p.s.i.g. a 0–300 p.s.i.g. Budenberg (Altrincham, Cheshire) direct reading gauge with 4-in. dial was used. For high pressures up to 3000 p.s.i.g. an Ashcroft-Durrance 0–6000 p.s.i.g. direct reading gauge (Dresser Europe S.A., London) with a 6-in. dial was fitted. For work at intermediate pressures an Ashcroft-Durrance 0–1000 p.s.i.g. direct reading gauge with 6 in. dial was more accurate.

Preparation of stationary phase. Silica gel has been firmly established as the most important general purpose adsorbent for LCC of petroleum products. The separations made by TLC in these laboratories are made almost exclusively on silica gel. One of the major objectives of this work was to compare separation of petroleum products by TLC and LCC and silica gel was selected as most suitable for this purpose. The principles established are without doubt applicable to other adsorbents and to liquid–liquid chromatography (LLC).

Experience in GC has shown the importance of narrow-range particle sizes in making columns of high performance. The existence of an optimum mean particle size for a given column length etc. has also been demonstrated in theory and in practice. Theoretical work[11] has shown that for LLC the smallest possible mean particle diameter, ideally zero, would give most rapid equilibrium between stationary and moving phases and maximum efficiency. The practical limitations are in preparation

of the material and in the increasing flow resistance of beds of fine particles. HUBER[12] finds particles of less than 25 μ difficult to pack and GIDDINGS[13] has discussed the effect of formation of aggregates of fine particles into macro particles in the packed bed.

For this study silica gel was prepared in narrow ranges from 28–36 μ up to 100–125 μ.

To prepare this material it was necessary to use a wet sieving technique. (Elutriation was also considered and might be used with advantage.) The Fritsch Pulverisette 3 (A. Fritsch OHG, Idar Oberstein) sieve shaker fitted with wet-sieving head was used. The charge of 100 g wide particle range material was first sieved on the finest mesh (28 μ) to remove fines and the retained material (*ca.* 50 g) was re-sieved on a nest of sieves to give the required size ranges. Table I gives the yield of various sizes obtained from silica gel for TLC (E. Merck A.G., Darmstadt). For preparation of silica gel from other sources preliminary grinding was required. This was conveniently carried out with the Pulverisette 2 Automatic Mortar Grinder (A. Fritsch OHG). The yield of a given size range was necessarily very low and the process of grinding and sieving is very wasteful of material. Table II gives typical recovery from grinding of "Davison 923" silica gel (74–149 μ). The final preparation of the silica gel included drying at 160° and deactivation with water, usually 4 % w/w to increase the linear capacity, according to SNYDER[14].

TABLE I

YIELDS OF SIZE GRADED MATERIAL FROM SILICA GEL FOR TLC (E. Merck A.G., Darmstadt)

Size range (μ)	*Weight* (%)
< 28	62.0
28–36	9.2
36–44	13.2
44–56	14.8
> 56	nil

TABLE II

YIELDS OF SIZE GRADED MATERIAL FROM GRINDING "DAVISON 923" SILICA GEL (74–149 μ) FOR 1 MIN

Size range (μ)	*Weight* (%)
< 28	34.3
28– 36	4.3
36– 63	9.0
63– 80	12.3
80–100	19.0
100–125	11.6
> 125	9.3

Solute detection. Three methods of solute detection were used in the course of the present work. For the first experiments a Barber-Colman (Rockford, Ill.) flame-ionisation detector, with a chain transport mechanism for eluate from the column, through a solvent evaporator to the ionisation detector, was used.

This detector, as received, could not be used at a sensitivity higher than a nominal 10^{-9} A FSD ($\times$ IK). Much of the background noise was traced to the two "Teflon" pulleys used to support the transport chain. These were either contaminating the chain or causing production of static electricity. As one pulley was stationary and the chain slid around it, static might well have been produced. These "Teflon" pulleys were replaced with pulleys of aluminium and both were made to revolve. The noise level was reduced approximately 50-fold and a stable base line was obtained at a sensitivity of 10^{-10} A FSD in the absence of solvent. In the presence of solvent residual noise was traced to particles of silica gel adhering to the chain. It has not proved

possible to achieve a noise-free performance from the detector and it has been used most effectively for detection of milligram amounts of non-volatile solutes. A detector similar in principle to the Barber Colman based on a design by SCOTT[15] and marketed by W. G. Pye, (Cambridge) has been evaluated. This detector exhibited a stable sensitivity one hundred-fold better than the Barber Colman and would be more suitable for LCC.

In view of the difficulties with the Barber-Colman instrument column performance was roughly evaluated with coloured solutes and direct measurement of the solute band width. For accurate measurement of column efficiency fractions were collected and the concentration of solute determined by U.V. absorption at 294 nm using a Unicam SP 600 and 0.5 mm cells. The concentration in absorbance units was plotted against solvent volume and the efficiency calculated in the usual way.

RESULTS

Criteria for an efficient LC column–preliminary studies

Packing technique. The packing of the column in LCC has received little attention. The usual procedure is to fill the column with dry adsorbent whilst tapping the side of the column to induce local vibration. After this consolidation the dry bed is wetted with solvent. The retention of air between and within the particles leads to channelling and an improved technique is often used in which the adsorbent is added to the column in the form of a slurry in the solvent. Trapped air is excluded and the settling out of the adsorbent from the slurry leads to a more regular packing. However, in columns < 5 mm i.d. settling of slurries of the very fine particles used in this work is impracticably slow. HOWARD AND MARTIN[16] introduced a tamping device which consists of a rod with a perforated disc at the end. The disc just fits into the internal diameter of the column and increments of packing can be tamped down while solvent flows up through the holes in the disc. A more recent study of packing methods, including loose filling, tamping, tapping and wet filling, concludes that tapping and bouncing is most convenient and gives the best results with particles of 76 μ average diameter[6].

For the initial experiment in the current study glass columns were packed by tapping. Adsorbent was added in small increments. The efficiency was roughly estimated by direct observation of the broadening of a band of coloured solute progressing through the column. Tapping and bouncing the column during packing was found to be ineffective for the finer adsorbents, *e.g.* 28–36 μ. Experiments with a combination of tapping and bouncing and gentle tamping with a close fitting rod were much more promising and a 3-fold increase in efficiency for 28–36 μ particles resulted. The most effective packing method was found to be incremental addition of dry adsorbent to fill about 10 cm column length, gentle tapping and periodic bouncing and tamping by allowing the weight of a glass or metal rod to rest on the top of the packing while tapping continued.

Column diameter. Columns of internal diameter close to 4 mm were found to pack best. Columns of 6.5 mm I.D. gave about 4-fold increase in band width compared with a similar length of 4 mm I.D. column. Columns 2 mm I.D. gave band widths similar to the 4 mm columns but were much more difficult to pack. Unless the increments of adsorbent in packing were very small (< 1.5 cm) the packing tended to break

up into zones with spaces between. For other studies of the effect of column diameter on efficiency see refs. 4–6.

Sample size. With the SCOTT design of injection port described earlier, experiments with coloured solutes showed that samples up to 100 μl were evenly distributed, provided the sample viscosity was not too high. For higher viscosity samples, *e.g.* lubricants, a preliminary dilution with solvent was necessary. Dilution with an equal volume of solvent was adequate for most samples of this class. An increase in sample band width was observed with increase in sample size from 2 μl to 100 μl of from *ca.* 0.2 cm to *ca.* 1 cm. VAN DEEMTER *et al.*[17] have studied the contribution of sample volume to peak variance in GC and the injection band widths in the current LC study do not make an excessive contribution to the final band variance. A study of the effect of solute/adsorbent weight ratio on efficiency[6] concludes that 0.5–1 mg/g should not be exceeded. In general, samples which exceed 100 μl in volume and 2 mg/g solute/adsorbent weight ratio are likely to nullify the benefits of a well-packed column.

Particle size. Studies with coloured solutes showed that the advantage predicted by theory for reduction in mean particle diameters in LC were significant down to about 40 μ. Particles in the range 28–36 μ gave slightly less efficient columns than those of 44–56 μ, and an increase in particle diameter beyond 56 μ also resulted in some loss of efficiency. For the most exacting work requiring the highest efficiency particles in the 44–56 μ range are about optimum. For many applications the conclusion[6] that 74–125 μ (120–200 mesh) may represent a good compromise is quite valid.

Criteria for an efficient LC column–final evaluation

When the above preliminary studies were completed a series of experiments was conducted designed to lay down operational requirements for LC at the highest practically achievable efficiency likely to be useful in petroleum product separations. For this work stainless steel columns were used, 4.65 mm I.D. and 1 m length. The Orlita pump, 0–6000 p.s.i. range pressure gauge, pressure safety switch and the SCOTT injection port were all utilised. The layout of the final apparatus is illustrated schematically in Fig. 2. Fractions were collected and solute concentration determined by U.V. absorption measurement and efficiencies were calculated in the usual way.

The beneficial effect of tamping the packing revealed by the preliminary work

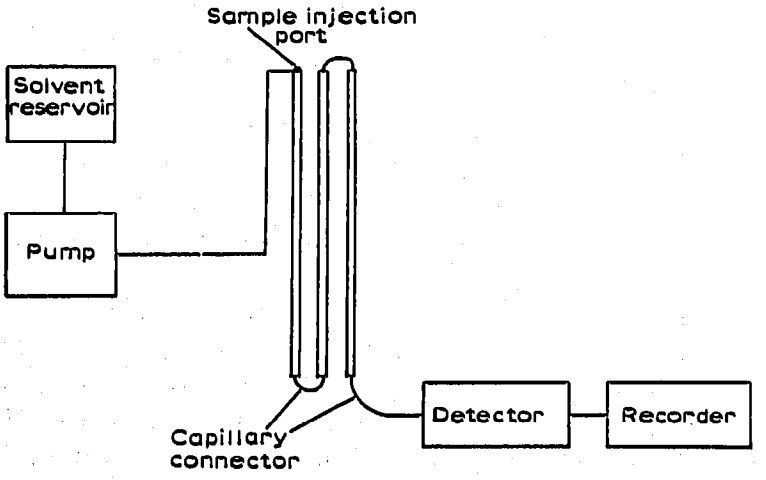

Fig. 2. Layout of liquid chromatograph.

TABLE III

VARIATION IN EFFICIENCY/VELOCITY RELATIONSHIP WITH PARTICLE DIAMETER AND PACKING TECHNIQUE

Column: 1 m; solvent: *n*-heptane; solute: azulene; adsorbent: silica gel (4 % w/w water). Sample: 10 μl 5 % w/w solution.

Particle size (μ)	*Packing technique*	*Solvent velocity* (*cm/sec*)	*Solvent pressure* (*p.s.i.g.*)	*HETP* (*mm*)
28–36	Tapping and bouncing	0.9	630	9.8
		0.082	56	3.5
	Tapping, tamping and bouncing	1.21	700	3.3
		1.15	650	3.5
		0.83	600	3.4
		0.10	85	1.8
		0.10	75	1.8
100–125	Tapping and bouncing	0.9	85	12.5
		0.075	8	3.9
	Tapping, tamping and bouncing	0.85	85	9.3
		0.45	42	7.2
		0.16	20	4.3
		0.092	10	1.8

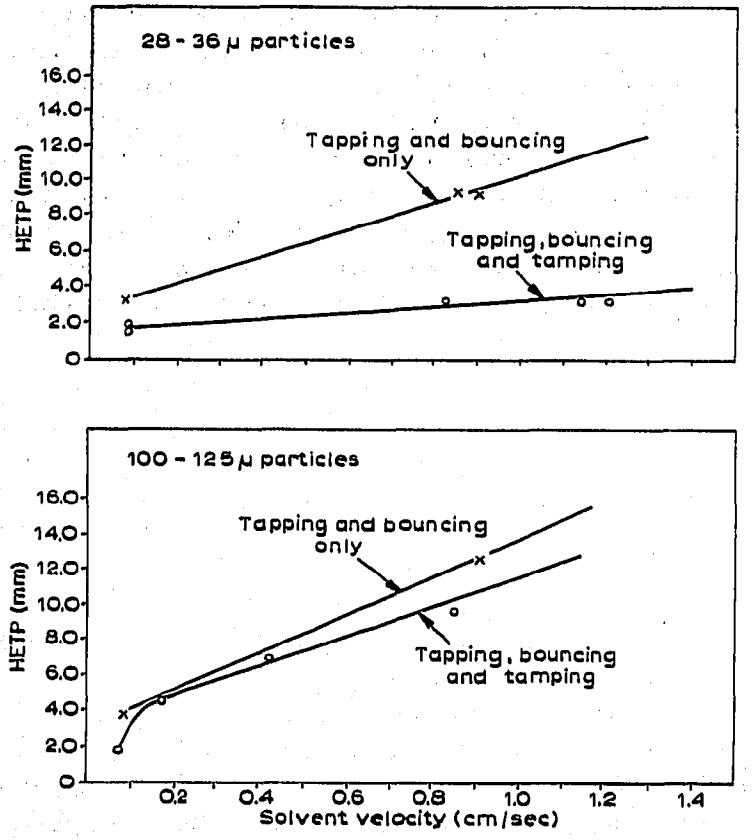

Fig. 3. Variation in efficiency/velocity relationship with particle diameter and packing technique.

was confirmed quantitatively. Efficiency determinations for 28–36 μ silica gel and 100–125 μ material (the extremes of the ranges previously evaluated) were made and the results are given in Table III and graphically in Fig. 3. The increase in efficiency with the 28–36 μ is more marked but some effect persists with the 100–125 μ range. The efficiencies obtained with the 100–125 μ range material were poor even when the tamping technique was used.

The optimum range of particle size was confirmed to be about 44–56 μ. Table IV lists the efficiencies obtained with 28–36 μ, 44–56 μ and 100–125 μ particles at a range of solvent velocities. The comparison is presented graphically in Fig. 4. More detailed study of ranges between 56 μ and 125 μ might be worth while. For a limited study of material in this range see ref. 6.

TABLE IV

EFFICIENCY/SOLVENT VELOCITY RELATIONSHIP WITH DIFFERENT PARTICLE DIAMETER RANGES

Columns: 1 m; solvent: *n*-heptane; solute: azulene; packing: tamping, tapping and bouncing; sample: 10 μl 5% w/w solution.

Particle diameter 28–36 μ		*Particle diameter 44–56 μ*		*Particle diameter 100–125 μ*	
Solvent velocity (cm/sec)	*HETP (mm)*	*Solvent velocity (cm/sec)*	*HETP (mm)*	*Solvent velocity (cm/sec)*	*HETP (mm) %*
1.21	3.3	—	—	—	—
1.15	3.5	—	—	—	—
0.83	3.4	0.86	2.4	0.85	9.3
—	—	0.44	1.7	0.45	7.2
—	—	0.22	1.5	0.16	4.3
0.10	1.8	—	—	0.092	1.8

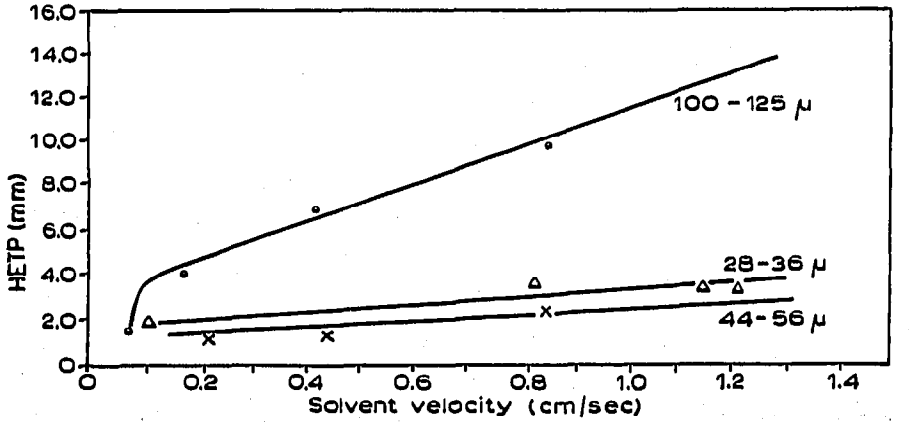

Fig. 4. Efficiency/solvent velocity relationship with different particle diameters.

A final experiment on column efficiency was performed with 44–56 μ particle size silica gel and *n*-pentane eluent. This allows a comparison with a similar column prepared by SNYDER[6]. Detailed experimental conditions are compared in Table V. Results of efficiency measurements at various solvent velocities are listed in Table VI and graphical comparison is made in Fig. 5. The combination of improved apparatus and packing technique has resulted in a 2-fold improvement in efficiency by comparison

TABLE V

EXPERIMENTAL CONDITIONS FOR FINAL EVALUATION OF COLUMN EFFICIENCY AND COMPARISON WITH SNYDER'S RESULTS

Variable	*This work*	*Snyder's work*[6]
Column length	1 m	122 cm (4 ft.)
Column diameter	0.46 cm	0.46 cm
Sample introduction	Injection (10 μl)	Switching valves (0.3 ml)
Adsorbent	Silica gel	Silica gel
Deactivation	4 % w/w water	4 % w/w water
Size range	44–56 μ	57 μ ± 20 %
Solvent	*n*-Pentane	*n*-Pentane
Solute	Azulene	Dibenzyl
Solute retention, $R°$	3.8 ml/g	4.4 ml/g
Packing	Tamping, tapping, bouncing	Tapping and bouncing
(HETP at solvent velocity 0.5 cm/sec)	(1.2 mm)	(2.2 mm)

TABLE VI

HETP VALUES AT VARIOUS SOLVENT VELOCITIES FOR (A) COLUMN PACKED WITH 44–56 μ SILICA GEL AND (B) SIMILAR COLUMN EVALUATED BY SNYDER[6]

44–56 μ packing		*Snyder's column*	
Solvent velocity (cm/sec)	*HETP (mm)*	*Solvent velocity (cm/sec)*	*HETP (mm)*
0.86	1.7	1.0	2.8
0.4	1.1	0.55	2.4
0.205	0.88	0.45	2.2
0.055	0.46	—	—

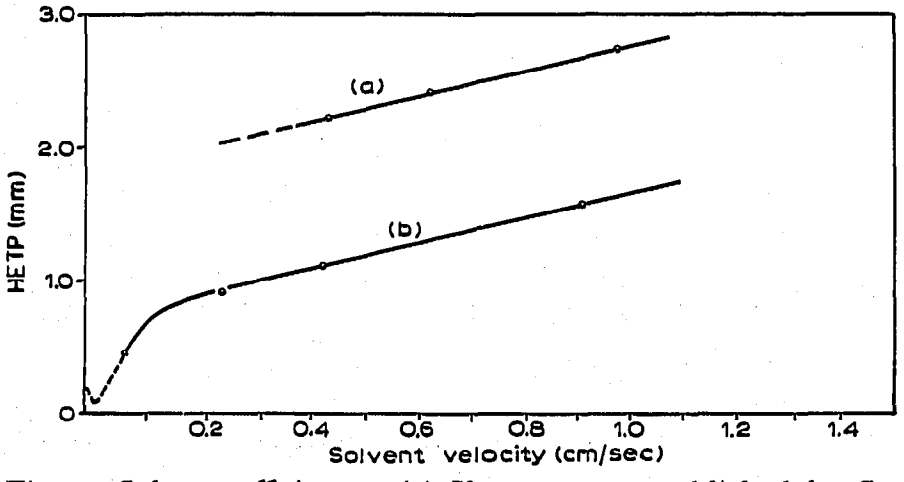

Fig. 5. Column efficiences. (a) Upper curve, published by SNYDER; (b) lower curve, 44–56 μ silica gel, 1 m column.

with SNYDER's results. No other values are available for further comparison but for related studies of the effects of solvent velocity and particle size on efficiency in LC see the work of HORVATH, PREISS AND LIPSKY[18] on ion-exchange resins and also refs. 4 and 5. The broken trace on the lower left of Fig. 5 shows the general shape of the

efficiency/solvent velocity curve at low values of solvent velocity. Efficiencies of $>$ 4000 plates/m are quite possible but as the rate of plate generation is only 30/min separations are very time consuming.

Comparison of LC in columns with TLC

Three separation problems, typical of petroleum product analysis, were tackled simultaneously by efficiency optimised LC and by TLC. These separations, which form a basis for evaluation of the relative merits of the techniques, were of (a) a mixture of phenol alkylation products, (b) a mixture of polynuclear hydrocarbons, and (c) a simulated lubricating oil. This last separation was made on a small-scale (mg) preparative basis. The following detailed observations were made:

Phenol alkylation product. The separation of the mixture of nonylphenols by TLC and LC as illustrated in Fig. 6a is of the densitometer trace of the TLC separation and shows peaks as follows: (1) impurities; (2, 3 and 4) dialkylphenols; and (5) monoalkylphenol. Fig. 6b is of the recorder trace of the separation of the mixture on a 1 m column with a gradient of ether in hexane as solvent. The column separation compares with that of the TLC plate in all respects. The LC separation took 30 min and the TLC separation 45 min. The resolution could be increased by using a longer column or a lower solvent velocity. Only in initial cost of equipment has TLC an advantage for separation of a single sample.

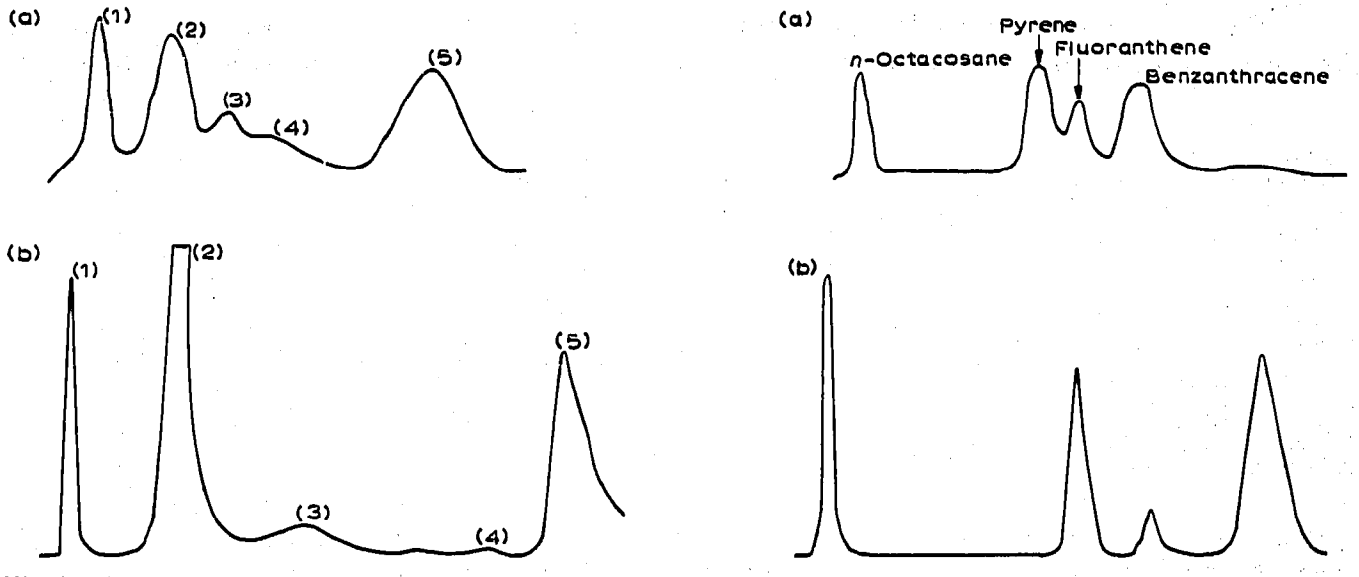

Fig. 6. Separation of alkylphenols by (a) TLC (toluene 45 min) and (b) 1 m column (hexane → ether 30 min).

Fig. 7. Separation of polynuclear hydrocarbons by (a) TLC (*n*-pentane double development 45 min), and (b) 1 m column (*n*-heptane 30 min).

Polynuclear hydrocarbons. The separation of *n*-octacosane, pyrene, fluoranthene and 1,2-benzanthrene was made by TLC in 45 min by double development of the plate with *n*-pentane. The densitometer trace of this separation is shown in Fig. 7a. The same mixture was separated by LCC in 30 min on a 1 m column with *n*-heptane as eluent (Fig. 7b). The column could separate the mixture as well as the plate in as little as 5 min and repeat analyses could be made continuously as in GC. The column length

could be increased by at least 10-fold to make much more difficult separations of polynuclear materials possible. For a further discussion see refs. 2 and 6.

Lubricating oil. The components of the simulated lubricating oil are: base oil, di-*tert.*-butylmethylphenol, *n*-octylphenol, zinc dialkyl dithiophosphate, and calcium sulphonate. The presence of calcium sulphonate in the mixture makes the separation difficult. Conventional wide-bore columns with the high sample loading usually employed are not very effective due to poor resolution and micelle formation. This type of mixture has been separated by preparative-layer chromatography (PLC) with some success. A separation by PLC was made and compared with a separation by LCC. The solvents used for step-wise elution of the components were *n*-hexane, toluene, 10 % acetic acid–hexane and 20 % aqueous ammonia (0.88)–isopropyl alcohol in that order. For the column separation a 1 m column was used with a 100 mg sample and solvent velocity of 0.5 cm/sec. Fractions were collected in combination with the Barber-Colman detector which revealed the presence of solutes in the eluant. Infrared spectra of the separated solutes were recorded and compared with the pure component to give a measure of effectiveness of separation under conditions which would apply in practical analysis. For samples of less than 2 mg the I.R. spectra were obtained either on thin-films or by multiple internal reflectance.

Recovery of solutes from the column was over 90 % with the exception of calcium sulphonate which was irreversible adsorbed. Recovery of solutes from PLC was poor and contamination with silica gel fines was a problem.

Fig. 8 shows (a) the I.R. spectrum of the sample of simulated lubricant and (b) the base mineral oil separated on the column. The absence of peaks due to additives

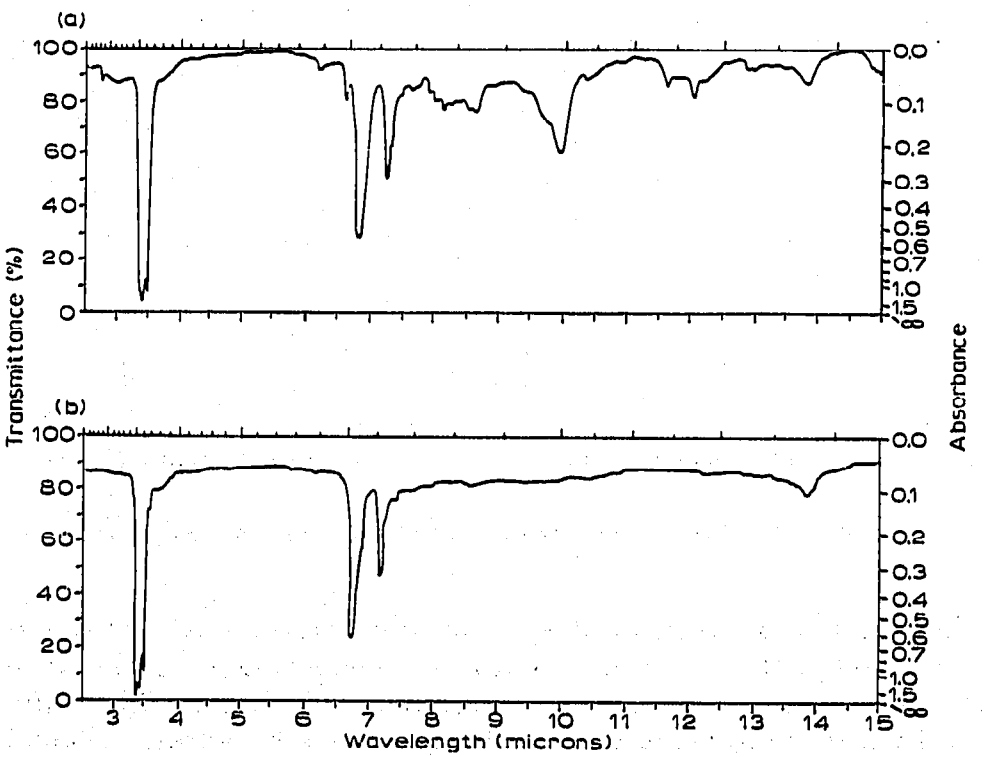

Fig. 8. Column separation of base oil from lubricant. (a) Lubricant blend; (b) base oil separated on column.

is noteworthy. The efficient column, with sample loadings up to 10 mg/g adsorbent, has avoided micelle formation and cleanly separated the additives from the oil.

Fig. 9a shows the I.R. spectrum of *n*-octylphenol, (b) the spectrum of compound separated by LCC from the lubricant, and (c) the spectrum of the component separated by PLC. A similar set of spectra were obtained for the ZDDP component.

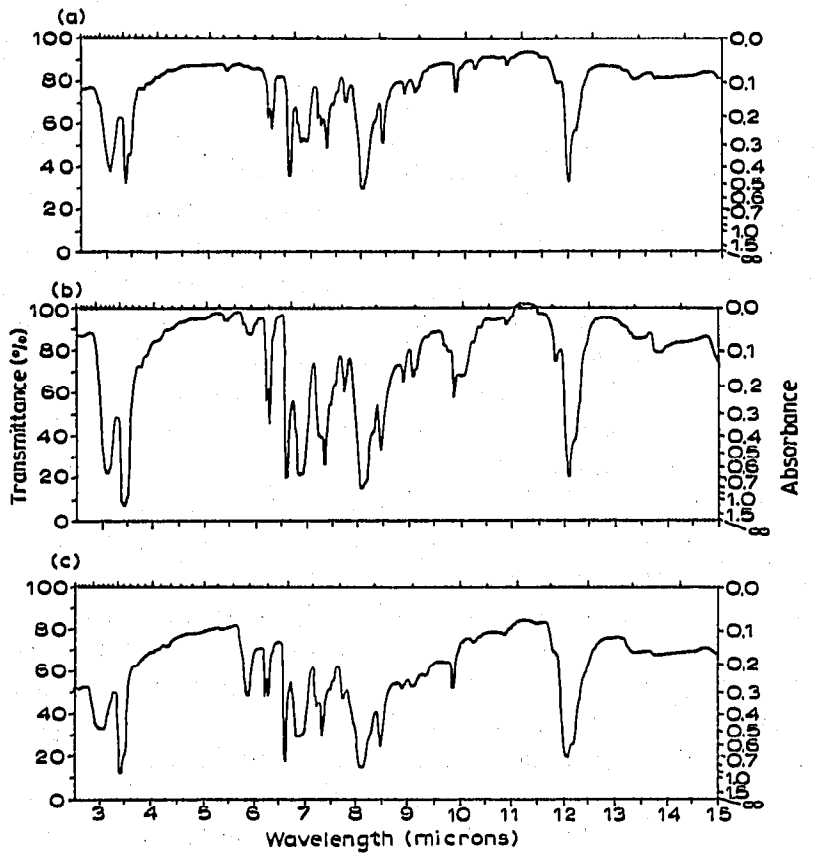

Fig. 9. Separation of *n*-octylphenol by column and TLC from lubricant blend. (a) *n*-Octylphenol; (b) separated by column; (c) separated by TLC.

The major points of comparison which emerged from these experiments were: (1) The techniques of PLC and LCC were equally effective. (2) Fraction collection was much simpler with LCC.

Fraction collection was simpler with LCC because as each solute emerged from the column it could be collected in a tared vessel, the solvent evaporated, the recovery measured and the I.R. spectra recorded with no intervening steps. With PLC even after development of the plate the steps of revealing the solute bands, scraping off solute and associated adsorbent, desorption, filtration (partially effective) and evaporation of desorbing solvent all remained to be carried out. The time spent on these exercises was much greater than that required for LCC separations. The time required to prepare a good column is not much greater than that required to prepare a plate for PLC.

Mention should be made of a technique for recovering material from TLC plates in amounts of about 50 μg. This technique described only recently[19] shows promise of eliminating the need for PLC with milligram amounts. In the new micro-

method the material on the TLC plate is revealed by complexing with iodine, a reversible process. A region of silica gel is drawn out around the spot in the shape of a "tear drop" and the solute is "chased' from this into 2–3 mg of dry potassium bromide. A disc is prepared from this and its I.R. spectrum recorded. Wherever a larger amount (mg) is required LCC would offer advantages for its separation and recovery.

DISCUSSION AND CONCLUSIONS

A number of principles for the practical application of LCC have emerged from this work. It should now be possible to choose the technique and conditions for an LC separation most appropriate to the problem. For many analytical separations TLC will not be replaced by LCC. TLC has the advantages of simplicity and cheapness. The wide range of specific revealing agents can afford important information about the presence of functional groups, elements and structural features in addition to visualising the material on the TLC plate. The ready accessibility of the whole plate avoids part of the sample being overlooked. This could happen in column work when a portion of the sample is not eluted. The possibility of making simultaneous analyses of many samples on one plate is also advantageous in TLC. Rapid screening of samples for a single component *e.g.* an additive can often be conveniently carried out.

The optimised LCC which has been described will greatly extend the range of TLC to more difficult and faster separations. Many separations which cannot be carried out by TLC could be made quite easily and rapidly on a good column. Such columns are not unduly difficult to make although care and attention to detail is essential in their preparation. The equipment required although more elaborate than the conventional crude systems hitherto used in LC is not unduly expensive or exotic. Adequate equipment was used in this work at a cost of under £ 1,500 including the Barber Colman detector, a cost comparable with a modern gas chromatograph.

The major advantage of optimised LCC lies in its flexibility. This is limited only by the practical limitations of increasing pressure requirements with increasing column length. Separations of complex mixtures of polynuclear hydrocarbons, potential carcinogens, can be envisaged and any separation currently being made could be improved by optimisation of operating conditions along the lines described. With the less complex separations involving a single non-polar solvent *e.g.* pentane or hexane a fully automated system could be planned and constructed for evaluation with no further fundamental experimentation.

For preparative separations it has been demonstrated that a good column can give good recovery and has marked advantages over PLC in convenience of solute detection and collection. The separation efficiency of both optimised LCC and PLC are markedly superior to the conventional wide-bore column; in addition optimised LCC is much faster than either PLC or conventional LCC.

Some of the references to related work which have been given, *e.g.* refs. 2 and 5, refer to studies in liquid–liquid (partition) chromatography (LLC). Some experiments have been made with LLC and will be described in a subsequent paper. LOCKE has reviewed much of the work done in LLC and made a number of contributions to the theory[20,21]. LLC has advantages of selectivity over adsorption LC and the additional advantage that columns should be reusable indefinitely. Most, if not all, the results of

the present work which has concentrated on silica gel adsorbent stationary phase, should apply to LLC with equal validity in making an efficient column system.

The judicious choice of the appropriate system, adsorption or partition, TLC or LCC, should lead to the most effective separation of most mixtures of organic and organo-metallic components with which we are faced. The next stage is the rapid accumulation of practical experience, in the application of the techniques now developed to real analytical problems.

REFERENCES

1 D. T. DAY, *Proc. Am. Phil. Soc.*, 36 (1897) 112.
2 J. C. GIDDINGS, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
3 T. W. SMUTS, F. A. VAN KIEKERK AND V. PRETORIUS, *J. Gas Chromatog.*, 5 (1967) 190.
4 R. P. W. SCOTT, D. W. J. BLACKBURN AND T. WILKINS, *J. Gas Chromatog.*, 5 (1967) 182.
5 J. F. K. HUBER AND J. A. R. J. HULSMAN, *Anal. Chim. Acta*, 38 (1967) 305.
6 L. R. SNYDER, *Anal. Chem.*, 39 (1967) 698, 705.
7 K. J. BOMBAUGH AND J. W. LITTLE, *J. Chromatog.*, 16 (1964) 47.
8 E. HAAHTI, T. NIKKARI AND J. KARKKAINEN, in A. GOLDUP (Editor), *Gas Chromatography*, Institute of Petroleum, London, 1964, p. 197.
9 R. J. MAGGS, *Column*, 2 (1967) 4.
10 L. R. SNYDER, in E. HEFTMANN (Editor), *Chromatography*, 2nd Ed., Reinhold, New York, 1967, p. 88.
11 J. C. GIDDINGS, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965, p. 281.
12 J. F. K. HUBER, private communication.
13 J. C. GIDDINGS, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965, Chap. 5, p. 195.
14 L. R. SNYDER, *Separation Sci.*, 1 (1966) 191.
15 R. P. W. SCOTT, A. T. JAMES AND J. R. RAVENHILL, in A. GOLDUP (Editor), *Gas Chromatography*, Institute of Petroleum, London, 1964, p. 197.
16 G. A. HOWARD AND A. J. P. MARTIN, *Biochem. J.*, 46 (1950) 532.
17 J. J. VAN DEEMTER, F. J. ZUIDERWEG AND A. KLINKENBERG, *Chem. Eng. Sci.*, 5 (1956) 271.
18 C. G. HORVATH, B. A. PREISS AND S. R. LIPSKY, *Anal. Chem.*, 39 (1967) 1422.
19 D. D. RICE, *Anal. Chem.*, 39 (1967) 1906.
20 D. C. LOCKE, in J. C. GIDDINGS AND R. A. KELLER (Editors), *Advances in Chromatography*, Vol. VII, Marcel Dekker, New York, 1968.
21 D. C. LOCKE, *Anal. Chem.*, 39 (1967) 921.