

Influence of pore structure of silica packing on HPLC column characteristics

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Received 16 November 1988

The influence of the inner structure of bare silica gel sorbents — especially of their pore volume — on the characteristics of the resulting HPLC columns was studied. The sorbents of various pore characteristics were tested and packed into the columns at optimized column packing conditions.

The results show that an increase in the sorbent pore volume leads to a slight increase of the column permeability and to a rather pronounced rise of the sample capacity expressed in terms of volume and concentration of a sample. Highly efficient and fairly stable columns can be obtained with strictly spherical silica gels even if their specific pore volume reaches the value of $2\text{ cm}^3\text{ g}^{-1}$.

Изучено влияние внутренней структуры чистых силикагельных сорбентов — в особенности, их пористости — на характеристики полученных на их основе колонн ВЭЖХ. Были испытаны сорбенты с различными пористыми характеристиками, которые затем были наполнены в колонны при оптимизированных условиях наполнения колонн.

Результаты показывают, что увеличение пористости сорбента приводит к некоторому улучшению проницаемости колонн, а также к довольно значительному возрастанию емкости по наносимому образцу, выражаемой через объем и концентрацию образца. Высоко эффективные и довольно стабильные колонны могут быть получены со строго сферическими силикагелями, даже если их пористость достигает величины $2\text{ см}^3\text{ г}^{-1}$.

Silica gels are at present the most often used column packings for high-performance liquid chromatography (HPLC). The HPLC silica gels available differ in the shape and size of particles, diameters, volume and shape of pores, pH of aqueous suspension as well as in the concentration and mutual position

of free silanol groups. All these parameters influence chromatographic properties of silica gels — both bare and modified. In our study we have directed our attention toward the effect of the inner structure of silica gel sorbents, especially their pore volume, on their chromatographic characteristics, such as retention parameters, efficiency, sample capacity, permeability and stability of the column packing.

The specific surface area S of sorbent will evidently increase in the materials of the same diameter and the same shape of pores with the increasing specific pore volume, *i.e.* with the number of pores. An increase in S involves an increase of the retention and selectivity of separation per unit of column volume [1—3]. Enlargement of the specific surface area of the column packing may guarantee the same extent of separation for smaller columns (shorter column has higher permeability); this means material and time saving in the analysis and reduction of the demands on pressure pumping output. On raising S of the column packing we may also expect an increase of the material capacity of sorbent per volume unit of the column under otherwise constant conditions (eluent, temperature, flow rate, dimension and shape of particles and packing density). The overall specific surface area of the packing in the column may be raised in various ways: by reducing the interparticle bed volume of the packing, using a sorbent with smaller pore diameters (and with higher specific surface area) or using a sorbent with larger pore volume. The last two possibilities are attractive for practical application but they have two limitations: if the pore diameter decreases too much, steric exclusion of larger molecules of the solute from the gel matrix starts to occur and, on the other hand, the enlargement of the sorbent pore volume is accompanied with a decrease of its mechanical stability.

An opinion has often appeared in the literature that silica gel with the specific pore volume of about $1.5 \text{ cm}^3 \text{ g}^{-1}$ containing only about 22 vol. % of matrix represents the limit of applicability and materials with larger specific pore volume and lower content of matrix are mechanically unstable [4—6].

The aim of this paper was to show on the basis of the evaluation of influence of the pore volume of silica gels on their chromatographic characteristics whether the limits reported at present from the point of view of the applicability of the high pore volume of silica gels for HPLC are justified as well as to evaluate potential limits of the development of the new types of silica gels in view of the total volume of their pores.

Experimental

Materials and reagents

Sorbents used: Silpearl (Kavalier, Votice); LiChrosorb SI-60 and SI-100 (Merck, Darmstadt); Separon SIX (Laboratorní přístroje, Prague); SG-7/G, silica gel [7, 8] (Polymer Institute CCR SAS, Bratislava).

Methanol, anal. grade (Lachema, Brno) was used as a dispersing and transport liquid for column packing. n-Hexane, anal. grade (International Enzymes, Windsor—Berkshire, England) was used as a mobile phase for testing packed columns. The testing mixture consisted of: benzene, naphthalene, biphenyl, nitrobenzene, all anal. grade (POCH Gliwice, Poland). Redistilled CCl_4 (Lachema, Brno) was used for estimating t_0 .

Methods

Argon adsorption and desorption isotherms were determined on a volumetric apparatus according to [9]. The specific surface area S was calculated from adsorption of argon according to BET [10]. The pore diameter D was found from the pore size distribution obtained from the sorption data according to [11, 12] and the pore volume V_{ps} from the sorption at relative pressure close to 1 [13]. V_{pe} was calculated from the bulk density ρ_s according to [4].

The concentration of the surface silanol groups ($c(\text{SiOH})$) was established using the method proposed by *Nondek* and *Vyskočil* [14] based on substitution reaction of the complex $(\text{CH}_3)_2\text{Zn}$ and THF with surface silanol groups at 373 K and the following determination of methane released.

pH of aqueous sorbent suspensions were measured using a pH-meter PHM-84 (Radiometer, Copenhagen) in 5 % aqueous suspension of silica gels [15, 16]. Sorbents were not washed before pH determination.

The particle size of sorbents used was determined both by microscopic analysis [17] measuring about 650 particles for each type of sorbent — average particle diameter d_{pM} and by chromatography using the dynamic method according to *Halász et al.* [18] — average particle diameter d_{pD} .

Stainless steel columns (100 mm \times 6 mm, Laboratorní přístroje, Prague) were packed in an apparatus described elsewhere [15] at optimum pressure P_{opt} , which provided maximum number of the theoretical plates. P_{opt} and other packing conditions were established *via* independent experiments [19].

Chromatographic measurements were carried out on a liquid chromatograph consisting of a reciprocating membrane, type VCM-300 (Vývojové dílny CSAS, Prague), sample valve, type 7120 (Rheodyne Co., Berkeley, USA), high-pressure manometer (Chirana, Stará Turá) with pulse damping capillary 30 cm long and 0.25 mm in diameter, UV detector, type UVM-4 (Vývojové dílny CSAS, Prague), and a linear recorder, type TZ 4200 (Laboratorní přístroje, Prague). The flow-rate of the mobile phase at current column testing was $1 \text{ cm}^3 \text{ min}^{-1}$ and during evaluation of the effect of the flow-rate on the efficiency of columns the flow-rate was $0.15\text{--}5 \text{ cm}^3 \text{ min}^{-1}$.

Results and discussion

Granulometric statical analysis using a microscope showed that the sorbents used practically do not contain particles smaller than $2 \mu\text{m}$. Particle diameter

distribution was relatively wide and varied between $\pm (2\text{--}3.5)\mu\text{m}$ from average values d_p ; here we should point out the general asymmetry of the distribution curve towards greater particle size (Fig. 1). The values of d_{pM} and d_{pD} in Table 1 are for the particular sorbents surprisingly close in spite of the problems arising from the particle size determination using the dynamic method. On the other hand, there are remarkable differences in d_p values among individual sorbents. We have to take this aspect into account in the comparison of their chromatographic characteristics. Similarly, the characteristics of the inner structure of sorbents expressed as S , D , and V_p also differ. For objective comparison, D should be kept constant with only S and V_p to vary. However, we had no suitable sorbents at disposal. In contrast, the samples LiChrosorb SI-60 and Separon SIX had rather close S values and different D values. Nevertheless, we expect that even such a limited selection of the samples allows to draw some conclusions.

Distributions of the pore dimensions were calculated from sorption data [11, 12, 20] and the individual curves are shown in Fig. 2b. Silpearl shows the

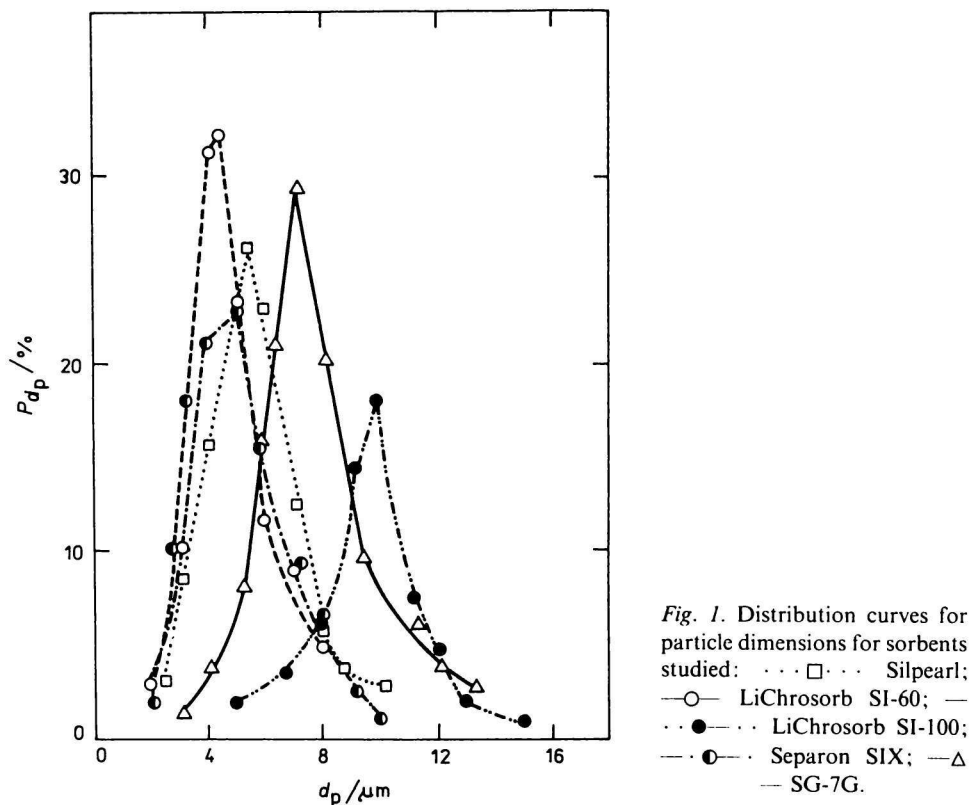


Table 1

Physical parameters for sorbents studied

Sorbent	Shape of particles	$\frac{d_{pM}}{\mu m}$	$\frac{d_{pD}}{\mu m}$	$\frac{S}{m^2 g^{-1}}$	$\frac{D}{nm}$	$\frac{V_{ps}}{cm^3 g^{-1}}$	$\frac{\rho_s}{g cm^{-3}}$	$\frac{V_{p\phi}}{cm^3 g^{-1}}$	$\frac{c(SiOH)}{\mu mol m^{-2}}$	pH
Silpearl	r	5.4	5.64	610	4.6	0.64	0.50	0.60	3.8	6.3
LiChrosorb SI-60	i	5.0	4.75	332	8.0	0.52	0.46	0.70	5.45	4.3
LiChrosorb SI-100	i	10.3	10.8	245	16.0	0.75	0.37	0.98	4.30	5.6
Separon SIX	s	4.9	4.78	298	13.0	0.51	0.32	1.22	5.80	6.0
SG-7G	s	7.5	7.33	421	16.0	1.35	0.23	1.90	4.55	4.6

s — spherical, r — spheroidal, i — irregular.

narrowest distribution of pore dimensions. As the average diameter of the pores of individual sorbents examined increases, the distribution curve broadens; silica gel SG-7G with average pore diameter of about 16 nm exhibits wide distribution.

Information on the shape of pores is obtainable from adsorption-desorption curves of nonpolar gasses [21]. Fig. 2*a* shows Ar adsorption and desorption

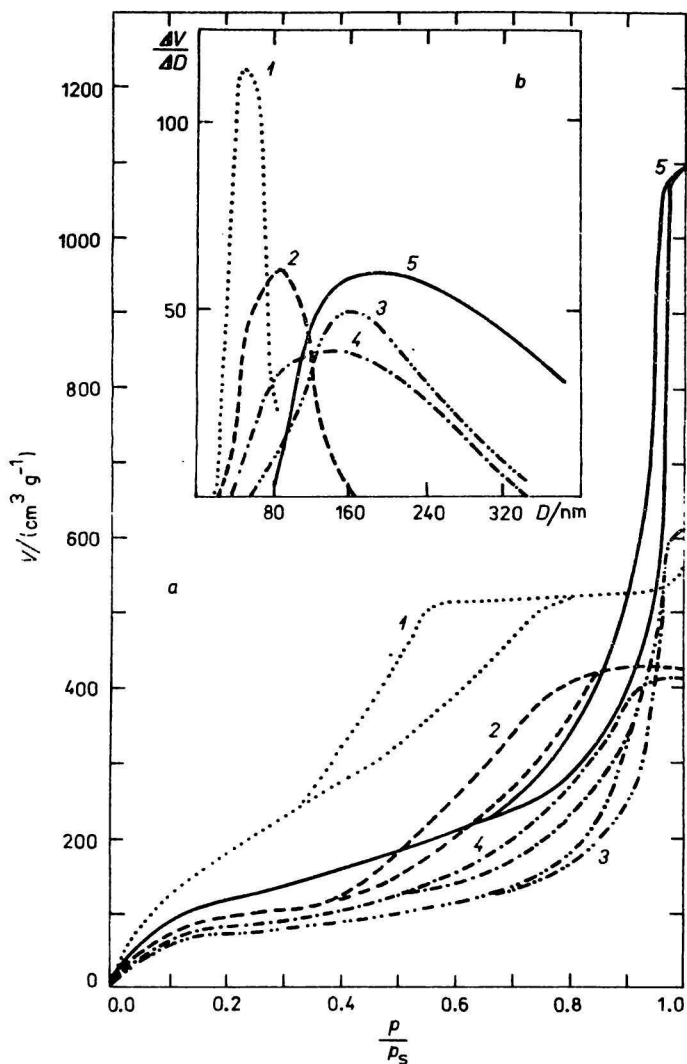


Fig. 2. *a*) Sorption isotherms of Ar. *b*) Distribution curves of pore dimensions.
1. Silpearl; 2. LiChrosorb SI-60; 3. LiChrosorb SI-100; 4. Separon SIX; 5. SG-7G.

curves for the sorbents studied. The course of these isotherms indicates that the shape of sorbent pores is approximately cylindrical. An exception is the isotherm of Silpearl which indicates a more or less conic shape of pores [21].

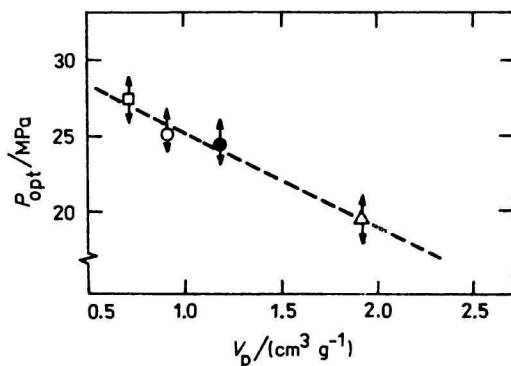


Fig. 3. A plot of the optimum packing pressure vs. specific pore volume V_p of sorbents. Packing liquid methanol, upward slurry. □ Silpearl; ○ LiChrosorb SI-60; ● LiChrosorb SI-100; △ SG-7G.

Fig. 3 shows the dependence of the optimum packing pressure P_{opt} on the pore volume of the sorbents examined. Sorbents with larger pore volume require lower optimum packing pressure. This conclusion is natural when considering packing in methanol by upward slurry technique [19]; higher pressure also means higher rate of the transport liquid which carries sorbent into the column during packing. Heavier particles require more rapid flow of the transport liquid to prevent settling and thus provide an optimum column bed. This relationship is to some extent influenced also by the diameter of sorbent particles, P_{opt} seems at first sight to be indirectly proportional to d_p . The largest particles of LiChrosorb 100 required, however, much higher P_{opt} than SG-7G.

Bristow and Knox [22] proposed to characterize the quality of HPLC columns by the parameters h_{min} , v_{opt} , E , ϕ , and f_{As} . The values of these parameters for individual columns are listed in Table 2. If we judge our columns from this point of view, we may consider them as "good" or even "very good".

A similar conclusion may also be drawn when we use classification of Colin *et al.* [23]. Commercial glass column filled with Separon SIX differed from other columns in length and in inner diameter. For this reason, the values in Table 2 are little higher than those for columns prepared by us. The high value of impedance E may serve as an evidence for the column not very stable, which negatively affects the repeatability of the retention data [17].

In the process of column testing, the choice of the proper testing mixture is

Table 2

Characteristics of the columns studied; solute — naphthalene

Sorbent	k'	h_{\min}	v_{opt}	E	φ	f_{As}
Silpearl	0.76	3.03	7.8	6520	710	1.14
LiChrosorb SI-60	0.68	3.14	5.3	9020	915	1.10
LiChrosorb SI-100	0.54	2.56	7.05	4010	612	1.25
Separon SIX	0.58	4.07	7.5	14485	875	1.13
SG-7G	0.54	2.2	5.9	3558	722	1.12

The data are average values from three—five columns with standard deviation $\sigma_{n-1} = \pm 10\%$.

important. We used for this purpose a mixture with components differing in the chemical and physical structure of molecules: benzene, naphthalene, biphenyl, and nitrobenzene.

When efficiency of the individual columns is comparable and the peak width of testing substances is similar, the separation selectivity of the first three components of the testing mixture correlates to a great extent with the specific surface area of sorbent and, as S increases, it is further improved [1, 2]. Fig. 4 shows a plot of the values of α for pairs of the testing mixture *vs.* specific surface

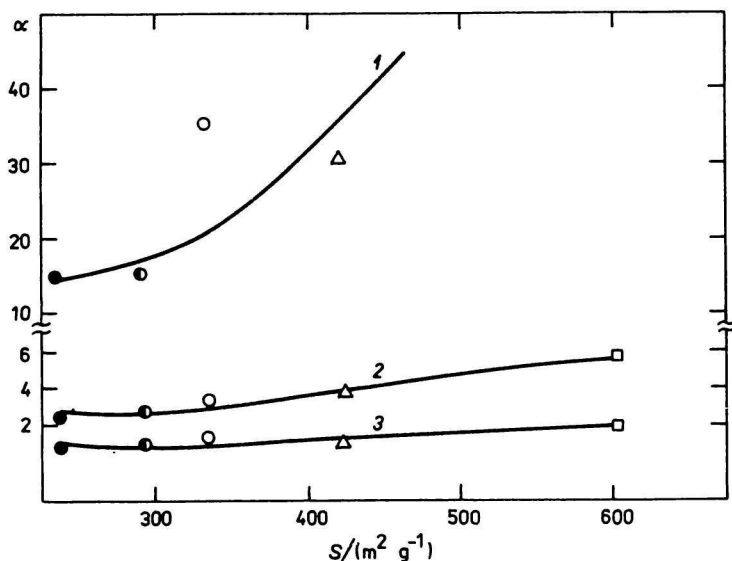


Fig. 4. A plot of the separation selectivity α vs. specific surface area. \square Silpearl; \circ LiChrosorb SI-60; \bullet LiChrosorb SI-100; \bullet Separon SIX; Δ SG-7G.

1. $k'(\text{nitrobenzene})/k'(\text{benzene})$; 2. $k'(\text{naphthalene})/k'(\text{benzene})$; 3. $k'(\text{biphenyl})/k'(\text{naphthalene})$.

area. It is natural that small pores of Silpearl will not already be accessible for molecules with the molar mass of several hundreds of g mol^{-1} and thus the advantage of the large specific surface will disappear. Macromolecules with the molar mass M of about 10^4 g mol^{-1} are completely excluded from Silpearl [17]. In contrast, SG-7G has, in spite of the large pore diameter, surface area large enough, which ensures sufficient separation selectivity for small molecules. The pores of this material are at the same time accessible for molecules of medium size with the molar mass of about 10^3 g mol^{-1} and only macromolecules with the molar mass of above 10^5 g mol^{-1} are entirely excluded [17]. We may then conclude that SG-7G is, from the point of view of the diameter of its pores and their distribution, more universal than *e.g.* Silpearl to be used in adsorption HPLC.

In addition to the specific surface, elution behaviour of the testing components in the mode of normal HPLC will also be influenced by the concentration of free surface silanol groups.

The presence of SiOH groups, which behave as Lewis acids, seems to affect also pH of aqueous suspensions of silica gels [15, 16] (*cf.* Fig. 5 and Table 1). Of course, the pH of such a system may largely be influenced also by residues of the salts and substances in sorbent if not sufficiently washed during preparation.

The value of t_0 strongly influences k' values. In the first approximation we have considered the values for the retention time of tetrachloromethane as t_0 . The sorbent with the smallest pore volume (Silpearl) shows the lowest t_0 value; as the volume of the pores of sorbent increases, the value of t_0 rises.

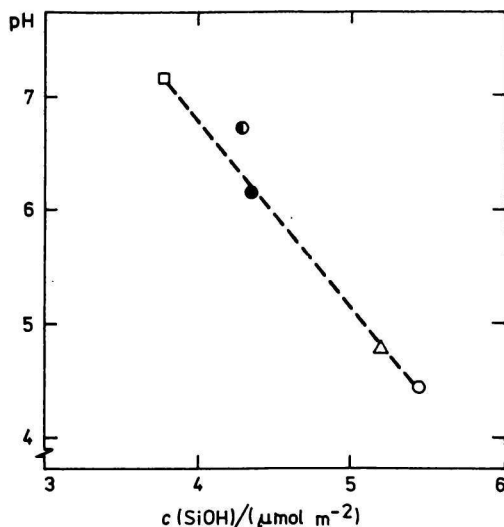


Fig. 5. The influence of the concentration of the surface silanol groups of the sorbents studied on the value of pH of 5% aqueous suspension. Denotation of sorbents as in Fig. 4.

Fig. 6 shows a decrease in the efficiency expressed as $h = H/d_p$ for sorbents of various pore volume with the increasing solute volumes injected (naphthalene) having the same concentration ($\rho_v = 8 \times 10^{-5} \text{ g dm}^{-3}$). The plot shows that with the increasing pore volume of the sorbents in the sequence from Silpearl to SG-7G, the region with the minimum and insignificant rise of the h values increases until rapid increase of h because of the general overloading of the column. In the upper part of the curve $h = f(V_c)$, plateau occurs, which corresponds to almost complete loss of efficiency.

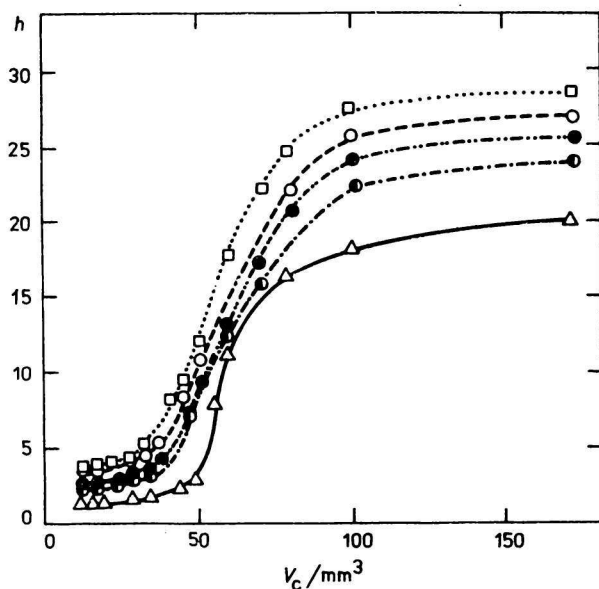


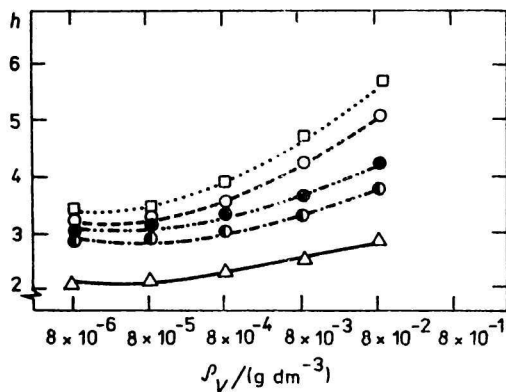
Fig. 6. A change in the efficiency as a function of the volume of naphthalene injected. Denotation of sorbents as in Fig. 1.

Fig. 7 shows the effect of the concentration of solute injected at constant volume (14 mm^3) on the values of h . Also here we can see that reduction of the efficiency with the increasing concentration of the solute injected is most pronounced for columns packed with the sorbent of low V_p . As V_p increases, the loss of efficiency is less expressive. This indicates that sorbents with greater pore volumes may advantageously be applied in gel chromatography and in preparative separations, when large loading of columns is applied.

Another criterion of the quality of columns may consist in their pressure stability as has been characterized by Unger *et al.* [6]. The effect of the increasing back pressure of eluent on the flow-rate through column is traced. The dependence is straight-line until significant deformation or partial fractionation and even crushing of silica particles of column packing occurs; this will be evident

as an inflection of the curve towards the pressure axis. On the basis of the shape of the curve, which is usually accompanied with significant hysteresis between the ascending and descending part of the curve, we may judge on the character of irreversible changes taking place within sorbent bed and its mechanical stability.

Fig. 7. The influence of the concentration of naphthalene on the efficiency of the column. Denotation of sorbents as in Fig. 1. The volume of the sample injected was 14 mm^3 .



An opinion often occurs in the literature that the pressure resistance of sorbents is indirectly proportional to their pore volume; *i.e.* sorbents with high porosities are pressure and mechanically unstable and thus not suitable as good packings for HPLC columns [4, 6].

We examined pressure resistance of the column packed with SG-7G sorbent according to the method described by Unger *et al.* [6]. Fig. 8 shows a plot of the back pressure of eluent *vs.* its flow-rate. The curve shows that the packing in

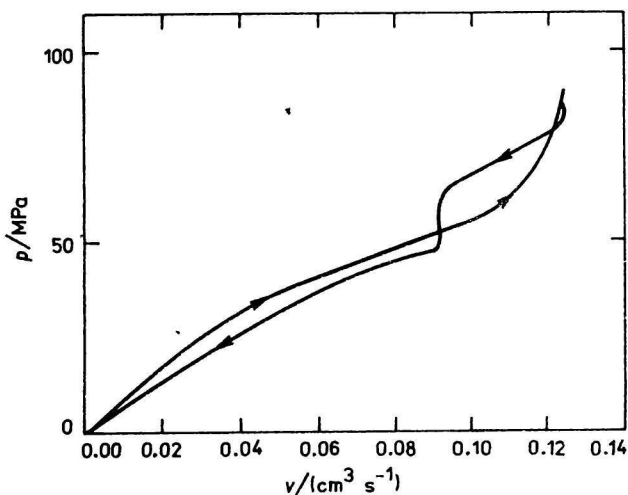


Fig. 8. Pressure resistance of the column packed with silica gel SG-7G. Arrows indicate direction of the dependence at pressure increase or decrease.

column will resist pressures of at least 60 MPa without any problem and at the highest pressure used (90 MPa, which is more than double the maximum pressures used in current HPLC) only slight bed compression occurred without any particle destruction. This has also been confirmed by microscopic checking of the shape of the particles after having been taken out of the column.

The measurements show that within certain limits, the particle strength and the mechanical stability of the particles are influenced more by the mode of preparation than by the resulting pore volume of the sorbent. It has to be said, however, that the matrix of the sorbent SG-7G has been specially treated during its preparation.

Conclusion

An examination of the characteristics of the pore structure and the surface of the silica gel sorbents in relation to their properties as packings of the highly efficient columns in the high-pressure liquid chromatography has shown that the higher volume of the pores positively affects the sample capacity as well as permeability of the column. Highly effective, stable and pressure-resistant columns may also be prepared from sorbent packings with the specific pore volume as high as $2 \text{ cm}^3 \text{ g}^{-1}$.

Acknowledgements. The authors thank Dr. T. Bleha for his interest in discussion and Drs. L. Nondek, P. Hložek, and K. Šebeková for their assistance in some measurements.

Symbols

$c(\text{SiOH})$	surface concentration of silanol groups	$\mu\text{mol m}^{-2}$
D	mean pore diameter	nm
d_p	mean particle diameter	μm
d_{pM}	mean particle diameter from microscopic measurement	μm
d_{pD}	mean particle diameter obtained by dynamic method [18]	μm
E	separation impedance	
f_{As}	peak asymmetry coefficient	
H	height equivalent	μm
h	reduced plate height	
h_{\min}	minimum reduced plate height on $h = f(v)$ curve	
k'	capacity factor of solute	
p	adsorption pressure	MPa
p_s	saturated pressure	MPa

p/p_s	relative pressure	
P_{opt}	optimum packing pressure	MPa
S	specific surface area	$\text{m}^2 \text{g}^{-1}$
t_0	elution time of an unretained solute	s
v	flow-rate	$\text{cm}^3 \text{s}^{-1}$
V	volume of adsorbed gas by the sorbent surface	$\text{cm}^3 \text{g}^{-1}$
V_c	volume of solute	cm^3
V_p	specific pore volume	$\text{cm}^3 \text{g}^{-1}$
V_{ps}	specific pore volume from adsorption data	$\text{cm}^3 \text{g}^{-1}$
V_{pg}	specific pore volume from density measurements	$\text{cm}^3 \text{g}^{-1}$
α	separation selectivity	
v	reduced eluent velocity	
v_{opt}	reduced eluent velocity at h_{min}	
ρ_s	bulk density	$\text{cm}^3 \text{g}^{-1}$
ρ_v	solute concentration	g dm^{-3}
φ	column resistance factor	

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Translated by A. Rebrová