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CHROMATOGRAPHIC CHARACTERISTICS OF POLYMER-BASED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY PACKINGS

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SUMMARY

Rigid macroporous polystyrene-based and polyacrylamide-based packings have been developed for reversed-phase high-performance liquid chromatography. Data on pore size distribution, total pore volume and surface area have been obtained for comparison with silica-based packings. Columns containing these polymer-based packings have good permeability with maximum working pressures of 5000 p.s.i. and 3500 p.s.i. for polystyrene-based and polyacrylamide-based packings, respectively. Experimental results for capacity factors of solutes show that the retention behaviour for the polystyrene-based packing differs from that for the polyacrylamide-based packing. The dependence of column efficiency on the flow velocity of the mobile phase as a function of solute type and temperature has been studied. Plate height is reduced considerably at higher temperature. This is tentatively interpreted in terms of a reduction in solute dispersion due to improved mass transfer in the stationary phase.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (HPLC) is generally performed with a silica packing coated with an octadecyl (C_{18}) chemically bonded phase[1,2]. A silica packing has the limitation that its use with a mobile phase having a high pH is not recommended because of dissolution of the silica surface[3].

Polymer-based packings may be produced to give much greater stability with eluents over a wide range of pH. Consequently, crosslinked polystyrene packings, constituted from styrene and divinylbenzene, have been studied recently for HPLC separations[4–10]. The neutral non-polar polystyrene surface will function as a stationary phase for reversed-phase separations with aqueous eluents. However, the presence of aromatic groups must generate somewhat different retention behaviour from that for alkyl-bonded silica. Thus, it might be expected that crosslinked polystyrene and phenyl-bonded silica packings might have more similar retention characteristics.

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Therefore, a polymer-based packing comparable to a C_{18} silica packing necessitates the bonding of a C_{18} stationary phase to the surfaces of macroporous polymer beads having high rigidity. We have demonstrated that crosslinked polyacrylamide packings, constituted from acrylamide and N,N'-methylenebisacrylamide, may be coated with a C_{18} chemically bonded phase. Furthermore, experimental results for capacity factor and separation factor (or selectivity) show that this polyacrylamide-based packing will provide reversed-phase HPLC separations of non-polar, polar, basic, and acidic compounds, similar to those observed for a C_{18} silica packing[11].

In this paper, a comparison is made between polystyrene-based (PLRP-S) and polyacrylamide-based (PLRP-C_{18}) packings with emphasis on such properties as surface area, pore size, chemical type, and stability. Results for column efficiency are presented in order to study the effect of solute type and temperature on plots of plate height *versus* linear flow velocity.

EXPERIMENTAL

The PLRP-S packing, which has received brief HPLC examinations previously[11,12], was produced by copolymerisation of styrene and divinylbenzene in a suspension process according to methods described elsewhere[13,14]. The PLRP-C_{18} material is a polyacrylamide-based packing containing N-octadecylamide groups. Macroporous polyacrylamide particles were produced in an inverse suspension process by copolymerising acrylamide and N,N'-methylenebisacrylamide with the crosslinking agent as the major monomeric component. The inverse suspension polymerisations were performed according to the conditions described elsewhere[15]. The bonded phase was prepared by first forming strongly nucleophilic amide anions on the surface of the porous polyacrylamide particles by the use of strong base[16], followed by treatment with *n*-octadecyl bromide. The polymer particles were separated by air classification, and optical and scanning electron micrographs indicated spherical particles with a narrow particle size distribution. Mean particle diameter d_p was estimated from the particle size distribution determined by Coulter Counter. Surface areas were calculated from nitrogen adsorption isotherms, provided by Quantachrome, NY, U.S.A. The dry polymer-based particles were dispersed in methanol, and this slurry was packed into a column (150 × 4.6 mm I.D.) at pressures up to 3500 p.s.i. (1 p.s.i. ≡ 6894.8 N/m^2). Size-exclusion separations were performed in order to compare pore size distributions of polymer-based packings with columns containing silica packings d_p = 5 μm, namely Hypersil ODS 100 Å (200 × 4.6 mm I.D.), Hypersil Phenyl 100 Å (150 × 4.6 mm I.D.), and Hypersil WP Octyl 300 Å (250 × 4.6 mm I.D.), supplied by Hewlett-Packard, Avondale, PA, U.S.A. The permeability of a column containing a silica packing was studied with a reversed-phase silica cartridge system (CP^{tm} Spher C_{18}, d_p = 8 μm) supplied by Chrompack U.K., Millharbour, London, U.K.

The chromatographic apparatus consisted of a Knauer pump Model 64 (Knauer, Berlin, F.R.G.), a Rheodyne Model 7125 injection valve (20 μl loop), supplied by HPLC Technology, Macclesfield, U.K., a thermostatted oven (Applied Chromatography Systems) supplied by HPLC Technology, and a Pye-Unicam variable-wavelength UV detector Model LC3 operated at 254 nm, supplied by Pye-Unicam, Cambridge, U.K. Injection volumes were 5 μl and solute concentrations were

typically below 40 $\mu g\ cm^{-3}$. Reagents (Fisons Scientific Equipment Division, Loughborough, U.K.) for the mobile phases were acetonitrile (HPLC grade), methanol (AR grade), tetrahydrofuran (general purpose grade), and water (HPLC grade). The solutes were phenol (AR grade), aniline (AR grade), diethyl phthalate (SLR grade), and toluene (AR grade), all supplied by Fisons Scientific Equipment Division, and polystyrene standards (Polymer Laboratories, Church Stretton, U.K.). Values of plate number (N) and plate height (H) were calculated from experimental chromatograms by the width-at-half-height method.

RESULTS AND DISCUSSION

Porosity and surface characteristics of packings

In size-exclusion chromatography, the separation mechanism may be shown to depend on the hydrodynamic diameter of a solute, and so a molecular size calibration curve may be related to the pore size distribution and pore volume of a packing[17]. For example, mean pore diameters determined for silica packings by X-ray scattering were shown to lie within the pore size distribution determined by mercury porosimetry and within the distribution of hydrodynamic diameters determined by size-exclusion chromatography[18].

Molecular weight calibration curves for Hypersil packings with a mean pore diameter of 100 Å are shown in Fig. 1. The shift in retention volumes between the calibration curves for the two silica packings is explained by the occurrence of sec-

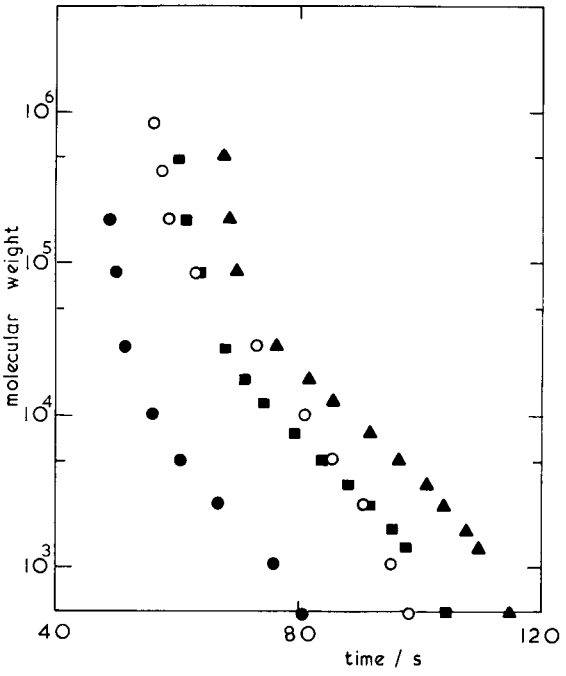

Fig. 1. Size-exclusion chromatography calibration curves for polystyrene standards in tetrahydrofuran at ambient temperature. ○, PLRP-S 100 Å; ●, PLRP-C_{18}; ■, Hypersil ODS 100 Å; ▲, Hypersil Phenyl 100 Å (retention volumes normalised to columns 150 × 4.6 mm I.D. at a flow-rate of 1 $cm^3\ min^{-1}$).

ondary separation mechanisms, such as adsorption and partition[17], resulting from the different bonded phases and possibly the degree of coverage of the silica surface by the bonded phase. Despite this shift, it is clear that the useful molecular weight separation range in Fig. 1 is similar for the two silica packings and similar to the molecular weight separation range for the PLRP-S packing. It is proposed, therefore, that these three packings have a similar mean pore diameter of about 100 Å, and so the polystyrene packing is designated PLRP-S 100 Å. In Fig. 2 the similar molecular weight calibration curves for the two packings indicate similar pore size distributions; therefore, this polystyrene packing is designated PLRP-S 300 Å.

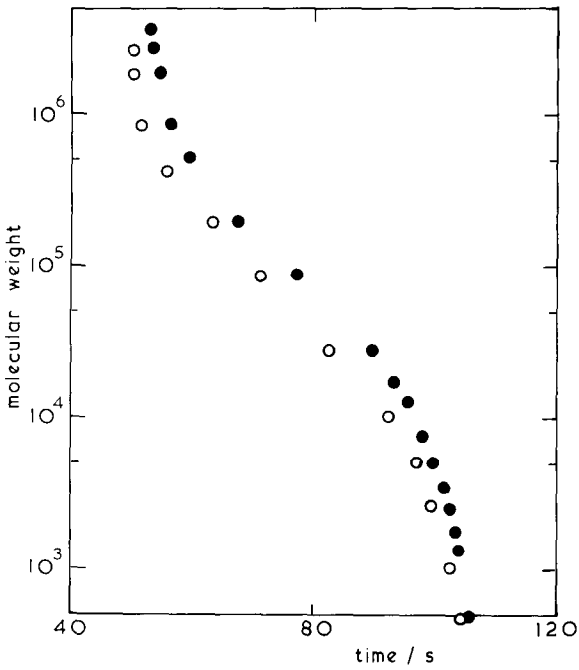

Fig. 2. Size-exclusion chromatography calibration curves for polystyrene standards in tetrahydrofuran at ambient temperature. ○, PLRP-S 300 Å; ●, Hypersil WP Octyl 300 Å (retention volumes normalised to columns 150 × 4.6 mm I.D. at a flow-rate of 1 cm^3 min^{-1}).

For simple pore geometries in a macroporous packing, the surface area, total pore volume, and mean pore diameter may be related. Typical HPLC silica packings with mean pore diameters of 300 and 100 Å will have surface areas calculated from gas adsorption isotherms in the range 200–300 m^2 g^{-1} (ref. 19). From nitrogen adsorption isotherms for the PLRP-S packings, much higher surface areas above 350 m^2 g^{-1} were determined, as shown in Table I. These high values may be explained by the proposal that the crosslinked polystyrene matrix contains microporosity which is accessible to nitrogen[17] but which is not present in a silica packing. The similar calibration curves for PLRP-S and silica packings in Figs. 1 and 2 suggest that this microporosity is not penetrated by the smallest solutes separated in HPLC. The molecular weight calibration curve for the PLRP-C_{18} packing in Fig. 1 indicates

TABLE I

POROSITY AND SURFACE CHARACTERISTICS OF POLYMER-BASED PACKINGS

Packing	d_p *(μm)*	*Surface area** *($m^2\ g^{-1}$)*	*Total pore volume** *($cm^3\ g^{-1}$)*	*Average pore radius** *(Å)*
PLRP-S 100 Å	9	451	0.99	44
PLRP-S 300 Å	7.5	362	1.18	65
PLRP-C_{18}	9	62	0.32	103

* From nitrogen adsorption isotherms.

lower pore volume than for the PLRP-S and silica packings. This is confirmed by the data given in Table I. Whilst the average pore radius for the PLRP-S 100 Å packing in Table I is in agreement with the prediction based on calibration curves in Fig. 1, the values in Table I for PLRP-S 300 Å and for PLRP-C_{18} are, respectively, lower and higher than expected.

HPLC separations generally require the use of high pressures. The stability of the column packing may be monitored by examining the dependence of column pressure on eluent flow-rate, as shown in Fig. 3 for three polymer-based packings which exhibit similar behaviour at pressures up to 2000 p.s.i. A reasonable linear dependence is observed for the PLRP-S packings, suggesting that little particle distortion occurs at pressures up to 5000 p.s.i. The PLRP-S 100 Å packing only permits the use of a higher eluent flow-rate at fixed column pressure because its d_p value (see Table I) is higher than that for the PLRP-S 300 Å packing. At flow-rates below 2.5 $cm^3\ min^{-1}$ in Fig. 3, the PLRP-C_{18} packing follows the same dependence as the PLRP-S 100 Å packing, and both these packings have the same mean particle size (see Table

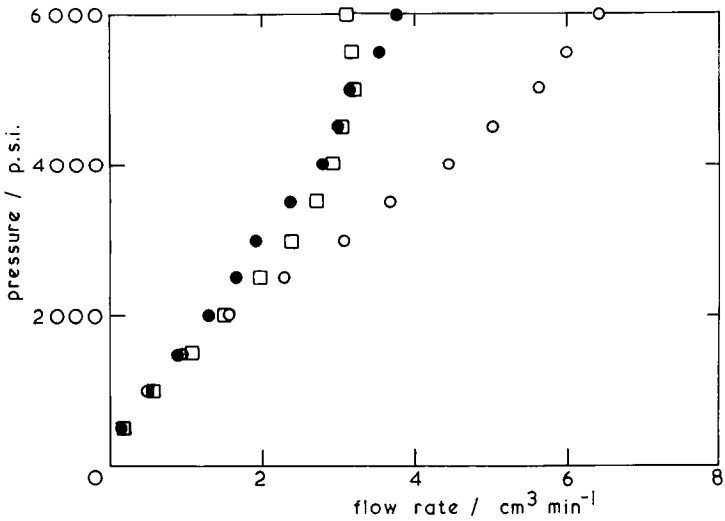

Fig. 3. Pressure *versus* flow velocity for columns containing polymer-based packings with methanol–water (70:30) as eluent at ambient temperature; ○, PLRP-S 100 Å; ●, PLRP-S 300 Å; □, PLRP-C_{18}.

I). Above 2500 p.s.i., particle compression occurs for the PLRP-C_{18} packing, as demonstrated by the curve in Fig. 3, whereas the dependence of pressure on flow-rate for the PLRP-S 100 Å packing decreases at flow-rates > 2 cm^3 min^{-1}. The observations at low flow-rates in Fig. 3 are confirmed by calculating the permeability (K) for the polymer-based packings with an eluent at a linear flow velocity $u = 0.2 \cdot 10^{-2}$ m s^{-1}. Permeability is defined by the equation[20]

$$K = u \eta L/\Delta p \quad (1)$$

where η is the viscosity of the eluent, assumed to be $1.5 \cdot 10^{-3}$ N s m^{-2} for methanol–water (70:30), L is the column length, and Δp is the pressure drop across the column. The values of K for the PLRP-S 100 Å, PLRP-S 300 Å, and PLRP-C_{18} packings, were calculated to be $3.5 \cdot 10^{-14}$ m^2, $3.4 \cdot 10^{-14}$ m^2, and $3.7 \cdot 10^{-14}$ m^2, respectively. These data are similar to the result of $K = 3.09 \cdot 10^{-14}$ m^2 obtained for a reversed-phase silica packing (CP^{tm} Spher C_{18}, $d_p = 8$ μm)[21].

Data for capacity factor (k') have been determined to show that the PLRP-C_{18} packing provides a retention behaviour for a range of solutes similar to that observed for conventional silica-based packings with a C_{18} chemically bonded phase[11]. Here, we use data for k' for selected solutes to demonstrate that the retention behaviour of the PLRP-C_{18} and PLRP-S 100 Å packings are considerably different. Values for k' were determined from

$$k' = (t_R - t_0)/t_0 \quad (2)$$

where the retention time t_R is for the solute and t_0 is assumed to be the retention time for an unretained peak which is obtained by injecting a liquid mixture with a volume composition different from that of the eluent, namely methanol–water (10:90) for the eluent methanol–water (70:30) and acetonitrile–water (10:90) for the eluent acetonitrile–water (90:10). Results for k' are presented in Tables II and III, where $k' < 1$ for many elutions. Although the reliability of these k' values is therefore dependent on the accuracy of the determination of t_0, the results in Tables II and III provide a relative comparison of solutes separating on the PLRP-C_{18} and PLRP-S packings. With the PLRP-S 100 Å packing and the eluent methanol–water (70:30), k' values were always above 2, for example $k' = 2.2$ (aniline) and $k' > 10$ (diethyl phthalate), *i.e.* much higher than the results in Table II. Clearly, because of the different surface characteristics of the two polymer-based packings, elution of solutes at fixed k' requires major alterations to the composition of the mobile phase.

TABLE II

CAPACITY FACTOR FOR PLRP-C_{18} PACKING WITH METHANOL–WATER (70:30) AS ELUENT AT 323 K

Solute	k'
Phenol	0.58
Aniline	0.58
Diethyl phthalate	0.54
Toluene	1.24

TABLE III

CAPACITY FACTOR FOR ELUTIONS WITH ACETONITRILE–WATER (90:10) AS ELUENT

Solute	*k' (PLRP-S 100 Å)**	*k' (PLRP-S 300 Å)***	*k' (PLRP-S 300 Å)**
Phenol	0.22	0.20	0.18
Aniline	–	0.33	0.28
Diethyl phthalate	0.53	0.48	0.37
Toluene	1.05	1.12	0.83

* Elutions at 323 K.
** Elutions at ambient temperature.

Column efficiency

Plots of plate height *versus* linear flow velocity for four solutes separated with PLRP-S 300 Å at ambient temperature are shown in Fig. 4. These results may be compared with the data in Fig. 5 which were obtained at 323 K. It is evident that whereas values of H for phenol and aniline at $u = 1$ mm s^{-1} in Fig. 4 are almost identical to the values in Fig. 5 a significant decrease in H at this flow velocity is obtained for diethyl phthalate and toluene on raising the temperature. At both temperatures, plate height is solute dependent, with k' increasing in the order phenol, aniline, diethyl phthalate, toluene (see Table III). The data for k' for all solutes with the PLRP-S 300 Å packing appear to decrease as the temperature is raised, though with values of $k' < 1$ any changes will be susceptible to the accuracy of t_0 in eqn. 2. It would appear therefore that the decrease in H for diethyl phthalate and toluene on raising the temperature of the PLRP-S 300 Å packing might be related to the

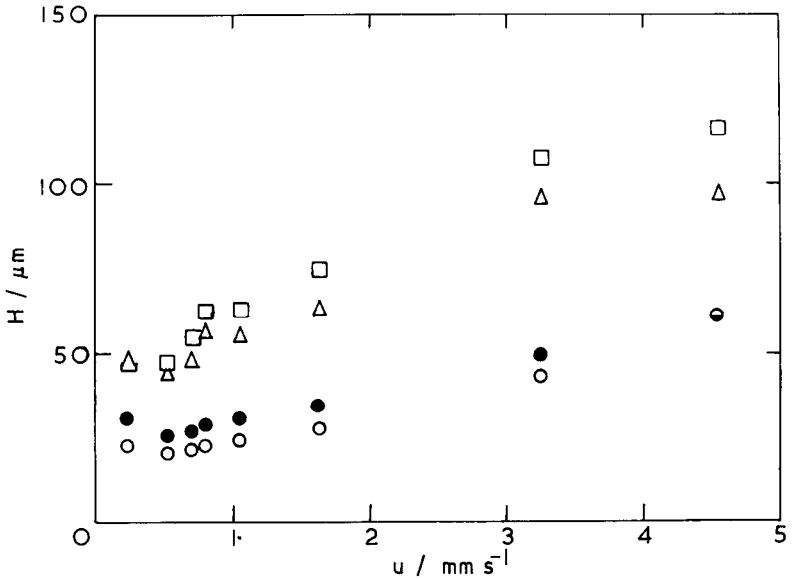

Fig. 4. Dependence of plate height on flow velocity for PLRP-S 300 Å with acetonitrile–water (90:10) as eluent at ambient temperature. ○, Phenol; ●, aniline; △, toluene; □, diethyl phthalate.

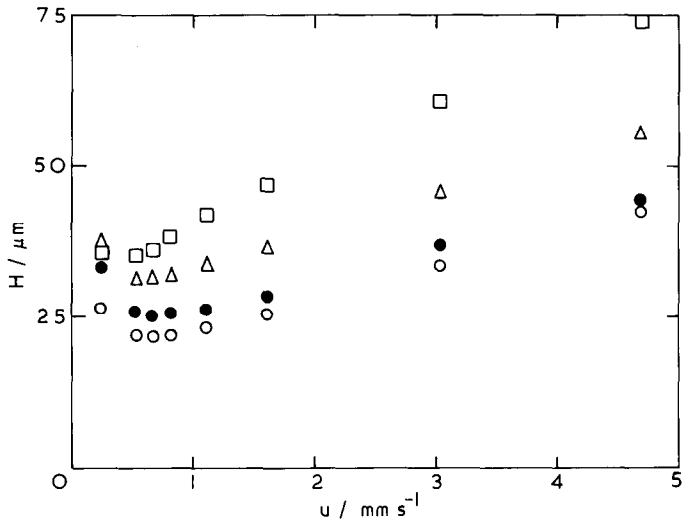

Fig. 5. Dependence of plate height on flow velocity for PLRP-S 300 Å with acetonitrile–water (90:10) as eluent at 323 K. ○, Phenol; ●, aniline; △, toluene; □, diethyl phthalate.

larger decrease in k' than was shown by phenol and aniline in Table III. In order to interpret this observation, the dependence of H on solute dispersion mechanisms in terms of the theory of Giddings[22] may be considered. At the linear flow velocities in Figs. 4 and 5, a major contribution to H arises from solute dispersion due to slow mass transfer in the stationary phase, given by

$$H \text{ (mass transfer)} = \frac{k'}{(1 + k')^2} \frac{d_p^2 u}{30 D_s} \quad (3)$$

where D_s is the diffusion coefficient of solute in the stationary phase. Whilst eqn. 3 predicts that H will depend on $k'/(1 + k')^2$, the change in H for diethyl phthalate and toluene on raising the temperature (see Figs. 4 and 5) would appear to be larger than would be expected from the change in values of k' for PLRP-S 300 Å in Table III. Consequently, it is possible that a decrease in H might also arise from an increase in D_s at the higher temperature. In order to study this diffusion effect, experiments

TABLE IV

TEMPERATURE DEPENDENCE OF PLATE NUMBER FOR TOLUENE WITH CONSTANT k' = 2.9 WITH COLUMN CONTAINING PLRP-S 300 Å WITH ACETONITRILE–WATER AS MOBILE PHASE AT A FLOW VELOCITY 1 $cm^3\ min^{-1}$

Temperature (K)	*Acetonitrile–water*	*N (plates m^{-1})*
310	70:30	14 900
316	68:32	17 100
323	65:35	18 200
336	60:40	22 900

have been performed at various temperatures by changing the composition of the mobile phase so that k' for toluene as solute was held constant. The results are shown in Table IV. The increase in efficiency at constant k' with increasing temperature is presumed to be significant, since the influence of the viscosity of the mobile phase on N is expected to be small because a decrease in liquid viscosity on raising the temperature will be balanced by increasing the concentration of water in the eluent (water having a higher viscosity than acetonitrile). It is concluded that in this polystyrene-based packing restricted diffusion of a solute in the stationary phase can occur for some solutes and that this mass transfer dispersion mechanism can be reduced by raising the temperature.

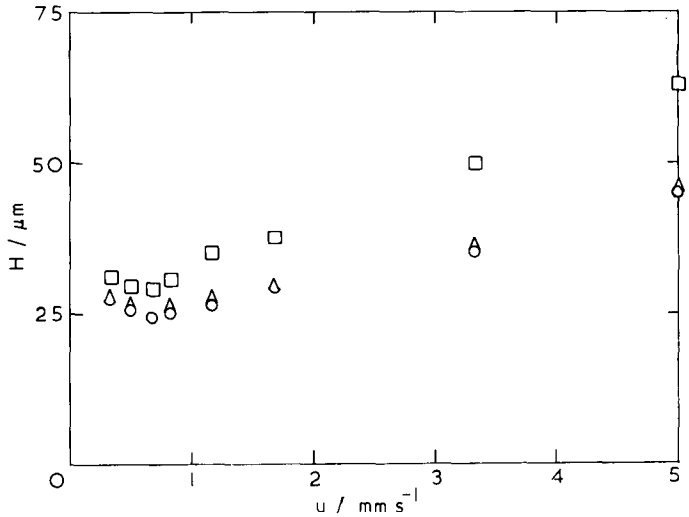

Fig. 6. Dependence of plate height on flow velocity for PLRP-S 100 Å with acetonitrile–water (90:10) as eluent at 323 K. ○, Phenol; △, toluene; □, diethyl phthalate.

Plots of plate height *versus* linear flow velocity for three solutes separated with PLRP-S 100 Å at 323 K are shown in Fig. 6. The relative placement of the plots for H *versus* u for phenol, toluene and diethyl phthalate is similar in Figs. 5 and 6, and similar values of k' are observed (see Table III). Plots of plate height *versus* linear flow velocity for four solutes separated with PLRP-C_{18} at 323 K are shown in Fig. 7. It is evident that values of H for PLRP-C_{18} are higher than the data in Figs. 5 and 6. The relative placement of the plots for H *versus* u for the four solutes in Fig. 7 is different from the trend displayed by PLRP-S 300 Å. The value of H for diethyl phthalate is much higher than the values for phenol and aniline at $u = 1$ mm s^{-1} in Fig. 7, although all three solutes have similar values for k' (see Table II). It is therefore concluded that a combination of the different surface for the PLRP-C_{18} stationary phase and the different composition of the mobile phase produces a change in retention behaviour and solute diffusivity in the stationary phase. In particular, whilst diethyl phthalate appears to be susceptible to restricted diffusion for all three polymer-based packings, the contribution of this mass transfer dispersion mechanism to plate height at 323 K is largest for the PLRP-C_{18} packing.

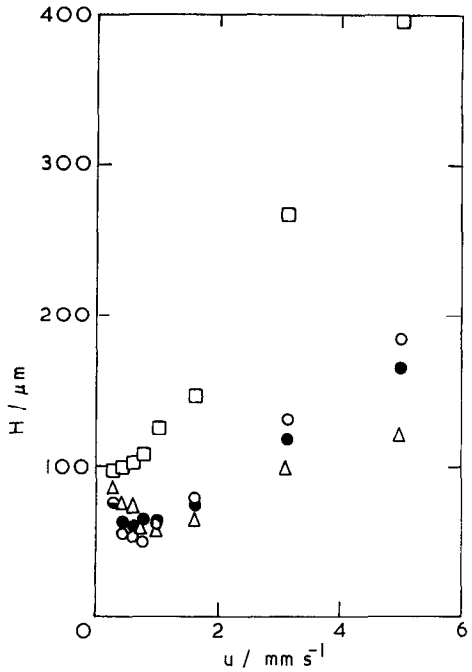

Fig. 7. Dependence of plate height on flow velocity for PLRP-C_{18} with methanol–water (70:30) as eluent at 323 K. ○, Phenol; ●, aniline; △, toluene; □, diethyl phthalate.

CONCLUSIONS

Polystyrene-based and polyacrylamide-based packings, which are rigid and macroporous and have pore size distributions similar to silica-based packings, provide efficient separations by reversed-phase HPLC. The polyacrylamide-based packing has a lower pore volume than the polystyrene-based packings. The permeability for columns containing these polymer-based packings is similar to silica-based packings. The polystyrene-based packings may be used at pressures up to 5000 p.s.i., whereas the polyacrylamide-based packing has a maximum working pressure of 3500 p.s.i. Data for capacity factor here and in ref. 11 demonstrate that retention behaviour on the two types of polymer-based packing is quite different. Column efficiency for polymer-based packings is markedly improved by performing separations at higher temperatures, *e.g.* 323 K, which is tentatively explained by the proposal that the dispersion mechanism due to mass transfer of solute in the stationary phase decreases significantly on raising the temperature.

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