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GEL CHROMATOGRAPHY

V. DEPENDENCE OF SEPARATION EFFICIENCY ON EXPERIMENTAL CONDITIONS

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SUMMARY

The separation efficiency of gel chromatographic columns was investigated under a wide variety of experimental conditions. By use of reduced quantities it is possible to describe the separation efficiency for different gels and experimental conditions by a single equation. Deviations from this equation were found to be caused by an alteration of the diffusion coefficient within the gel with respect to the mobile phase. Factors governing the gel chromatography of *p*-oligophenylenes on poly(vinyl acetate) and poly(styrene) gels are generalized and discussed.

INTRODUCTION

Small, non-polymeric molecules offer almost ideal conditions for the study of various factors influencing the behaviour of molecules in the two-phase (gel–solvent) system used in gel chromatography.

Any zone of identical molecules will broaden on passing through the gel column. It would be advantageous to be able to define such peak spreading, which depends on the substance (its diffusion properties), experimental conditions and on the stationary phase used, by the separation efficiency as recommended for gas chromatography[1].

The stationary phase in gel chromatography is a crosslinked material having porous structure, and hence the separation process may be described as a network-limited partition[2]. Thus it seems to be highly probable that theoretical approaches derived for the separation efficiency of other chromatographic processes should also be applicable in gel chromatography.

THEORY

Using reduced quantities the well known VAN DEEMTER's equation[3] may be expressed as:

$$h = a + b/\nu + c\nu \qquad (1)$$

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where the reduced plate height $h = H/d_p$;

H = height equivalent to a theoretical plate (HETP);

d_p = particle diameter;

the reduced velocity of flow $v = vd_p/D$;

v = linear velocity of elution;

D = diffusion coefficient of the solute in the mobile phase.

The term a describes the regularity of the packing. With well packed columns a has a value of 2 to 3. Even if the gel particles used are practically monodisperse, the resulting packing arrangement is irregular as shown by DEBBAS AND RUMPF[4].

b reflects the peak broadening caused by diffusion, this term will diminish with increasing flow velocity. In many gel chromatographic separations the b-term can be neglected.

c originates in nonequilibrium conditions. It probably contains two different factors:

(1) The lack of equilibrium in the exchange process.

(2) The nonequilibrium in the mobile phase itself.

These two contributory factors should be additive. If (2) can be neglected, the c-term will vanish if no exchange between the mobile and the stationary phase occurs, *e.g.* on eluting substances that are totally excluded from the gel. So in this case we may expect a constant value for the reduced HETP if the reduced velocity is higher than 5.

The third right hand term of eqn. (1) contains the diffusion coefficient in the stationary phase (the permeation coefficient P) which is related to the diffusion coefficient in the mobile phase by

$$P = \gamma D \qquad \text{where } \gamma < 1$$

As the permeation coefficient is controlled by obstruction and partition ("solubility")[5], the value of the permeation coefficient differs from the diffusion coefficient in the mobile phase.

WALTON[6] derived an equation accounting for the behaviour in ion-exchange chromatography. His proposal is based on the film diffusion theory, also leading to a linear dependence at high flow-rates.

Another interesting theoretical approach for the separation efficiency was reported by GIDDINGS AND MALLIK[7] resulting in the equation

$$H = B/v + Cv + \sum_i \frac{1}{1/A_i + 1/C_{mi}v} \tag{2}$$

where A_i, B, C, C_{mi} are constants,
which can be expressed in reduced terms as

$$h = b'/v + c'_s v + \sum_i \frac{1}{1/a'_i + 1/c'_{mi}v} \tag{2a}$$

C_{mi} originates from nonequilibrium in the mobile phase. The characteristic feature of eqn. (2) is that the irregularities of packing (contained in A_i) and the nonequilibrium term in the mobile phase are complementary. The resulting "coupled" form gives a lower contribution to h than either term alone. If the experimental conditions are

such that it is possible to neglect the $1/C_{mt}$ terms, eqn. (2) degenerates to the VAN DEEMTER equation. However, the final term of eqn. (2) involves the greatest uncertainty as far as quantitative predictions of HETP are concerned.

The general shape of the curves representing the reduced separation efficiency according to VAN DEEMTER and GIDDINGS is shown in Fig. 1.

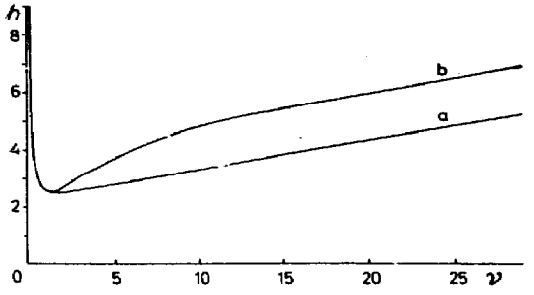


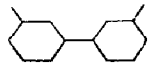
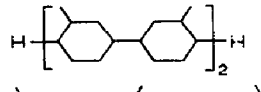
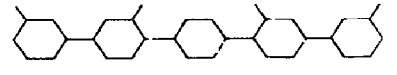
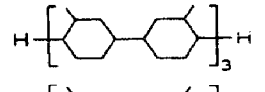
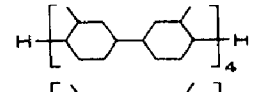
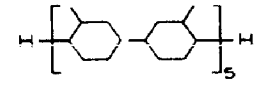
Fig. 1. General shape of the curves according to the equation of (a) VAN DEEMTER and (b) GIDDINGS.

EXPERIMENTAL

The present work was carried out with methyl substituted *p*-oligophenylenes. These substances are chromatographically pure and their hydrodynamic behaviour is well characterized[8,9]. The compounds used, as well as their diffusion coefficients, are listed in Table I.

TABLE I

METHYL SUBSTITUTED *p*-OLIGOPHENYLENES USED IN THESE INVESTIGATIONS AND THEIR DIFFUSION COEFFICIENT *D*

Formula	$D \cdot 10^5$ (cm^2/sec)
I 	2.7*
II** 	1.81
III 	1.09
IV 	0.92
V 	0.90
VI 	0.63
VII 	0.59

* This value is not determined by experiment. It is calculated to obtain the best fit.
** The lines denote methyl groups.

Poly(vinyl acetate) gels, crosslinked with butanediol divinyl ether or divinyl adipate, and poly(styrene–divinylbenzene) gels were prepared by suspension polymerization in the usual manner[10]. A typical example of vinyl acetate–divinyl adipate copolymerization is given below.

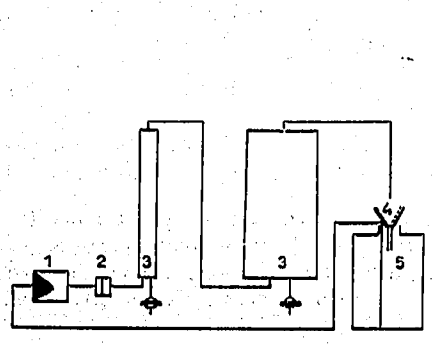
A 2-l round bottomed, four-necked flask, fitted with an efficient stainless-steel mechanical stirrer, a contact thermometer, an in- and output of nitrogen and a reflux condenser, is fitted with an electrical heating jacket. In the flask is placed a solution of 10 g poly(vinyl pyrrolidone), 0.35 g NaH_2PO_4 and 6.0 g Na_2HPO_4 in 1200 ml of distilled water and the stirrer is started at preset revolutions. The oxygen is eliminated from the system by a stream of N_2. A solution of 0.5 g azodiisobutyronitrile and 7.49 g divinyl adipate in 200 g of vinyl acetate is slowly added under continuous stirring. After 20 min the heating is commenced and the temperature is kept at 70° for 5–6 h. The reaction mixture is cooled and the contents are filtered through a crude sieve into a 5-l beaker, decanted 8–10 times with water, filtered by suction and the suspension of polymerisate obtained was dried at 35° and 15 mm Hg vacuum.

In order to obtain a narrow distribution of particle sizes the gels were fractionated. Fractions of particle size larger than approximately 0.07 mm were separated by wet sieving on standard sets of sieves. Fractions of smaller particles were obtained by sedimentation in an apparatus shown schematically in Fig. 2. The particle size of the gels in the dry state ($\bar{d}_{pd}$) was measured by the usual microscopic technique. The gel bed volume of the dry gel (V_d) and in the swollen state (V_s) was determined. The same statistical packing in both cases can be assumed. Thus the particle diameter in the swollen state is represented by:

$$\bar{d}_p = \bar{d}_{pd} \sqrt[3]{V_s/V_d}$$

Subsequent microscopic measurements of the particle size in the swollen state, as well as the fact that the reduced HETP was found to be independent of the particle size for a set flow-rate confirmed the validity of this equation.

The packing of the columns was carried out in the apparatus shown in Fig. 3.



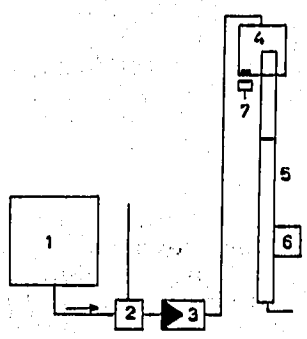


Fig. 2. Equipment for size separation of gel particles by sedimentation. 1 = Pump; 2 = sintered glass filter; 3 = series of sedimentation vessels with increasing diameter; 4 = filter; 5 = solvent reservoir.

Fig. 3. Device for packing of the columns. 1 = Solvent reservoir; 2 = degasser; 3 = pump; 4 = vessel for the gel suspension; 5 = column; 6 = vibrator; 7 = magnetic stirrer.

A fixed flow of degassed solvent was maintained during the packing by means of a pump. The gel suspension was magnetically stirred in a metallic, high-pressure

container. An upright tube, 7 cm long and of the same diameter as the column connected to it, was fitted inside to the bottom. Such an arrangement assures that a very diluted and homogeneous gel suspension settles down the column.

The column was vibrated during this procedure. The whole system must be free of air bubbles during the packing. The final pressure of the solvent has to be about 3–5 atm higher than the hydrodynamic pressure which develops during actual chromatographic measurements at the highest flow-rates used. A standardized packing lasts about 36–48 h.

The experimental arrangement for the separations is shown in Fig. 4; the steel columns were 1.2 cm × 100 cm. To avoid the dead volumes we have used the injection device and column end fittings which are also shown in Fig. 4. A U.V.-spectrometer (Uvicord) served as the detector; the volume of the eluate was measured by means of a calibrated syphon coupled with a light barrier.

RESULTS AND DISCUSSION

Packing procedure

To ensure reproducibility of packing several packing procedures[11–13] were investigated.

The most simple and most effective method seems to be to lengthen the column by a tube of the same diameter and to pour in the suspension. The solvent is pumped at a slow rate while the gel particles gradually settle down. Curve 1 (Fig. 5) shows the resulting separation efficiency obtained with a column filled in this way. However the reproducibility of such a procedure is bad.

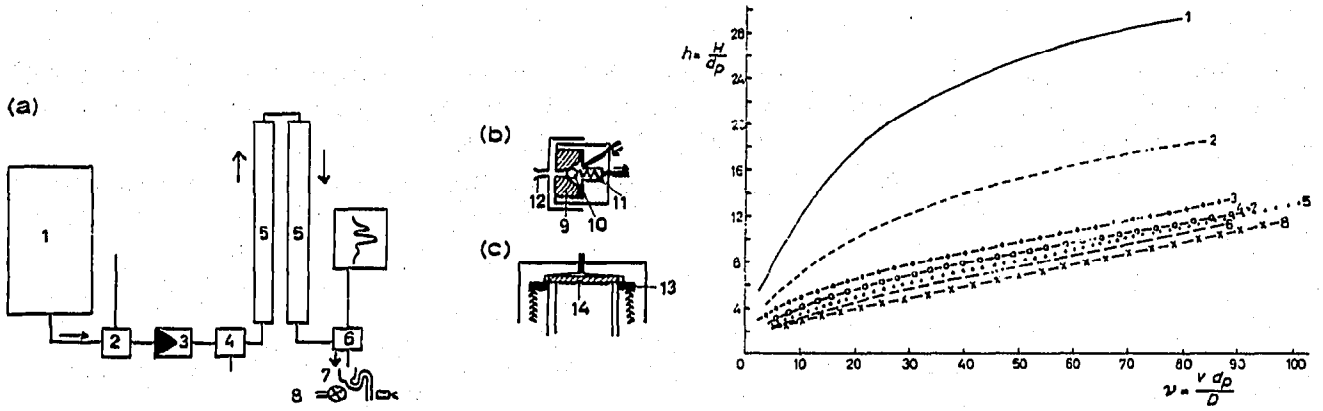


Fig. 4. Schematic representation of the apparatus: (a) for separation; (b) injection part and (c) column end fittings. 1 = Solvent reservoir; 2 = degasser; 3 = pump; 4 = injection part; 5 = column; 6 = detector; 7 = siphon; 8 = light barrier; 9 = Teflon part; 10 = ball; 11 = spring with a steel core to avoid dead volume; 12 = fitting for syringe; 13 = Teflon ring, 14 = sintered metal filter.

Fig. 5 Reduced separation efficiency with different conditions of packing. (1) Normal sedimentation; (2) swelling the gel in the column; (3)–(8) using the equipment given in Fig. 3; (3) without vibration; (4) packing time 48 h, solvent flow 720 ml/h; (5) 48 h, 360 ml/h; (6) 48 h, 90 ml/h; (7) 24 h with and 24 h without vibration, 180 ml/h; (8) 24 h, 60 ml/h and 24 h, 120 ml/h.

Another possibility is to pack the column in the same way as described above but using a mixture of solvents which partially contracts the gel particles. The packed column is then closed and the gel is swollen to the full extent in the column by

replacing the mixture by a good solvent. However, in this case also the reproducibility and the absolute values of the separation efficiency (curve 2, Fig. 5) are not very good.

On filling the column by means of the packing device shown in Fig. 4 the curves obtained are completely reproducible if the same conditions of packing (*i.e.* flow rate and vibration) are maintained. By varying these conditions the procedure could be optimized (curves 3–8). Under the optimum conditions the relationship is represented by a straight line (curve 8), *i.e.* it satisfies the VAN DEEMTER equation over a wide range of flow rates. Irregularities in the packing cause a considerable deviation from the equilibrium in the mobile phase which is operating in conjunction with the corresponding A_t term of the GIDDINGS equation. At higher reduced flow velocities all the curves have the same slope of 0.1. The packing conditions of curve 8 were used throughout this work.

Influence of particle size

Using reduced dimensionless quantities it should be possible to relate different experiments in order to predict the ultimate obtainable performance of the method[7,14]. The plot of reduced quantities in Fig. 6 shows no dependence on the diameter of the

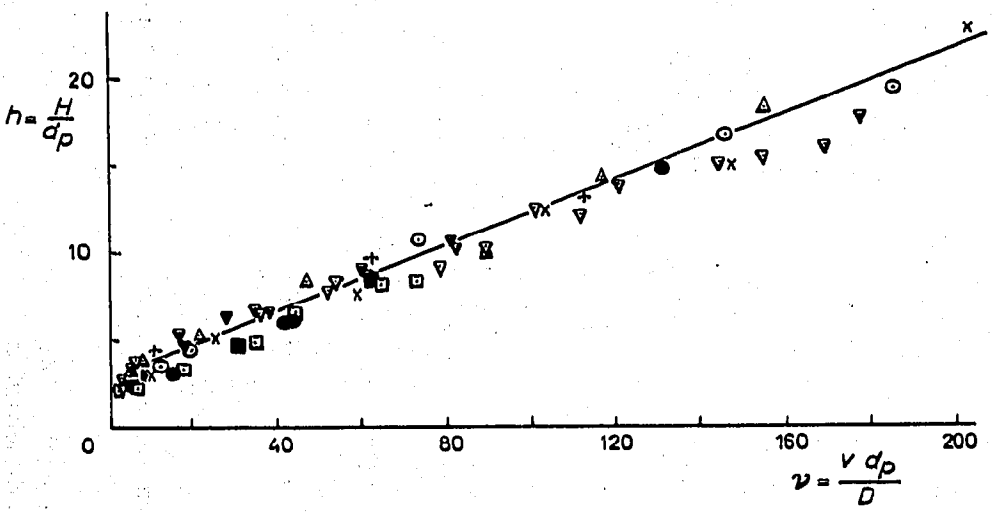


Fig. 6. Reduced separation efficiency of the system poly(vinyl acetate) gel (crosslinked with 5 mole-% butanediol divinyl ether); tetrahydrofuran; methyl substituted *p*-oligophenylenes. $\bar{d}_p$ = 0.0191 cm: (⊡) benzene; (▽) *m*-bitolyl, II; (△) quaterphenyl, III; (×) quinquephenyl, IV; (⊙) octiphenyl, VI. $\bar{d}_p$ = 0.0382 cm: (■) benzene; (▼) *m*-bitolyl, II; (▲) quaterphenyl, III; (+) quinquephenyl, IV; (●) octiphenyl, VI.

particles measured. However, we have to take into account the fact that with a small particle diameter (< 0.05 mm) it is more difficult to obtain a good packing. The reason for this might originate from a relatively strong tendency of particles to form aggregates, or from the difficulty of getting fractions of small particles with a comparable relative size distribution. For example in the fraction with mean $\bar{d}_p$ = 0.3 mm the standard deviation was 0.019 mm (6.3 %), while in the fraction of $\bar{d}_p$ = 0.024 mm the standard deviation was 0.005 mm (21 %).

The chemical nature of the gel

The reduced separation efficiency of poly(vinyl acetate) gel crosslinked with 5 mole-% of butanediol divinyl ether is shown in Fig. 6. In Fig. 7 the same diagram

TABLE II

RELATIVE ZONE VELOCITY ($R = V_0/V_e$) FOR OLIGOPHENYLENES WITH DIFFERENT GELS AND THEIR SEPARATION BEHAVIOUR

No.	*Gel**	$\bar{d}_p$ *(cm)*	*R***						*Separation behaviour****
			I	*II*	*III*	*IV*	*V*	*VI*	
52	VAC–macroporous	0.00862	0.457	0.478	0.502	—	0.516	0.528	i
19	VAC–BDVE (5%)	0.0191	0.473	0.557	0.656	0.697	—	0.827	i
46	VAC–DVA (1.6%)	0.0180	0.488	0.564	0.645	—	—	0.793	i
48	VAC–DVA (4.1%)	0.0170	0.538	0.650	0.784	—	—	0.938	d
41	VAC–DVA (8.8%)	0.0218	0.611	0.772	→ 1†	—	—	1.0	d
49	VAC–DVA (34%)	0.0195	→ 1†	1.0	1.0	—	—	1.0	d
29	St–DVB (1.6%)	0.0112	0.432	0.459	0.511	—	—	0.615	i
32	St–DVB (4.1%)	0.0187	0.504	0.552	0.634	—	—	0.764	d
27	St–DVB (8.2%)	0.0169	0.578	0.664	0.779	—	—	0.909	d
40	St–DVB (25.5%)	0.0099	0.725	→ 1.0†	1.0	—	—	1.0	d

* VAC = poly(vinyl acetate) gel; BDVE = butanediol divinyl ether; DVA = divinyl adipate; St = poly(styrene) gel; DVB = divinylbenzene; (amount of crosslinking agent in mole-%).

** No. of the substance given in Table I.

*** i = independent of the solute; d = dependent on the solute.

† Elution volume dependent on the flow rate of the solvent.

for a poly(vinyl acetate) gel crosslinked with 1.6 mole-% of divinyl adipate and a poly(styrene) gel crosslinked with 1.6 mole-% of divinylbenzene is given. These gels have a low crosslinking density and give an identical straight line within the limits of error. This curve does not depend on the molecular weight of the substances measured or the type of weakly crosslinked gel and has a slope of approximately 0.1. From the results shown in Figs. 6 and 7 it follows that the influence of the C term in eqn. 2 has

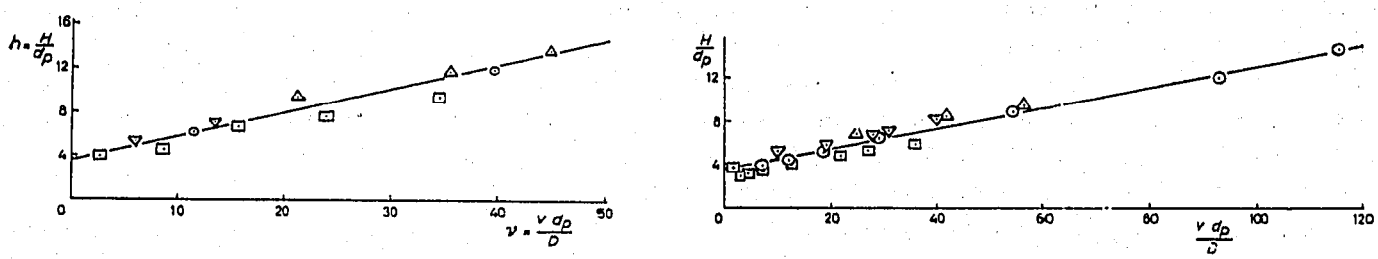


Fig. 7. Reduced separation efficiency of (a) poly(vinyl acetate) gel crosslinked with 1.6 mole-% divinyl adipate $\bar{d}_p$ = 0.0180 cm and (b) poly(styrene) gel crosslinked with 1.6 mole-% divinylbenzene $\bar{d}_p$ = 0.0112 cm. (⊡) Benzene; (▽) *m*-bitolyl, II; (△) quaterphenyl, III; (⊙) octiphenyl, VI.

been underestimated[7]. In contrast to theoretical prediction C is independent of the reduced zone velocity R (see the following section and Table II).

As the partition on these gels had been established we can conclude that the phase boundary between the mobile and stationary phase is extremely ill-defined in this case.

Crosslinking density of gels

Gels with higher crosslinking density show that the reduced separation efficiency is remarkably dependent on the substance measured (Fig. 8). For a given gel the slope of the curves increases with increasing molecular weight of the solute. The higher the crosslinking density the more pronounced is this effect. Figs. 7b, 9a and 9b show

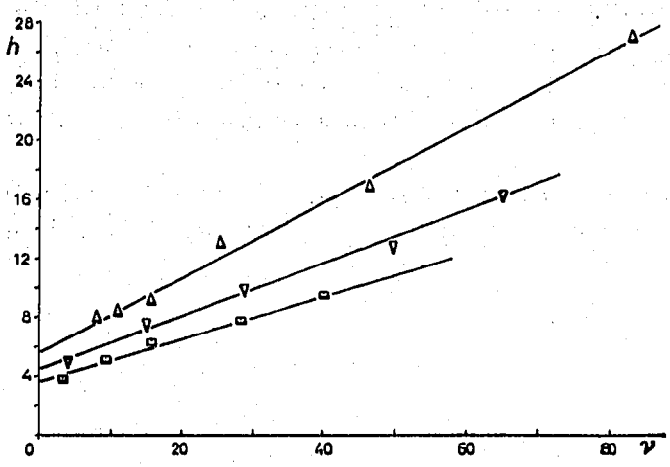


Fig. 8. Reduced separation efficiency of a poly(vinyl acetate) gel crosslinked with 4.1 mole-% divinyl adipate; $\bar{d}_p$ = 0.017 cm. (⊡) Benzene; (▽) *m*-bitolyl, II; (△) quaterphenyl, III.

the separation efficiency of a series of poly(styrene) gels prepared by copolymerization of styrene with different amounts of divinylbenzene. Poly(vinyl acetate) gels crosslinked with divinyl adipate behave in the same manner. These examples clearly

demonstrate the influence of the varying permeation coefficient on the separation efficiency.

The restriction of diffusion in the stationary phase is not caused by an obstruction in the gel network only. The restriction effect should be particularly high if the diameter of pores available and of the molecules of the solute are of a comparable size, and thus we might expect a dependence of the reduced separation efficiency on

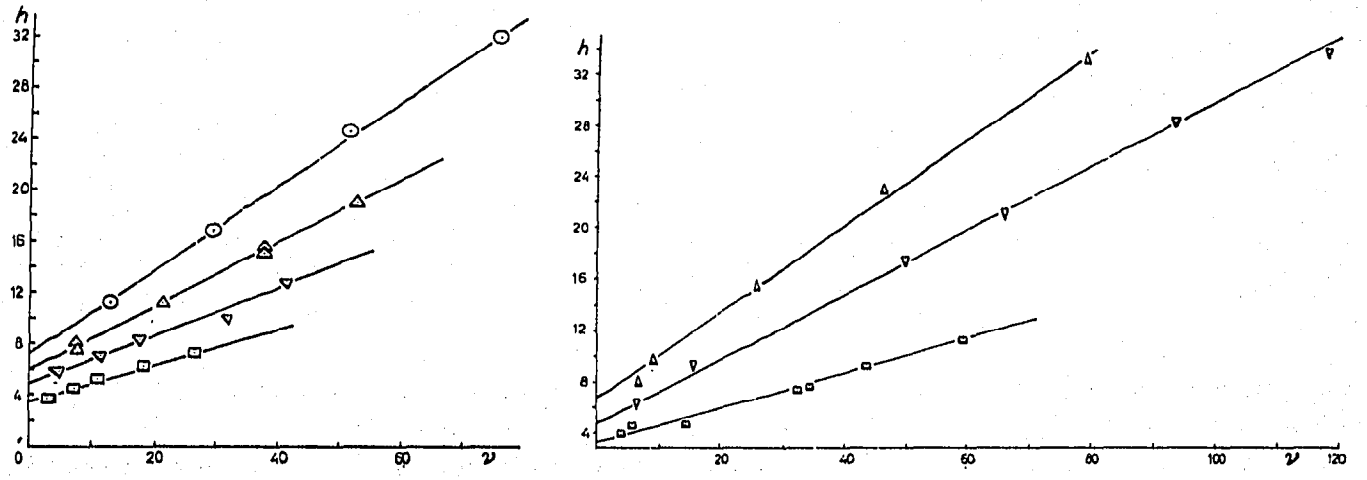


Fig. 9. Reduced separation efficiency of (a) poly(styrene) gels crosslinked with 4.1 mole-% divinyl benzene, $\bar{d}_p$ = 0.0134 cm, and (b) 8.2 mole-% divinylbenzene, $\bar{d}_p$ 0.0169 cm. (⊡) Benzene; (▽) *m*-bitolyl, II; (△) quaterphenyl, III; (⊙) octiphenyl, VI.

the substances if the molecular weight of the solute approaches the excluded molecular weight, in other words if the elution volume is close to the void volume of the column. As shown in Table II this is not the case. The ratio of the velocity of the zone and the mobile phase, known as the relative zone velocity R, may also be expressed as V_0/V_e. In case of gels 19, 29, 46, 52 the reduced separation efficiency is independent of the substances, whereas the other gels show this influence quite clearly. See for example, the independent behaviour of gel 19 (up to $R = 0.827$) compared with the pronounced dependence of gel 32 (from $R = 0.55$ onwards) on the molecular size of the oligophenylenes used.

Using fractions of the gel with small particle diameter it is possible to determine the reduced separation efficiency in the range of its minimum value (Fig. 10). The

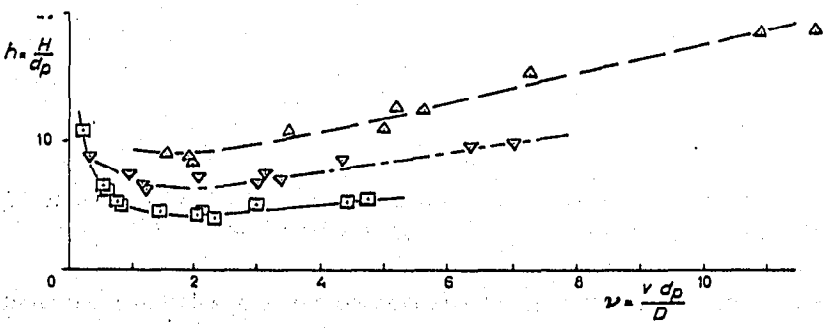


Fig. 10. Reduced separation efficiency of a poly(styrene) gel crosslinked with 8.2 mole-% divinylbenzene, $\bar{d}_p$ = 0.00237 cm. (⊡) Benzene; (▽) *m*-bitolyl, II; (△) quaterphenyl, III.

difference in the slope of the curve in Fig. 9b and Fig. 10 indicates a more irregular packing of the smaller particles.

Nonequilibrium conditions

If the crosslinking density of gels is very high some of the solutes used in our investigations are completely excluded from the stationary phase and even the substances with the lowest molecular weight (benzene) have an elution volume V_e close to the void volume V_0. As high crosslinking densities have a pronounced effect on the permeation coefficient of the substances which can enter into a gel, a steep slope for the reduced HETP is to be expected. Moreover the substances excluded completely from the gel should give a constant value for the reduced HETP at varying flow rates.

Eluting benzene on a column filled with poly(styrene) gel crosslinked with 34 mole-% divinyl adipate we find that the elution volume is dependent on the elution velocity as well. As shown in Fig. 11 the shape of the curves agrees well with the

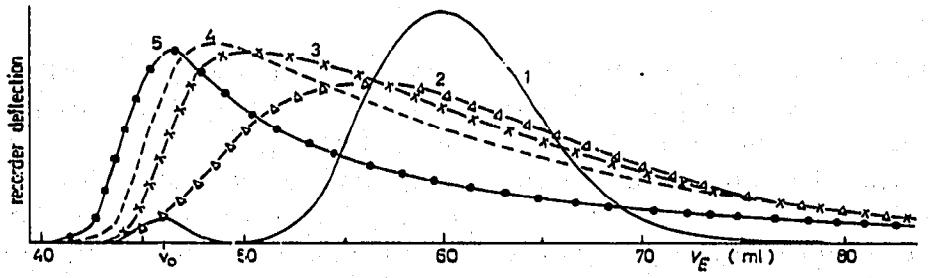


Fig. 11. Elution diagram of benzene on a poly(vinyl acetate) gel crosslinked with 34 mole-% divinyl adipate ($\bar{d}_p$ = 0.0134 cm) for different reduced flow rates. (1) ν = 2.26; (2) ν = 14.9; (3) ν = 26.4; (4) ν = 34.3, (5) ν = 57.2.

predictions of VINK[15] and LAURENT AND LAURENT[16] for a departure from equilibrium conditions. With increasing flow rate, the maximum is moving towards V_0 and the curves are broad and unsymmetrical. The same behaviour was found with other gels and substances (Table II, marked by → 1). This phenomenon observed can be explained in terms of a kinetically controlled gel chromatographic process. The flow of

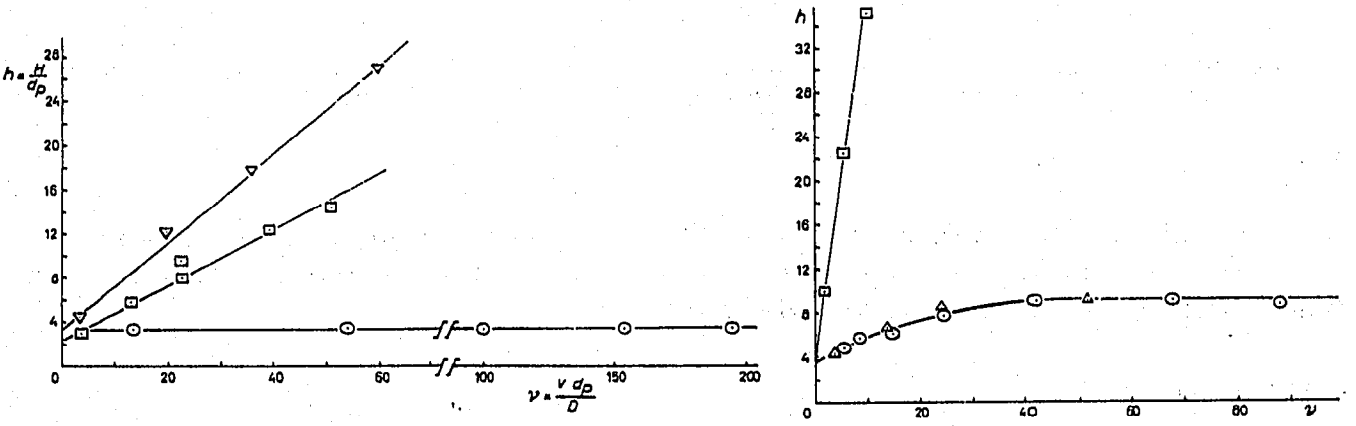


Fig. 12. Reduced separation efficiency of a poly(vinyl acetate) gel crosslinked with 8.8 mole-% divinyl adipate, $\bar{d}_p$ = 0.0218 cm. (⊡) Benzene; (▽) *m*-bitolyl, II; (⊙) octiphenyl, VI.

Fig. 13. Reduced separation efficiency of a poly(styrene) gel crosslinked with 25.5 mole-% divinylbenzene, $\bar{d}_p$ = 0.0099. (⊡) Benzene; (△) quaterphenyl, III; (⊙) octiphenyl, VI.

the solvent becomes so fast that the exchange process between stationary and mobile phase is no longer established.

Substances which do not enter the gel show a reduced separation efficiency which may be described by the VAN DEEMTER equation (Fig. 12) or by the GIDDINGS equation (Fig. 13).

The independence of the reduced HETP on the elution velocity over the whole experimental range (Fig. 12) demonstrates that it is possible to produce nonequilibrium conditions in the mobile phase ($1/C_{mt}$ in eqn. 2) that are negligible. If the column shows nonequilibrium conditions, their contribution is not additive with those of the irregularities of the packing (the a-term in eqn. (1) but complementary as predicted by eqn. (2). As the summation term in eqn. (2) involves only the diffusion coefficient in the mobile phase, the reduced separation efficiency should be independent of the substances in these cases. This assumption was experimentally confirmed (Fig. 13).

Macroporous gels

Since the introduction of the macroporous gels as stationary phases by MOORE[17], their most important application is in the gel chromatography of polymers. From the results given it is obvious that the crosslinking density of the polymer network must be sufficiently high so that the entrance of even low molecular weight substances is prevented. In this case the exchange process operates between the solvent of the mobile phase and the solvent in the pores only. The diffusion coefficient in the mobile and the stationary phase should be the same.

Fig. 14 shows the reduced separation efficiency for a macroporous poly(vinyl acetate) gel copolymerized with 22.5 mole-% of divinyl adipate and in the presence of 66 vol-% n-heptanol. The resulting straight line is independent of the substances and agrees quantitatively with the line in Figs. 6 and 7.

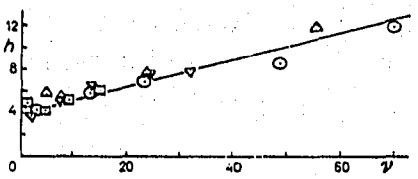


Fig. 14. Reduced separation efficiency of a macroporous poly(vinyl acetate) gel, $\bar{a}_p$ = 0.00862 cm. (□) Benzene; (▽) m-bitolyl, II; (△) quaterphenyl, III; (⊙) octiphenyl, VI.

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