Journal of Chromatography, 122 (1976) 243–258

CHROM. 9148

OPTIMISATION OF LIQUID CHROMATOGRAPHIC PERFORMANCE ON COLUMNS PACKED WITH MICROPARTICULATE SILICAS

T. J. N. WEBBER and E. H. McKERRELL

Shell Research Limited, Woodstock Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent (Great Britain)

SUMMARY

A modular packing system for packing analytical and semi-preparative scale columns has been constructed. Columns were packed with microparticulate silicas using different slurry media. Optimum results were obtained with carbon tetrachloride as the slurry medium using a packing pressure of 20.7 MPa (3000 p.s.i.g.) and a slurry concentration in the range 1–10% w/v. Various methods of sample introduction were investigated. Highest efficiencies were obtained using syringe injection, but comparable performance can be achieved with valve injection if a split-stream technique is used. Plate heights of between 2.5 and 3.5 particle diameters were attained with 11-, 6- and 3.7-μm particle size silicas in 0.10-m × 4.5-mm-I.D. columns. The use of wider-bore, 9.5-mm-I.D., columns gave plate heights of 2 particle diameters and up to 30 effective plates per second at a linear velocity of 1.5 mm/sec.

INTRODUCTION

The realisation of optimal performance from columns packed with microparticulate silicas is dependent on many factors, such as the slurry packing procedure employed, the column dimensions and the method of sample introduction.

Many previous workers[1–13] have reported results with 10- and 5-μm particle size silicas, although the quoted efficiencies have varied widely. It can only be concluded that this is due to differences in the efficacy of various packing procedures and to such factors as extra-column effects and methods of sample introduction. The use of sub-5-μm materials should result in the achievement of higher efficiencies[2,4,14], but few results on the performance of these materials have been reported[9,13].

At present there are few comparative data on the effects of some factors that can influence column performance, whilst there is diversity of opinion on other factors, such as the packing procedure. Several slurry-packing procedures have been developed over the past five years, including the balanced density technique[1,3,7,8] developed by Majors, Kirkland's stabilised slurry procedure[2,4,12] and the viscosity method of Asshauer and Halász[7]. The use of low-viscosity single-component solvent systems has also been advocated; these include hexane[8], chloroform[8], methanol[15] and carbon tetrachloride[10,11]. Recommended packing pressures have ranged from 13.8–69.0 MPa

(2,000 to 10,000 p.s.i.g.)[1,2,5,8,15,16], whilst the slurry concentrations have varied between 5 and 30% w/v[1,3,4,7,10,11].

The method of column termination and sample introduction is of great importance[4,10], as poor design here can result in a very adverse effect on the resultant column performance. Kirkland[4] demonstrated that the lowest plate heights were obtained by injection of the sample directly into the top and centre of the column packing using a microsyringe. This technique is not suitable for routine operation because of disturbance to the column bed, though Kirkland subsequently showed that results equivalent to on-column injection could be obtained by using a stainless-steel screen on the column inlet. Coq *et al.*[10] demonstrated the desirability of injecting the sample directly onto the column packing. The use of valve injection is attractive for routine analytical operation though few comparative data on syringe and valve injection methods are available, while no procedure for optimising valve injection appears to have been reported.

The effect of column bore on resultant efficiency has been the subject of several papers following Knox and Parcher's development of the "infinite diameter" concept[17]. DeStefano and Beachell[18,19] subsequently demonstrated with the use of large particle size silicas (Zipax <37 μm and Porasil A 35–75 μm) that improved column performance could be obtained using 10.9-mm-bore columns in place of 2.1-mm-bore columns. Few data have been reported, however, on the use of columns of wider bore than 5 mm packed with microparticulate silicas.

The purpose of this work was to further study a number of the variables involved in the production and operation of high-performance microparticulate silica columns with the aim of achieving optimal and reproducible performance.

EXPERIMENTAL

Chemicals

The chromatographic solvents, isopropanol, dichloromethane and hexane, were of reagent grade and were used without further treatment. Methanol of analytical grade was used as received whilst reagent grade carbon tetrachloride was distilled in a glass apparatus before use.

Adsorbents

The silicas used were Partisil 10, Partisil 5 and Partisil 3 (Whatman, Maidstone, Great Britain), the latter being an experimental material.

Column construction

Columns were made from 316 grade seamless stainless-steel 6.35-mm-O.D. (1/4-in.) × 4.5-mm-I.D. and 12.7-mm-O.D. (1/2-in.) × 9.5-mm-I.D. tubing (Tube Sales, Southampton, Great Britain) of the requisite length. This tubing was rotated in a lathe at 1000 rpm and the internal surface honed and polished by means of deburring brushes or Flex-hones (Nicro (Leamington), Stroud, Great Britain) of suitable diameter. The ends of the column were machined flat and the tubing was degreased in chloroform in an ultrasonic bath.

The 6.35-mm-O.D. (1/4-in.) columns were terminated as shown in Fig. 2 with a 6.35-mm-O.D. (1/4-in.) stainless-steel frit of 2-μm-pore size (Whatman) fitted into

a zero dead volume (ZDV) 6.35-mm (1/4-in.) to 1.6-mm (1/16-in.) reducing union (Whatman), whilst the 12.7-mm-O.D. (1/2-in.) columns were terminated as shown in Fig. 5. Compression tube fittings (Swagelok or Gyrolok) were used throughout.

Column packing

Fig. 1 shows a schematic representation of the packing equipment. The Haskel DST-122C pump (Olin Energy Systems, Sunderland, Great Britain) was fitted with a solvent reservoir and mounted on a framework which also supported the packing assembly. Nova Swiss (Olin Energy Systems) high-pressure components and fittings were used throughout. Scale drawings of the two designs of packing assembly are shown in Figs. 2 and 3. The system shown in Fig. 2, which was used with slurry concentrations up to 10% w/v, was constructed of three main components, *viz.*, a top section, interchangeable middle sections 40 mm, 90 mm, and 190 mm in length giving slurry capacities of 20 ml, 50 ml, and 100 ml, respectively, for packing columns of different bores and/or lengths, and interchangeable bottom sections fitted with the appropriate couplings for packing 6.35-mm-O.D. (1/4-in.), 12.7-mm-O.D. (1/2-in.), and 25.4-mm-O.D. (1-in.) columns. The system shown in Fig. 3 was used with slurry concentrations greater than 10% w/v.

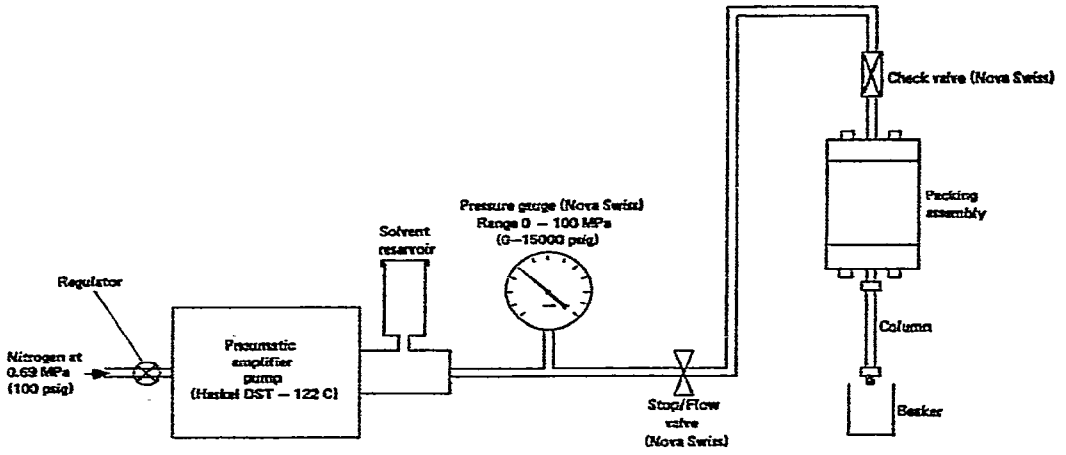

Fig. 1. Schematic representation of packing equipment.

For packing 0.10-m × 4.5-mm-I.D. columns the slurry was prepared by weighing 1.3 g of silica into a test tube and adding 15 ml of the slurry medium —carbon tetrachloride, methanol or 0.001 *M* ammonium hydroxide. The slurry was shaken and the test tube placed in an ultrasonic bath for 1–2 min. The packing assembly (Fig. 1) was disconnected at the check valve and the top section of the slurry vessel (Fig. 2) was removed. The column blank was capped with a 1.6-mm (1/16-in.) plug, connected to the bottom section and filled with slurry medium. The slurry was introduced into the reservoir, the test tube was washed out with a further 5 ml of slurry medium, the top section was bolted into position and the connecting tubing was filled with slurry medium by means of a syringe. The remainder of the system was filled with slurry medium by pumping through the one-way check valve. The stop/flow valve

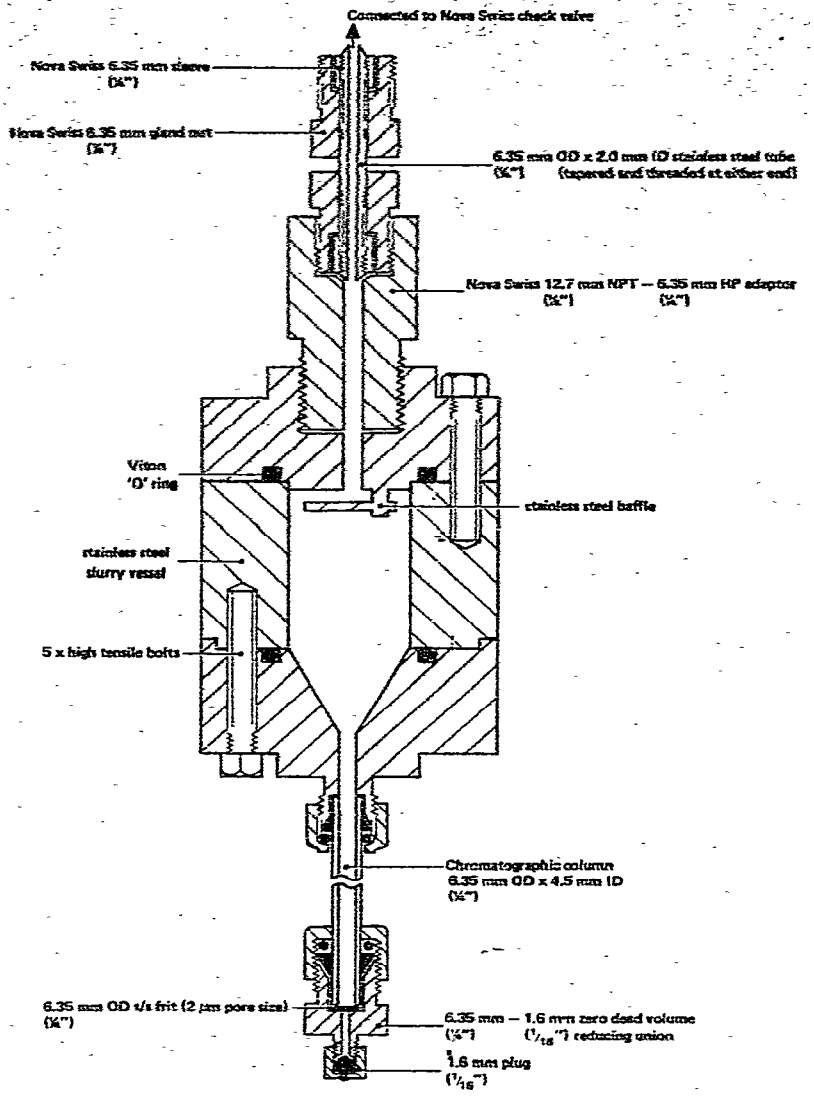

Fig. 2. Packing assembly with column attached. Scale, 1:1.8.

was then closed, the packing assembly was reconnected and the required packing pressure was generated by adjusting the air control regulator on the Haskel pump. The 1.6-mm (1/16-in.) plug was removed, the column was packed by opening the stop/flow valve and a further 100 ml of slurry medium were passed through the column before it was disconnected. The 0.10-m × 9.5-mm-I.D. columns were packed in a similar manner to the above using a slurry consisting of 4.0 g of silica in 45 ml of slurry medium.

The top 2.5 mm of packing were removed using a pre-set reamer and a disc (4.5 mm O.D. or 9.5 mm O.D.) of 8-μm-pore size stainless-steel woven mesh (Sankey

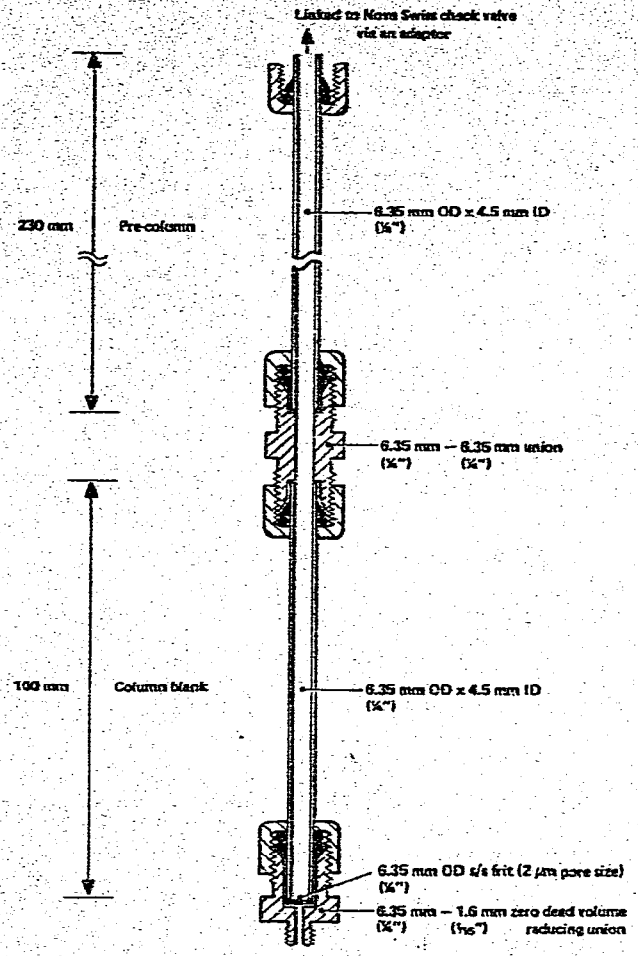

Fig. 3. Extended tube packing assembly.

Green Wire Weaving, Warrington, Great Britain) pressed into position on top of the packing. A 4-mm-thick plug of 35-μm-pore size PTFE (Field Instruments, Richmond, Great Britain) of the appropriate diameter was then pressed into the column and the excess was removed with a razor blade to leave a flush finish at the top of the column. The columns packed using carbon tetrachloride and methanol as the slurry medium were conditioned before use by washing with 100 ml of the mobile phase, whilst those packed using 0.001 *M* ammonium hydroxide were washed with 100 ml methanol, 50 ml diethyl ether, followed by 100 ml of the mobile phase.

For packing slurries of higher concentration an extended tube packing assembly (see Fig. 3) was used. A 26% w/v slurry, prepared as described previously using 1.3 g silica in 5 ml slurry medium, was poured into the pre-column. The assembly was linked to the check valve and the column was packed, terminated and equilibrated as described previously.

Liquid chromatographic equipment

The LC equipment was laboratory assembled from commercial and workshop constructed components. The solvent delivery system consisted of a MPL Series II Micro-pump fitted with a size 2 high-pressure pump head (Metering Pumps, London, Great Britain). Pulse damping was effected by the combination of a Bourdon type pressure gauge and a coiled length (25 m × 0.25 mm I.D.) of stainless-steel capillary tubing (Scientific Glass Engineering, London, Great Britain). A Cecil CE 212 variable-wavelength UV monitor (Cecil Instruments, Cambridge, Great Britain) fitted with a 8-μl flow cell was used as the detector. The standard metal cell compartment lid was replaced with a lid (see Fig. 4) constructed from Heron grade Tufnol (Tufnol, Birmingham, Great Britain), made in two overlapping sections to allow easy access to the cell compartment, and held in position by magnetic strip. The 4.5-mm-I.D. columns were fitted directly into the cell compartment (see Fig. 4) and linked to the 8-μl flow cell by 35 mm of capillary PTFE tubing (1.6 mm O.D. × 0.3 mm I.D.), whilst the 9.5-mm-I.D. columns were connected with capillary PTFE tubing which terminated at the base of the stainless-steel frit (see Fig. 5).

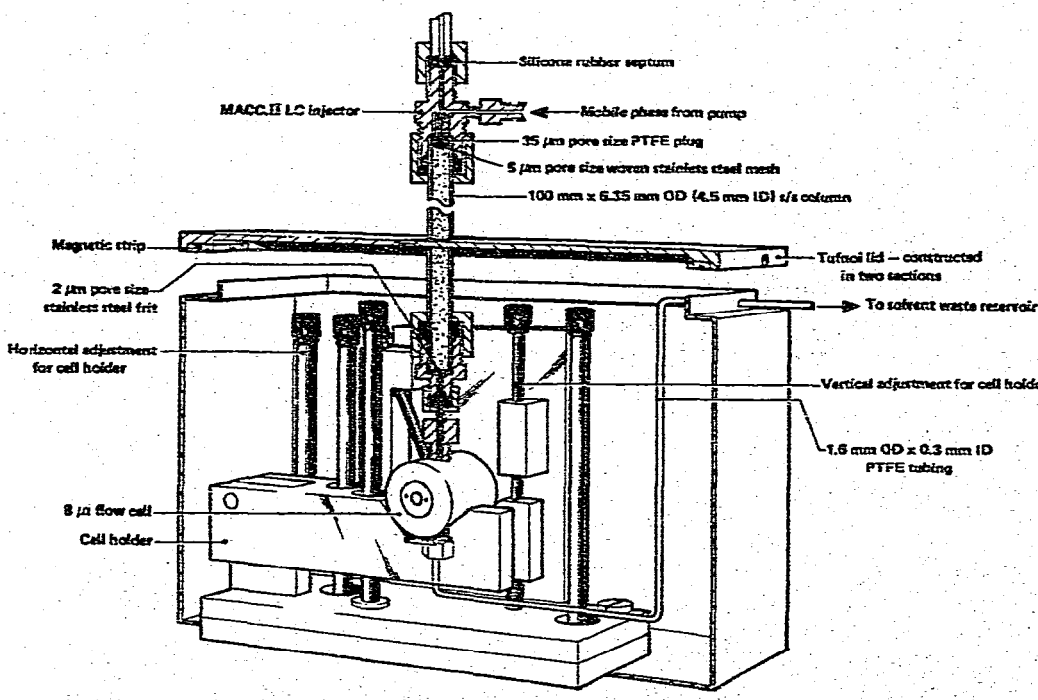

Fig. 4. Sectional view of cell assembly with column in position.

Two methods of sample introduction were used with the 4.5-mm-I.D. columns: (i) syringe injection in combination with a MACC II LC Injector (Phase Separations, Queensferry, Great Britain), as shown in Fig. 4. With this system the syringe needle was pushed through the PTFE plug and the injection was made with the needle tip touching the stainless-steel mesh; (ii) valve injection using a Specac four-port sample

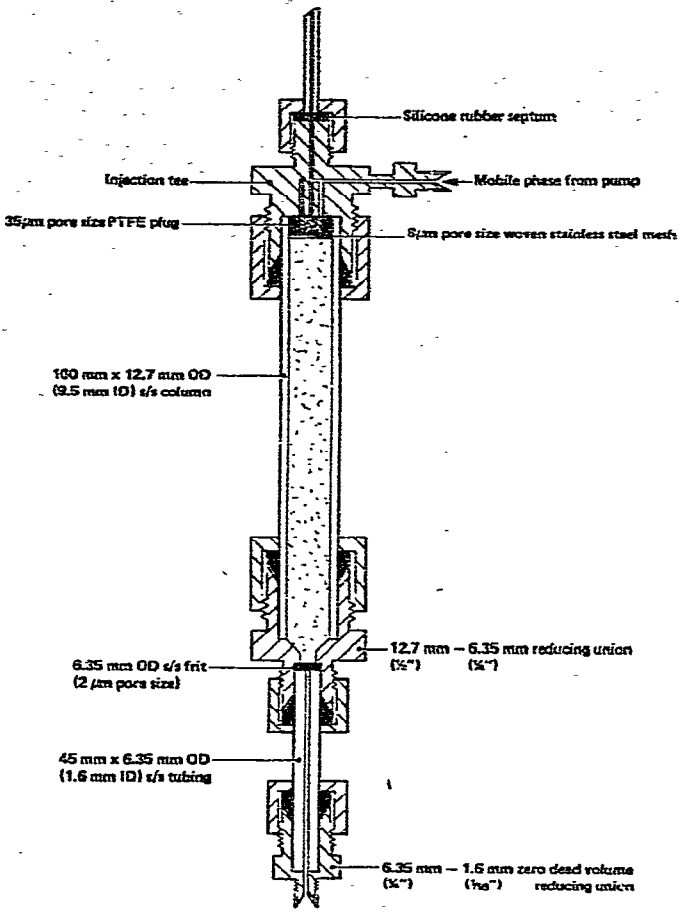

Fig. 5. Column fittings for a column of 12.7 mm (½ in.) O.D.

introduction valve (Spectroscopic Accessory Company, Sidcup, Great Britain) in combination with the split stream arrangement shown in Fig. 6. With the 9.5-mm-I.D. columns sample introduction was accomplished by means of an injection tee (see Fig. 5) of similar design to the MACC II injector.

Investigation of column parameters

The effects of the various parameters on column performance were determined by measuring the column efficiencies achieved when chromatographing a test mixture of *o*-, *m*- and *p*-nitroaniline on 0.10-m × 4.5-mm-I.D. columns using IPA–CH_2Cl_2–hexane (1:50:49) as the mobile phase, a Cecil CE 212 monitoring at 254 nm as the detector, and the syringe injection technique shown in Fig. 4, unless otherwise stated.

The results from a comparison of the use of carbon tetrachloride, methanol and 0.001 *M* ammonium hydroxide as the slurry media are shown in Table I. For all subsequent investigations carbon tetrachloride was used as the slurry medium. Tables II and III illustrate the effects of packing pressure and slurry concentration on resul-

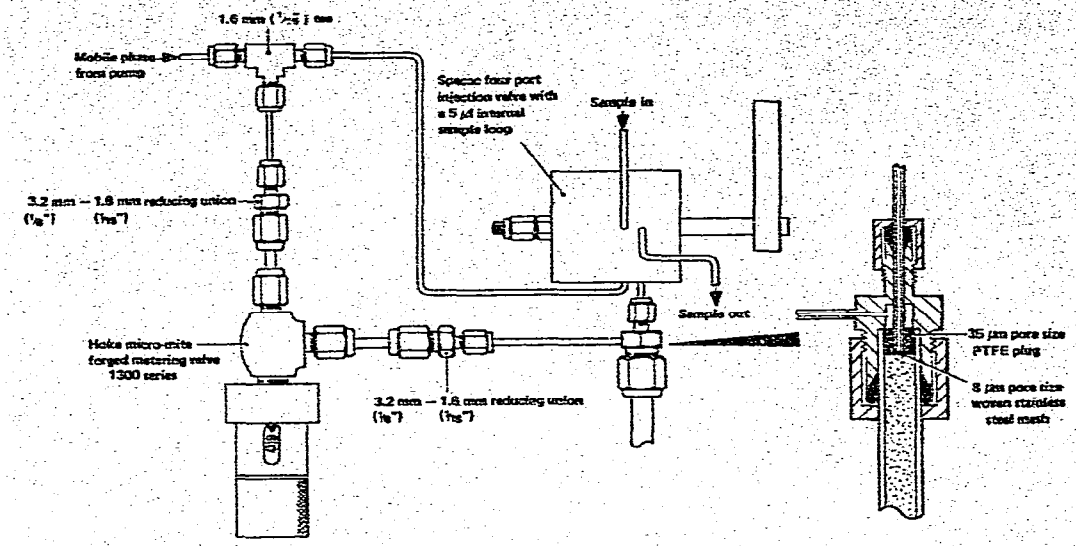

Fig. 6. Split stream arrangement for valve injection.

tant column efficiency. The chromatographic performances achieved with 10-, 5- and 3.7-μm-particle size silica are shown in Table V, whilst the effects of sample introduction technique and column bore size are illustrated in Tables IV and VI, respectively.

Measurement of column performance

The values of k', t_R, and N quoted in the tables are the mean values obtained from three injections of the test mixture. The measurement of N was based on the half-height equation[20], whilst the definitions for k' and N_{eff}/sec have been published previously[1,2].

RESULTS AND DISCUSSION

The design of the packing assembly (Fig. 2) was based on that of Kirkland[21], but it was constructed on a modular basis using interchangeable sections to allow both analytical and preparative-scale columns to be packed.

The studies of slurry medium, packing pressure, and slurry concentration (Tables I–III) were carried out at linear velocities between 3 and 4 mm/sec, resulting in mobile phase flow-rates for 0.10-m × 4.5-mm-I.D. columns of ~2 ml/min. This linear velocity was selected as it is typical of that used with the 0.10-m columns that are routinely employed in this laboratory, giving a good compromise between column efficiency and speed of analysis, *i.e.*, between plate height, H, and the number of effective plates per second.

The activity of the silica was controlled by the addition of 1 % v/v isopropanol to the mobile phase. The reproducibility of column activity and selectivity thereby obtained is demonstrated by the repeatability of the k' values in Tables I–VI. Isopropanol was used in preference to water as in general it is easier to use and gives

shorter column equilibration times, although the use of water as modifier is known to give higher efficiencies and greater selectivity in some instances[4,11].

The comparison of three slurry media (see Table I) clearly demonstrates the superior performance obtained with carbon tetrachloride. This slurry medium was used for all further studies. Addition of silica to carbon tetrachloride gave a translucent, gelatinous suspension which settled only slowly and with no visible evidence of particle size fractionation. This contrasted with the use of methanol and 0.001 *M* ammonium hydroxide, where rapid sedimentation occurred, leaving a milky suspension of "fines". This particle size segregation was probably the cause of the less efficient and non-gaussian shaped peaks obtained with columns packed using these slurry media.

TABLE I

EFFECT OF SLURRY MEDIUM ON RESULTANT COLUMN EFFICIENCY

Packing material, Partisil 5; column packing pressure, 31.0 MPa (4500 p.s.i.g.); slurry concentration, 8.7% w/v. (a) and (b) refer to individual 0.10-m × 4.5-mm-I.D. columns.

Operating conditions		*Slurry medium*								
		Carbon tetrachloride			*Methanol*			*0.001 M ammonium hydroxide*		
Operating pressure (MPa)	(a)	5.9 (850)*			5.9 (850)			6.9 (1000)		
	(b)	5.9 (850)			5.9 (850)			6.6 (950)		
Flow-rate (ml/min)	(a)	2.14			2.14			2.11		
	(b)	2.14			2.07			2.07		
Linear velocity (mm/sec)	(a)	3.47			2.82			3.21		
	(b)	3.70			3.24			3.14		
Parameter		*Solute***								
		o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
k'	(a)	0.60	2.03	2.45	0.62	2.12	2.54	0.62	2.07	2.49
	(b)	0.66	2.21	2.60	0.66	2.13	2.59	0.62	2.03	2.44
t_R (min)	(a)	0.77	1.46	1.66	0.96	1.84	2.09	0.84	1.59	1.81
	(b)	0.74	1.43	1.61	0.86	1.62	1.86	0.87	1.62	1.84
N	(a)	5400	5700	5600	2700	3500	3900	3600	3900	4200
	(b)	4900	5400	5700	2600	3100	3200	3500	4100	4400
H (μm)	(a)	18	17	18	37	29	26	28	26	24
	(b)	20	18	17	39	32	31	29	24	23
N_{eff}/sec	(a)	16	29	28	7	15	16	10	19	20
	(b)	17	30	31	8	15	15	10	19	20

* The figures in brackets represent pressures in p.s.i.g.

** *o-*, *m-* and *p*-nitroanilines.

A study of the effect of packing pressure (Table II) using a slurry concentration of 8.7% w/v in carbon tetrachloride gave less definitive results, with column performances being comparable for packing pressures between 20.7 and 41.4 MPa. Although the use of higher pressures might be expected to give a denser packing bed and hence

TABLE II

EFFECT OF PACKING PRESSURE ON RESULTANT COLUMN EFFICIENCY

Packing material, Partisil 5; slurry concentration, 8.7% w/v in CCl_4. (a) and (b) refer to individual 0.10-m × 4.5-mm-I.D. columns.

Operating conditions		*Packing pressure (MPa)*					
		*10.3 (1500)**	*20.7 (3000)*	*31.0 (4500)*	*41.4 (6000)*	*62.1 (9000)*	*82.8 (12000)*
Operating pressure (MPa)	(a)	4.5 (650)	5.2 (750)	5.9 (850)	6.9 (1000)	9.3 (1350)	10.7 (1550)
	(b)	4.5 (650)	5.2 (750)	5.9 (850)	7.6 (1100)	7.9 (1150)	10.0 (1450)
Flow-rate (ml/min)	(a)	2.31	2.14	2.14	2.14	2.11	2.11
	(b)	2.31	2.14	2.14	2.14	2.11	2.11
Linear velocity (mm/sec)	(a)	3.62	3.40	3.47	3.58	3.21	3.21
	(b)	3.79	3.47	3.70	3.47	3.40	3.14

Parameter		*Solute***																
		o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
k'	(a)	0.58	1.97	2.35	0.59	1.92	2.35	0.60	2.03	2.45	0.68	2.14	2.58	0.64	2.21	2.64	0.66	2.23	2.67
	(b)	0.59	1.99	2.38	0.67	1.68	2.31	0.66	2.21	2.60	0.71	2.20	2.66	0.67	2.19	2.63	0.64	2.15	2.58
t_R (min)	(a)	0.73	1.37	1.55	0.78	1.43	1.64	0.77	1.46	1.66	0.78	1.45	1.66	0.86	1.67	1.90	0.87	1.68	1.91
	(b)	0.70	1.32	1.49	0.80	1.28	1.58	0.74	1.43	1.61	0.82	1.54	1.76	0.82	1.55	1.77	0.87	1.67	1.90
N	(a)	4600	4800	4700	5900	5700	5600	5400	5600	5600	4700	5000	5000	4000	3900	3900	3200	3100	3000
	(b)	4200	4300	4200	5100	5800	5700	4900	5400	5700	5800	5600	5600	3200	3200	3100	2400	2700	2800
H (μm)	(a)	22	21	21	17	18	18	19	18	18	21	20	20	25	26	26	31	32	33
	(b)	24	23	24	20	17	18	20	18	18	17	18	18	31	31	32	41	37	36
N_{eff}/sec	(a)	14	26	25	17	29	28	16	29	28	16	27	26	12	18	18	10	15	14
	(b)	14	24	23	17	30	29	17	30	31	20	29	28	10	16	15	7	13	13

* The figures in brackets represent pressures in p.s.i.g.

** *o-*, *m-* and *p*-nitroanilines.

columns of higher efficiency, a marked reduction in efficiency was obtained at pressures >41.4 MPa. Comparison of the particle size distribution curve of the original silica with that of the silica from a column packed at 82.8 MPa showed no increase in "fines" in the latter, the presence of which might result in lower efficiencies. There was, however, some evidence of particle agglomeration, which could result in channelling.

Variation in slurry concentration over the range 1.4–8.7% w/v (Table III) was found to have a negligible effect on column efficiency. The standard packing assembly could not be used with slurries of high concentration because of their viscosity and an extended tube assembly (Fig. 3), based on the design of Cox[22], was employed. Columns packed with this assembly using a slurry concentration of 26% w/v were less efficient than those packed using lower slurry concentrations, though their performance was adequate for many separations.

Plots of linear velocity (u) against plate height (H) for 4.5-mm- and 9.5-mm-I.D. columns had shown that the lowest plate heights were obtained at linear velocities between 1.0 and 2.0 mm/sec and increased again as the linear velocity was further

TABLE III

EFFECT OF SLURRY CONCENTRATION ON RESULTANT COLUMN EFFICIENCY

Packing material, Partisil 5; column packing pressure, 31.0 MPa (4500 p.s.i.g.), using CCl_4. (a) and (b) refer to individual 0.10-m × 4.5-mm-I.D. columns.

Operating conditions		*Slurry concentration (% w/v)*											
		1.4			*2.9*			*8.7*			*26.0**		
Operating pressure (MPa)	(a)	5.9 (850)**			5.5 (800)			5.9 (850)			5.9 (850)		
	(b)	5.5 (800)			5.5 (800)			5.9 (850)			6.2 (900)		
Flow-rate (ml/min)	(a)	2.11			2.11			2.14			2.14		
	(b)	2.14			2.07			2.14			2.14		
Linear velocity (mm/sec)	(a)	3.51			3.40			3.47			3.70		
	(b)	3.47			3.30			3.70			3.62		
Parameter		*Solute****											
		o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
k'	(a)	0.63	2.14	2.54	0.66	2.19	2.60	0.60	2.03	2.45	0.66	2.19	2.61
	(b)	0.63	2.16	2.55	0.64	2.13	2.54	0.66	2.21	2.60	0.61	2.11	2.52
t_R (min)	(a)	0.78	1.49	1.68	0.82	1.57	1.77	0.77	1.46	1.66	0.75	1.43	1.62
	(b)	0.78	1.52	1.71	0.83	1.58	1.79	0.74	1.43	1.61	0.74	1.43	1.62
N	(a)	5800	5600	5600	6100	5700	5900	5400	5700	5600	4700	4900	4800
	(b)	5800	5300	5500	5600	5700	5800	4900	5400	5700	4300	4300	4200
H (μm)	(a)	17	18	18	16	17	17	18	17	18	21	20	21
	(b)	17	19	18	18	17	17	20	18	17	23	23	24
N_{eff}/sec	(a)	19	29	29	20	29	29	16	29	28	17	27	26
	(b)	19	27	28	71	28	28	17	30	31	14	23	22

* Packed using the extended tube packing technique.

** The figures in brackets represent pressures in p.s.i.g.

*** *o-*, *m-* and *p*-nitroanilines.

reduced. The effects of sample introduction, bore size, and particle size were examined at linear velocities in the above range, firstly, because optimum values of H were obtained and secondly, because deficiencies in the sample introduction system are emphasised at low values of u.

Detailed attention to the minimisation of extra-column effects and to the method of sample introduction is very important when using short, high-performance columns. The modification to the Cecil CE 212 (Fig. 4) allowed the column to be connected close to the flow cell, thereby reducing dead volume to a minimum. It also resulted in greater temperature stability, permitting the detector to be operated on the 0.01 A f.s.d. range without the need for temperature control of either the column or the cell compartment. Detector noise was typically 0.0001 A.

Various methods of sample introduction (Table IV) were examined using the same 0.10-m × 4.5-mm-I.D. column. Syringe injection onto the top of the PTFE plug (system a) resulted in less efficient chromatography than when the sample was injected directly onto the stainless-steel mesh (system b). This was probably the result of sample diffusion in the PTFE plug in the former method. In system b the PTFE plug acts as a diffuser for the mobile phase, thereby minimising lateral diffusion of the sample on injection, whilst it has the added advantage of being an in-line filter, which can be readily replaced without disturbing the packing bed.

Use of an injection valve with the outlet terminated at the top of the PTFE plug gave inferior results to those of syringe injection (system a, Table IV). A split-stream technique (Fig. 6) was therefore developed which was similar in principle of

TABLE IV

EFFECT OF SAMPLE INTRODUCTION TECHNIQUE ON RESULTANT COLUMN EFFICIENCY

Introduction techniques: (a) Syringe —injected onto top of PTFE plug. (b) Syringe —injected through PTFE plug onto stainless-steel mesh. (c) Injection valve —with capillary tube through PTFE plug onto stainless-steel mesh, no split flow. (d) Injection valve —with capillary tube through PTFE plug onto stainless-steel mesh, with split flow. Column, 0.10 m × 4.5 mm I.D.; packing material, Partisil 5; column packing pressure, 31.0 MPa (4500 p.s.i.g.); slurry concentration, 8.7% w/v.

Operating conditions	*Introduction technique*											
	a			*b*			*c*			*d*		
Operating pressure (MPa)	2.8 (400)*			2.8 (400)			2.8 (400)			2.8 (400)		
Flow-rate (ml/min)	1.08			1.08			1.09			1.09		
Linear velocity (mm/sec)	1.43			1.41			1.44			1.38		
Parameter	*Solute***											
	o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
k'	0.61	1.99	2.39	0.60	1.99	2.39	0.60	2.01	2.41	0.57	1.92	2.31
t_R (min)	1.88	3.48	3.95	1.89	3.52	3.99	1.85	3.48	3.94	1.90	3.54	4.01
N	5100	6100	6100	6800	6700	6400	5200	5400	4500	6100	6300	6100
H (μm)	20	16	16	15	15	16	19	19	22	16	16	16
N_{eff}/sec	6	13	13	8	14	13	7	12	10	7	13	12

* Figures in brackets represent pressures in p.s.i.g.

** *o-*, *m-*, *p*-nitroanilines.

operation to the syringe injection system b. With the metering valve closed and all the mobile phase directed through the injection valve (system c), the chromatography obtained from injection of the test mixture was poor with marked peak tailing being observed. In system d the metering valve was adjusted so that part of the solvent flow was directed to the top of the column, whilst the balance was passed through the injection valve. Good peak shapes were obtained and although the efficiencies were slightly lower than those of system b, some diffusion of the sample may have occurred in the 1-mm-bore tubing linking the injection valve to the top of the column. This split-stream technique has obvious application in semi-preparative and preparative LC systems, where the use of valve injection in conjunction with external sample loops is almost essential.

The increase in efficiencies that is obtained with decrease in particle size is shown in Table V and Fig. 7. Plate heights of between 2.5 and 3.5 particle diameters were attained with each material at a linear velocity of ~1.5 mm/sec, whilst the number of effective plates per second increased with reduction in particle size.

TABLE V

EFFECT OF MEAN PARTICLE SIZE (d_{50}) ON RESULTANT COLUMN EFFICIENCY

Column, 0.10 m × 4.5 mm I.D.; column packing pressure, 31.0 MPa (4500 p.s.i.g.); slurry concentration, 8.7% w/v.

Operating conditions	*Packing material*								
	Partisil 10 (d_{50} = 11 μm)			*Partisil 5* (d_{50} = 6 μm)			*Partisil 3* (d_{50} = 3.7 μm)		
Operating pressure (MPa)	1.0 (150)*			2.8 (400)			5.2 (750)		
Flow-rate (ml/min)	1.24			1.08			1.00		
Linear velocity (mm/sec)	1.95			1.41			1.54		
Parameter	*Solute***								
	o-	*m-*	*p-*	*o-*	*m-*	*p-*	*o-*	*m-*	*p-*
k'	0.60	2.04	2.41	0.60	1.99	2.39	0.61	2.00	2.37
t_R (min)	1.37	2.61	2.92	1.89	3.52	3.99	1.74	3.24	3.64
N	3300	3100	3100	6800	6700	6400	7300	8100	8300
H (μm)	30	32	32	15	15	16	14	12	12
N_{eff}/sec	6	9	9	8	14	13	10	19	19

* Figures in brackets represent pressures in p.s.i.g.

** *o-*, *m-*, *p*-nitroanilines.

The use of wider-bore, 9.5-mm-I.D., columns packed with Partisil 5 and Partisil 3 resulted in marked increases in efficiencies (Table VI) over the comparable 4.5-mm-I.D. columns (Table V). Theoretically both 4.5-mm- and 9.5-mm-I.D. columns of 0.10-m length are of "infinite diameter", since their dimensions meet the requirements of the equation[17,19] $d_c > (2.4\, d_p L)^{\frac{1}{2}}$, where d_c is the internal diameter of the column, d_p is the average particle diameter, and L is the column length. As a consequence, the 4.5- and 9.5-mm-I.D. columns should have given the same performance. The "infinite

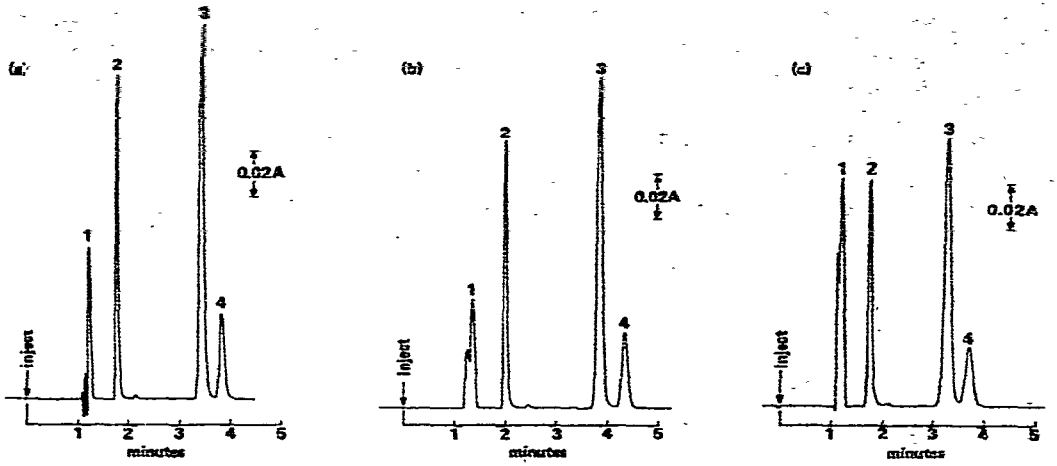

Fig. 7. Separation of *o*-, *m*-, and *p*-nitroanilines on (a) Partisil 3, (b) Partisil 5, and (c) Partisil 10. Column, 100 mm × 4.5 mm I.D.; mobile phase, IPA—dichloromethane—hexane (1:50:49); flow-rate, 1 ml/min; ΔP = (a) 5.2 MPa (750 p.s.i.g.), (b) 2.4 MPa (350 p.s.i.g.), and (c) 0.7 MPa (100 p.s.i.g.). Injection (3 μl) contained 300 ng of each isomer. 1 = Chloroform; 2 = *o*-nitroaniline; 3 = *m*-nitroaniline; 4 = *p*-nitroaniline.

TABLE VI

EFFICIENCIES ACHIEVED USING 0.10-m × 9.5-mm-I.D. COLUMNS

Column packing pressure, 31.0 MPa (4500 p.s.i.g.); slurry concentration, 8.7% w/v.

Operating conditions	*Packing material*					
	Partisil 5 (d_{50} = 6 µm)			*Partisil 3 (d_{50} = 3.7 µm)*		
Operating pressure (MPa)	3.4 (500)*			9.3 (1350)		
Flow-rate (ml/min)	5.0			4.5		
Linear velocity (mm/sec)	1.71			1.48		
Parameter	*Solute***					
	o-	*m*-	*p*-	*o*-	*m*-	*p*-
k'	0.68	2.05	2.47	0.55	1.79	2.14
t_R (min)	1.63	2.97	3.39	1.75	3.14	3.53
N	9500	9800	9300	12100	13500	13100
H (µm)	10	10	11	8	7	8
N_{eff}/sec	16	25	23	15	29	29

* Figures in brackets represent pressures in p.s.i.g.
** *o*-, *m*-, *p*-nitroanilines.

diameter" concept, however, assumes point injection of the sample centrally on to the packing material; this is not achievable experimentally. It also assumes uniform packing across the column whilst in practice the column bed probably consists of a dense central core with a less dense region near the walls of the column. The superior per-

formance of the 9.5-mm-I.D. columns might be explained, therefore, by the fact that the solute does not reach this region of less dense packing.

Columns of increased length, 0.20 m × 4.5 mm I.D., were successfully packed using the above procedure and gave twice the number of plates as were obtained for the 0.10-m columns. Initial attempts to pack 0.40-m × 4.5-mm-I.D. columns proved unsuccessful in that little increase in efficiency over the 0.20-m columns was obtained. This might be caused by a decrease in packing rate as the column is packed, resulting in a decreasing density of packing over the top half of the column.

CONCLUSIONS

The packing system described allows analytical and semi-preparative columns to be packed with equal facility. Carbon tetrachloride is the preferred slurry medium, since slurry preparation is simple, the settling rate is slow, and columns can be rapidly equilibrated with mobile phase after packing. Optimum packing pressure is reached at 20.7 MPa (3000 p.s.i.g.), thus obviating the need for special high-pressure pumps, whilst slurry concentrations in the range 1–10% w/v are satisfactory. This procedure has been successfully used in this laboratory for two years and the reproducibility of performance for different columns is normally within 10%.

The inlet to the column is terminated with a disc of woven stainless-steel mesh capped with a plug of porous PTFE and the highest efficiency is attained using a micro-syringe to inject the sample directly onto the mesh. Comparable performance can be obtained with valve injection if a split stream technique is used. This technique has obvious application in the design of sample introduction systems for preparative-scale columns.

Plate heights of between 2.5 and 3.5 particle diameters can be attained with 11-, 6- and 3.7-μm particle size silicas on 0.10-m × 4.5-mm-I.D. columns. The use of wider-bore, 9.5-mm-I.D., columns gives plate heights of around 2 particle diameters, together with up to 30 effective plates per second at a linear velocity of 1.5 mm/sec.

The production of high-performance columns and the realisation of optimum performance does not require specialised, expensive equipment. Detailed attention, however, must be directed to the many criteria involved in the production and operation of these columns.

ACKNOWLEDGEMENTS

The authors thank their colleagues who helped in this work, especially Mr. A. G. Wyles for carrying out much of the experimental work and Mr. T. S. Floyd for his advice and expertise in the construction of the packing assembly.

REFERENCES

1 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
2 J. J. Kirkland, *J. Chromatogr. Sci.*, 10 (1972) 593.
3 W. Strubert, *Chromatographia*, 6 (1973) 50.
4 J. J. Kirkland, in S. G. Perry (Editor), *Gas Chromatography 1972—Montreux*, Applied Science Publishers, Barking, 1973, p. 39.
5 R. E. Majors, *J. Chromatogr. Sci.*, 11 (1973) 88.

6 J. J. Kirkland, *J. Chromatogr.*, 83 (1973) 149.
7 J. Asshauer and I. Halász, *J. Chromatogr. Sci.*, 12 (1974) 139.
8 R. M. Cassidy, D. S. LeGay and R. W. Frei, *Anal. Chem.*, 46 (1974) 340.
9 B.-A. Persson and B. L. Karger, *J. Chromatogr. Sci.*, 12 (1974) 521.
10 B. Coq, C. Gonnet and J.-L. Rocca, *J. Chromatogr.*, 106 (1975) 249.
11 C. Gonnet and J. L. Rocca, *J. Chromatogr.*, 109 (1975) 297.
12 M. Caude, LeX. Phan, B. Terlain and J.-P. Thomas, *J. Chromatogr. Sci.*, 13 (1975) 390.
13 K. K. Unger, R. Kern, M. C. Ninou and K.-F. Krebs, *J. Chromatogr.*, 99 (1974) 435.
14 J. H. Knox and M. Saleem, *J. Chromatogr. Sci.*, 7 (1969) 614.
15 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
16 J. J. Kirkland, *Chromatographia*, 8 (1975) 661.
17 J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41 (1969) 1599.
18 J. J. DeStefano and H. C. Beachell, *J. Chromatogr. Sci.*, 8 (1970) 434.
19 J. J. DeStefano and H. C. Beachell, *J. Chromatogr. Sci.*, 10 (1972) 654.
20 E. Grushka, *Anal. Chem.*, 46 (1974) 510A.
21 J. J. Kirkland, *J. Chromatogr. Sci.*, 9 (1971) 206.
22 G. B. Cox, C. R. Loscombe, M. J. Slucutt, K. Sugden and J. A. Upfield, *J. Chromatogr.*, 117 (1976) 269.