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CHROMOSORB 101 AS A PACKING MATERIAL FOR REVERSED-PHASE CHROMATOGRAPHY

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SUMMARY

A procedure for preparing a Chromosorb 101 liquid chromatographic column is described. Some column parameters such as the permeability, efficiency and peak asymmetry factor were calculated. The elution of some benzene and phenol derivatives from the polymeric column with different mobile phases was performed. The results showed that some gas chromatographic adsorbents such as Chromosorb 101 can be used as packings for high-performance liquid chromatographic columns. The behaviour of these styrene copolymers resembles that of a C_{18} silica bonded phase.

INTRODUCTION

The majority of the packing materials currently employed in high-performance liquid chromatography (HPLC) consist of porous silica chemically bonded to various hydrocarbon groups, *e.g.*, alkyl, phenyl, amine, cyano, nitro. The modified silica provides effective stationary phases that are efficient, reproducible and versatile with respect to many separation problems. However, there are serious drawbacks in the use of these packings. Degradation of the phase occurs outside the range pH 2–7, due to hydrolysis of the siloxane bond at low pH and dissolution of the silica at high pH. Moreover the adsorbent surface of these packings, not completely hydrophobic because of the presence of residual silanols, is not always ideal (peak tailing) for reversed-phase separation[1], particularly for basic compounds.

Recently, macroporous poly (styrene–divinylbenzene) resins have been applied to a wide variety of separation problems in reversed-phase HPLC[2–16]. The Amberlite XAD resins are well known[2,3,6,10,11,14]. Some have been produced specifically for LC[9,12,16].

In previous works the gas chromatographic behaviour of some Chromosorb Century series and Porapak porous polymer supports was studied in order to evaluate their suitability as adsorbent beds for trace concentration of air pollutants [17–20]. We are now examining the same porous polymers as packings for HPLC columns.

In the present work a column of Chromosorb 101 was prepared and its chromatographic properties evaluated by comparison to commercially available columns.

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EXPERIMENTAL

Apparatus

The chromatographic measurements were carried out on a Spectra Physics Model 8700 liquid chromatograph equipped with a Model 770 sepctrophotometric detector, set at 254 nm. Chromatograms were recorded on a Spectra Physics SP4270 integrator. Samples were introduced with a Rheodyne Model 7125 injector, equipped with a 10-μl sampling loop.

Chemicals

HPLC quality solvents, methanol and acetonitrile, and distilled water were filtered through suitable filters in glass apparatus (Millipore). All eluents were degassed by agitating them in an ultrasonic bath and kept under a weak stream of helium. The solutes were reagent-grade chemicals. They were dissolved in methanol so as to give concentrations, ranging between 50 and 500 ppm, which produced suitable responses from the detector without overloading the column. The commercial column was a PRP-1 (Hamilton). Potassium nitrate was taken as an unretained solute, for determination of the column dead volume.

Preparation of Chromosorb 101 polymer and column packing

Despite their high chemical stability at extreme values of pH, and other advantages, such as rigidity, polymer packings have only recently gained wide acceptance in LC because of practical limitations in the handling of these materials. In order to prepare small and regular particles, many grinding and screening techniques have been considered[10,21–24].

Chromosorb 101 was obtained from a commercial source as 80–100 mesh particles. In order to obtain smaller ones, the starting material was ground to suitable particle size with a ball mill for 30 min. It was then suspended in methanol–water, ultrasonicated to disaggregate any clumps and allowed to stand at room temperature. The procedure was repeated several times, the "fines" being removed by decantation and larger particles by sedimentation. Since Chromosorb 101 is hydrophobic, it was necessary to wet it with methanol before dispersing the small particles in methanol–water. Both the grinding and screening steps were controlled by microscopic examination.

The sized polymer fraction was washed with methanol and dried at 50°C to remove water completely. Finally the polymer was cleaned by extraction with methylene chloride in a Soxhlet apparatus for 48 h, dried at 50°C and stored in a drier.

The particle size distribution curve was obtained by Coulter counter analysis: $d_{10\%}$, $d_{50\%}$ and $d_{90\%}$, characterizing the collected polymer fraction, were determined as 7, 10 and 13 μm, respectively. The modal particle diameter was 7 μm. Photographs of the polymer before and after the screening procedure are shown in Fig. 1.

A wet filling technique was employed for packing the column. The packing material, slightly wetted with methanol, was dispersed in a solution of 50% glycerine and 2.5% sodium chloride in water. A 15-ml volume of the packing solvent was used to disperse about 2 g of polymer. The slurry, shaken ultrasonically for 5 min, was stable for a long time (about 30 min). The stainless-steel column (15 cm × 4.6 mm I.D.) having two 2-μm fritted end fittings was filled with methanol, connected with

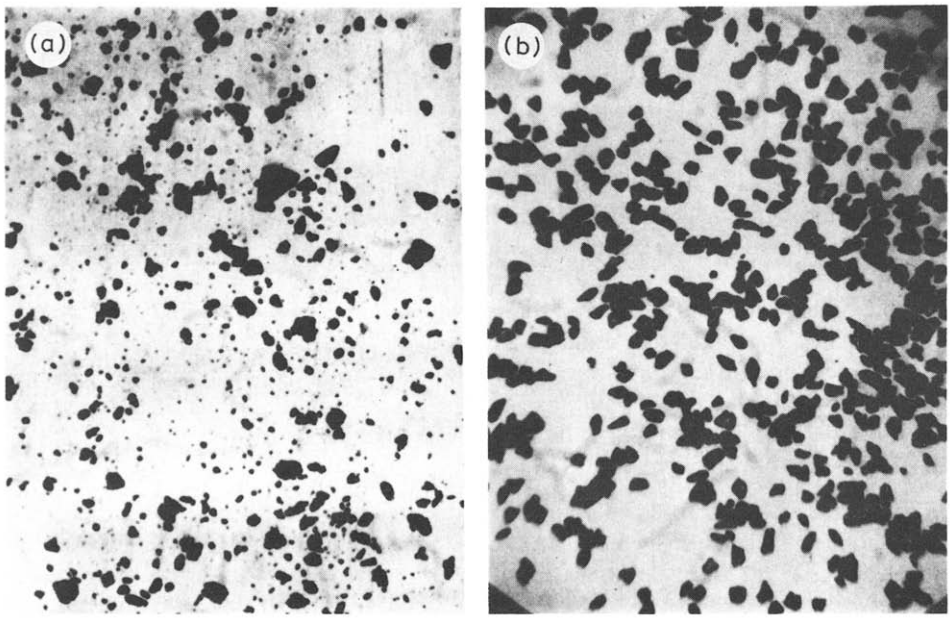

Fig. 1. Chromosorb 101 before (a) and after (b) screening.

a reservoir column and filled with a slurry of the polymer. The system was immediately pressurized by pumping methanol with the liquid chromatograph pump. The slurry was forced into the analytical column until a back-pressure of a 200–300 atm was achieved and maintained for a few minutes. After the pressure was released, the column bed being wet, the reservoir column was disconnected and the fritted end fitting placed at the top. Methanol and acetonitrile alternatively were employed as eluents for the column conditioning until the back-pressure became constant. Eluent changes were gradual to avoid damage due to possible differences in swelling of the packing[25]. Two columns were prepared for these experiments, named column 1 and column 2.

RESULTS AND DISCUSSION

The Chromosorb series polymers are well known packing materials designed for gas–solid chromatography. Chromosorb 101 is a porous styrene–divinylbenzene cross-linked polymer having a surface area of 11 m^2/g and an average pore diameter of 0.3–0.4 μm[19].

In order to evaluate the column performance, the following chromatographic parameters were determined: permeability, B^0, efficiency and asymmetry factor A_s. The permeability is calculated as $B^0 = \bar{u}\eta Lf/\Delta P$, where $\bar{u}$ is the linear flow-rate, η is the viscosity, L is the column length, f is the total porous fraction of the column and ΔP is the pressure drop. The values employed for viscosity were taken from the current literature[1]. The permeabilities were constant over the pressure range used, 100–2500 p.s.i. This suggests that the columns have good stability and no bed com-

pression occurs under the operating pressures. The values of B^0 calculated in methanol were $2.6 \cdot 10^{-10}$ cm^2 for column 1 and $3.7 \cdot 10^{-10}$ cm^2 for column 2. They are smaller than those predicted by the Kozeny–Carman equation[1] but still good and comparable with those obtained with similar columns[9,10]. Upon changing the eluent from methanol to acetonitrile, B^0 did not remain constant: for column 1, it was $2.0 \cdot 10^{-10}$ cm^2; probably a small reversible swelling of the particles took place. However, the permeabilities were independent of solvent sequences and reproducible after several experiments.

The theoretical plate heights, H, measured at flow-rates from 0.03 to 0.3 cm/s, were calculated for benzene ($k' = 1.03$). Fig. 2 shows plots of H *versus* linear velocity, $\bar{u}$. The shape of the curves is similar to that observed for other porous absorbents[9,10,26]. The best efficiencies were achieved at linear velocities ranging between 0.035 and 0.070 cm/s. Column 2 is better packed than column 1, though the number of theoretical plates, N, *i.e.*, 3000 per m for column 1 and 5000 per m for column 2, is lower than for similar commercial packing material under the same conditions, a commercial PRP-1 column gave a plate number of 13 000 per m. Probably the relatively low pressure employed during the packing procedure does not allow higher efficiencies: column 2 was more efficient because of the slightly higher pressure (about 300 atm) compared with column 1 (*ca.* 200 atm). In addition, irregular particle shape can have a significant effect, even if it does not seem as important as the packing procedure[10].

Because of its hydrophobic surface, the polymer exhibits non-polar interactions so that there is no noticeable peak tailing. The peak asymmetry factors, measured at 10% peak height for benzene, phenol and 4-ethylphenol, are reported in Table I. Such values are similar to those observed for other polymeric columns[10].

The interaction between various solutes and the polymer has been evaluated and expressed as in terms of the capacity factor, k'[1]. In Table II the values obtained in methanol and acetonitrile are reported: the k' values in acetonitrile are lower than

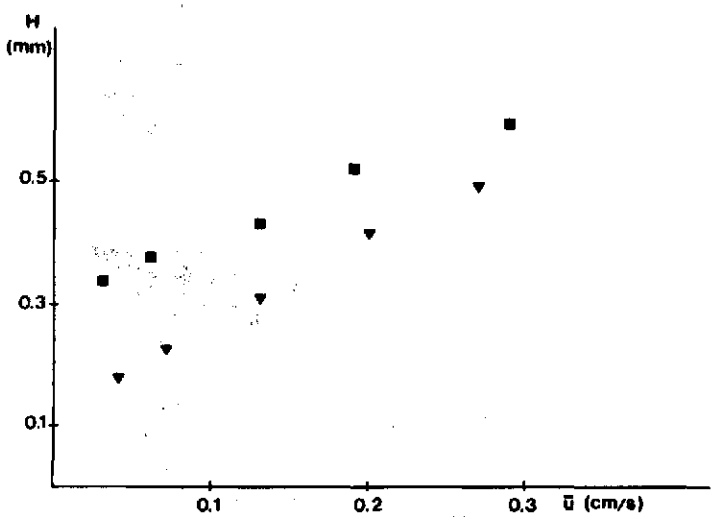

Fig. 2. Plate heights *vs.* linear flow-rate, on column 1 (■) and column 2 (▼).

TABLE I

PEAK ASYMMETRY FACTORS, A_s, ON CHROMOSORB 101

Mobile phase: methanol.

Compound	A_s	*Flow-rate (ml/min)*
Benzene	1.3	0.25
Phenol	1.1	0.25
4-Ethylphenol	1.2	0.50

those in methanol in accord with the solvent eluotropic forces in the reversed-phase system. The polymer shows a low affinity for hydroxyl-containing compounds and an enhanced retention of aromatic solutes.

Some authors[4,27–29] have discussed the retention mechanism of styrene–divinylbenzene gels and observed that such packings had separation properties similar to those of reversed bonded phases, *i.e.*, a C_{18} bonded phase. Logarithmic k'–k' plots are known to be useful for comparing the energetics of solute retention on selected column pairs[30]. A linear correlation with unit slope indicates identical thermodynamic behaviour for the two columns compared (homoenergetic retention). A linear

TABLE II

CAPACITY FACTORS ON CHROMOSORB 101

Compound	*Methanol*	*Acetonitrile*
Phenol	0.13	0.12
2-Methylphenol	0.21	0.15
3-Methylphenol	0.18	0.12
4-Methylphenol	0.19	0.14
2-Ethylphenol	0.24	0.17
3-Ethylphenol	0.22	0.14
4-Ethylphenol	0.25	0.17
2,3-Dimethylphenol	0.30	0.17
2,3,5-Trimethylphenol	0.37	0.21
2-Chlorophenol	0.23	0.18
3-Chlorophenol	0.22	0.17
4-Chlorophenol	0.23	0.17
2,3-Dichlorophenol	0.31	0.26
2,3,5-Trichlorophenol	0.56	0.44
2-Nitrophenol	1.00	0.22
Benzene	1.03	0.38
Toluene	1.39	0.49
Ethylbenzene	1.60	0.51
n-Propylbenzene	1.92	0.54
n-Butylbenzene	2.48	–
Chlorobenzene	1.44	0.53
Nitrobenzene	1.21	0.25
Naphthalene	3.26	0.86
Anthracene	10.2	2.06

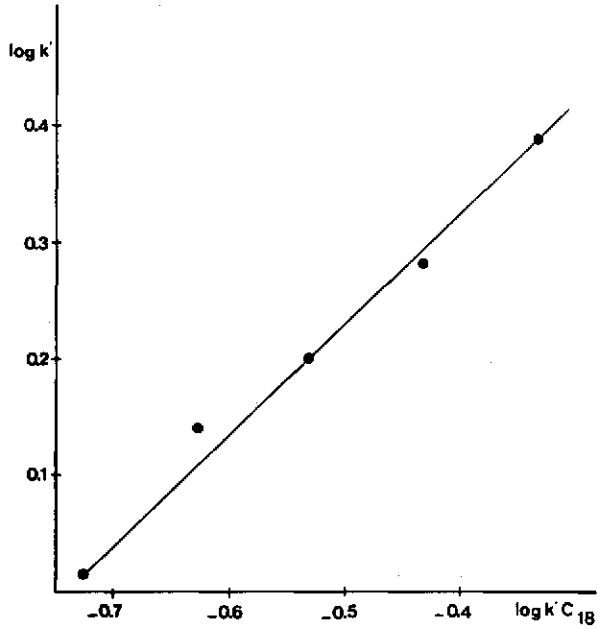

Fig. 3. Plots of log k' on Chromosorb 101 *vs.* log k' on C_{18} (RP-18). Solutes: alkylbenzenes. Mobile phase: methanol.

TABLE III

REGRESSION PARAMETERS FOR LOG k' ON CHROMOSORB 101 *VERSUS* LOG k' ON C_{18}

Compounds	*Slope*	*Intercept*	*Correlation coefficient*
n-Alkylbenzenes*	0.90 ± 0.06	0.69 ± 0.03	0.990
Phenols**	2.3 ± 0.2	0.86 ± 0.04	0.970

* Mobile phase: methanol.
** Mobile phase: methanol–water (75:25).

correlation with a slope different from unity suggests a similar physico-chemical basis for retention (homeoenergetic retention).

Log k'–log k' plots were made (Figs. 3 and 4) using a C_{18} bonded phase as a reference column for two classes of compounds, alkylbenzenes (RP-18, Merck)[31] and some substitued phenols (Jasco, FineSil C_{18})[32]. The regression parameters are reported in Table III. According to Horváth and co-workers[30], a correlation coefficient greater than 0.95 is acceptable as an index of linearity and the slope was considered to be 1 when the absolute difference between the observed slope and the unity was not statistically significant (95% confidence limit). The results show that Chromosorb 101 is homoenergetic with C_{18} for the elution of alkylbenzens, but is homeoenergetic for the elution of phenols.

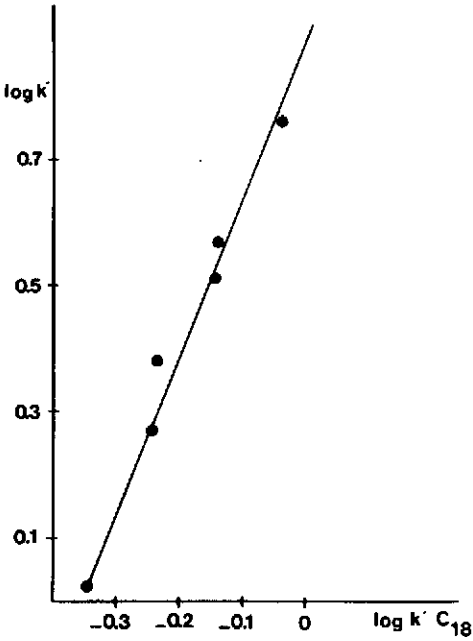

Fig. 4. Plots of log k' on Chromosorb 101 *vs.* log k' on C_{18} column. (Jasco, Fine Sil C_{18}) Solutes: substituted phenols. Mobile phase: methanol–water (75:25).

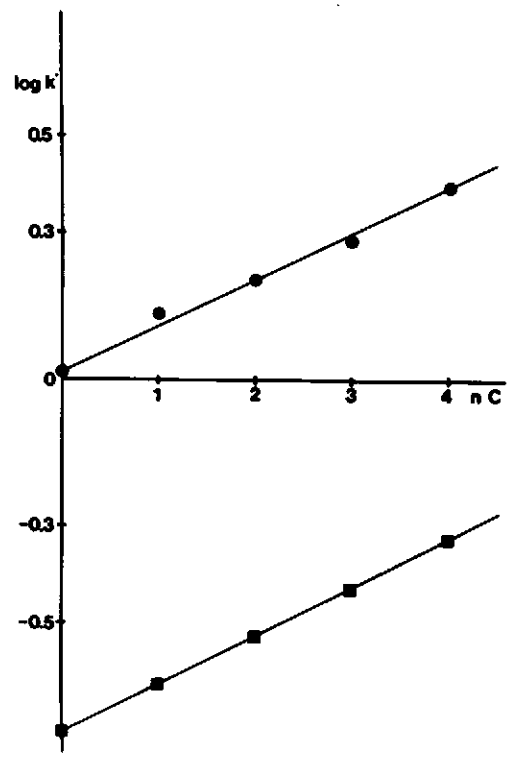

Fig. 5. Plots of log k' *vs.* the number of carbon atoms, n, for alkylbenzenes. Mobile phase: methanol. Columns: ●—●, Chromosorb 101; ■—■, C_{18} (RP-18).

The solute retention can be ascribed to solvophobic effects. The capacity factor varies linearly with the surfaces area of the hydrocarbon skeleton and, within an homologous series, with the number of methylene groups in the linear alkyl chain[30,31]. In Fig. 5 plots of log k' *vs.* the number of carbon atoms, (n) for alkylbenzenes on Chromosorb 101 and C_{18} (RP-18) are reported and the regression parameters are given in Table IV. It appears the retention increases similarly on the two columns. The interaction with benzene is stronger on Chromosorb 101. There-

TABLE IV

REGRESSION PARAMETERS FOR LOG k' ON CHROMOSORB 101 *VERSUS* THE NUMBER OF CARBON ATOMS FOR ALKYLBENZENES

Mobile phase: methanol.

Column	*Slope*	*Intercept*	*Correlation coefficient*
Chromosorb 101	0.090 ± 0.006	0.03 ± 0.01	0.990
C_{18}*	0.100	−0.73	

* See ref. 31.

fore, the retention seems largely controlled by non-specific interactions but, to some extent, by π–π interactions between aromatic compounds and the benzene rings of the polymer[16,27].

The experimental results show that styrene copolymers can be useful packing materials for HPLC columns. Their chromatographic behaviour resembles that of a C_{18} reversed phase and the mechanism of solute retention is mainly due to non-polar interactions between the solute molecules and the stationary phase.

The aim of current work is to improve column efficiences by optimizing the packing procedure and, since such polymers are stable over the whole pH range, to study the retention behaviour of ionogenic solutes.

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