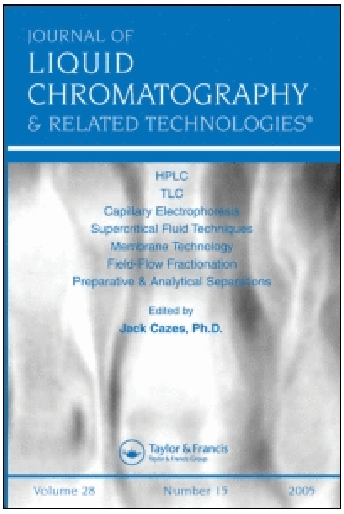

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of Pressure on Preferential Sorption within Column Packing: Possible Explanation of some Unexpected Results in GPC with mixed Eluents

T. Macko[a]; M. Chalányova[b]; D. Berek[a]
[a] Polymer Institute Center of Chemical Research Slovak Academy of Sciences, Bratislava, Czechoslovakia [b] Chemical Institute Comenius University, Brataslava, Czechoslovakia

To cite this Article Macko, T. , Chalányova, M. and Berek, D.(1986) 'Effect of Pressure on Preferential Sorption within Column Packing: Possible Explanation of some Unexpected Results in GPC with mixed Eluents', Journal of Liquid Chromatography & Related Technologies, 9: 6, 1123 — 1140

To link to this Article: DOI: 10.1080/01483918608075495

URL: http://dx.doi.org/10.1080/01483918608075495

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(6), 1123-1140 (1986)

EFFECT OF PRESSURE ON PREFERENTIAL SORPTION WITHIN COLUMN PACKING: POSSIBLE EXPLANATION OF SOME UNEXPECTED RESULTS IN GPC WITH MIXED ELUENTS

T. Macko[1], M. Chalányova[2], and D. Berek[1]
[1]Polymer Institute
Center of Chemical Research
Slovak Academy of Sciences
84236 Bratislava, Czechoslovakia
[2]Chemical Institute
Comenius University
84215 Brataslava, Czechoslovakia

ABSTRACT

It is shown that pressure affects the extent of preferential sorption of mixed eluent components on the surface of GPC column packing. In some systems, this may result in base line perturbations and possibly also in changes of size, shape and position of sample peaks when pressure is changed within column either due to variation in the flow rate or as a result of partial blocking of column end fittings and connecting capillaries. The described phenomenon could lead to erroneus GPC results. On the other hand, it may help to explain some unexpected and artificial results obtained in GPC separations with mixed eluents.

0148-3919/86/0906-1123$3.50/0

INTRODUCTION

For several reasons, two- and multicomponent solvents are systematically applied as eluents in gel permeation chromatography (GPC) (1). Mixed eluents are used often used also unwantedly if the solvents contain impurities such as water in tetrahydrofuran (2,3). Various secondary effects may accompany GPC with mixed eluents. Typically, the elution volumes are shifted due to both the changes in dimensions of macromolecules and the preferential sorption of one eluent component on the active column packing surface (3,4). Simultaneously, extra peaks (vacancy or solvent peaks) appear on chromatograms at high elution volumes brought about by preferential solvation of sample molecules in mixed eluents and by displacement effects (1,3,5,6).

In this paper, we discuss other group of apparent artifacts in GPC with mixed eluents, namely the base line perturbations and changes in sample peak characteristics induced by pressure (and flow rate) variations in chromatographic system.

EXPERIMENTAL

The assembly according to Fig. 1 was composed of a pump (Model FR-30, Knauer, Bad Homburg, FRG),

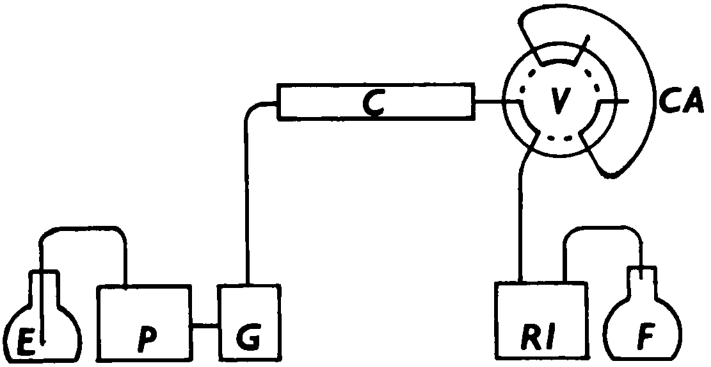

FIGURE 1 Scheme of the LC assembly: E = eluent container; P = pump; G = pressure gauge; C = column; V = valve in position a (———) and b (– – –); CA = capillary; RI = detector; F = vaste.

a pressure gauge with the overpressure limitation (Institute for Chemical Processes Fundamentals, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) stainless steel column 250 x 0.6 or 100 x 0.6 mm I. D. (Laboratory Instruments, Prague, Czechoslovakia), 6-port 3-way valve (Spectra-Physics, Santa Clara, CA, USA) and refractometric detector (Model RIDK 101, Laboratory Instruments, Prague, Czechoslovakia). When switching the valve V from position a to b, capillary CA (50 m x 0.25 mm I. D.) was inserted between the column and detector.

The column was packed alternatively either by silica gel CH-70 or by octadecyl silica gel Silipor C_{18} (both Lachema, Brno, Czechoslovakia) or by silica gel

Separon SI VSK (Laboratory Instruments, Prague, Czechoslovakia) or by silica gel Silpearl (Glass Works Kavalier, Votice, Czechoslovakia).

Eluent was either the azeotropic mixture benzene/methanol prepared by distillation at atmospheric pressure or mixtures tetrahydrofuran (THF)/water, chloroform/tetrachloromethane and 2-propanol/1,2-dichloroethane with various compositions, prepared by mixing single components. THF was purified according to (2). Water was treated with $KMnO_4$ and twice distilled from glass. Other solvents were used without purification. All organic solvents of analytical grade were purchased from Lachema, Brno, Czechoslovakia.

The experiments were done at room temperature.

RESULTS AND DISCUSSION

Base Line Perturbations

In the majority of GPC measurements, differential refractometer is used as detector. Inherently, its response depends on temperature and pressure (7, 8). In other words, any sudden change of temperature, pressure or elution rate in the GPC system produces unavoidably some base line perturbation. The GPC system containing mixed eluents are, however, sometimes extremely sensitive to small changes in operational parameters and an additional explanation must be looked for, e. g. the pressure dependence of preferential sorption of eluent components on the column packing surface (9 - 12).

We tested the influence of pressure variations on the base line stability in several packing - eluent systems by means of the assembly described in the experimental part. When valve V is switched, pressure is suddenly changed within column C; however, pressure within the measuring cell of differential refractometer remains constant and the elution rate variations are very small if the pumping system works precisely enough and the short volume pulse due to the compressibility of eluents can be neglected.

The typical examples of detector responses to the switching valve V are shown in Figs. 2 - 6, 8. The change of pressure within the column produces a pronounced base line perturbation of typical shape. The process is fairly reversible in almost all systems studied. This means that if an increase in pressure brings about a perturbation with positive sign of the detector response, the following pressure drop due to switching the valve V from position b to a results in the mirror image perturbation with the negative sign (Figs. 2 - 5, 8). Simultaneously, the process is fully repeatable in most of the systems studied, i. e. the

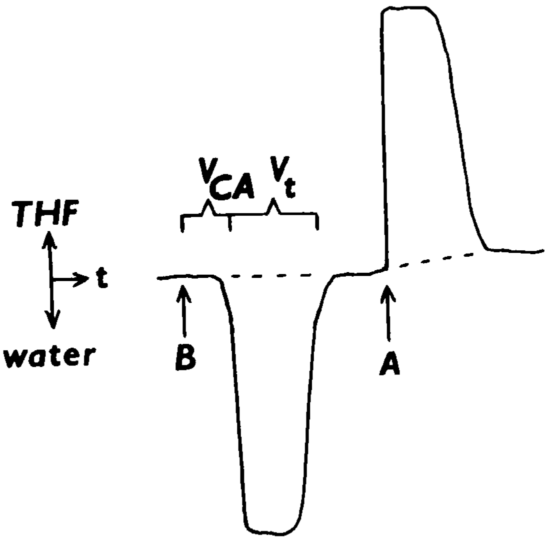

FIGURE 2 Example of the base line perturbations.
Column: Silica gel CH-70, 70-200 µm, 25x0.6 cm I.D.
Eluent: THF/water, 80/20, v/v.
Pressure change: 10.4 MPa.
Elution rate: 1.1 ml/min.
Detector sensitivity: 32x.
Symbols: A - In this moment valve V was switched from position b to a.
B - Valve V was switched from position a to b.
t - Elution time.
V_t- Volume of liquid within column C.
V_{CA} - Volume of capillary CA.
The sign of the detector response for the excess of particular eluent components is shown in the figure.

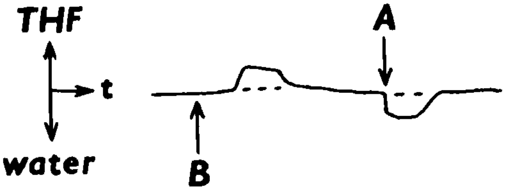

FIGURE 3 Example of the base line preturbations.
Column: Silipor C_{18}, 125-150 µm, 25x0.6 cm I.D.
Eluent: THF/water, 80/20, v/v.
Pressure change: 10.2 MPa.
Elution rate: 1.4 ml/min.
Detector sensitivity: 32x.
Symbols as in Fig. 2.

FIGURE 4 Example of the **base line** perturbations.
Column: Silica gel CH-70, 70-200 μm, 25x0.6 cm I.D.
Eluent: Benzene/methanol, azeotropic mixture.
Pressure change: 5.3 MPa.
Elution rate: 1.0 ml/min.
Detector sensitivity: 32x.
Symbols as in Fig. 2.

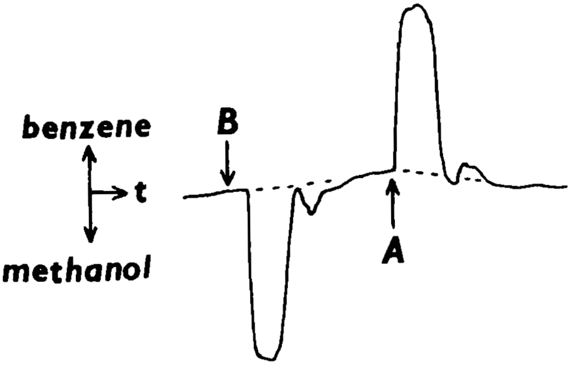

FIGURE 5 Example of the base line perturbations.
Column: Silipor C_{18}, 125-150 μm, 25x0.6 cm I.D.
Eluent: Benzene/methanol, azeotropic mixture.
Pressure change: 12.3 MPa.
Elution rate: 2.1 ml/min.
Detector sensitivity: 32x.
Symbols as in Fig. 2.

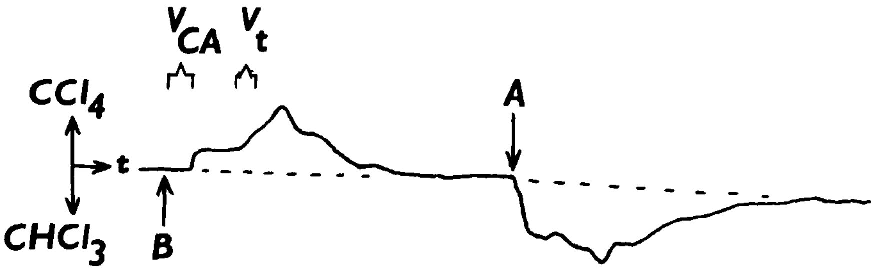

FIGURE 6 Example of the base line perturbations.
Column: Silpearl, 6 µm, 10x0.6 cm I.D.
Eluent: Chloroform/tetrachloromethane, 4.5/95.5, v/v.
Pressure change: 5.5 MPa.
Elution rate: 1.0 ml/min.
Detector sensitivity: 32x.
Symbols as in Fig. 2.

multiple switching the valve V produced the same results. The base line perturbations appear in the form of zones with widths corresponding to the total volume of liquid within column V_t. The only exception from the above rules present mixed eluents $CHCl_3$ plus CCl_4, where the reversibility and repeatability of base line perturbations were not perfect. Simultaneously, in this case the width of zones depended on the relative concentration of eluent components being often larger than V_t (Fig. 6).

If pressure is increased in the system i. e. if capillary CA is inserted into the eluent path, the base line perturbations appear with a delay corresponding to the volume of capillary CA (cf. Figs. 2 - 8).

The base line perturbations did not generally appear if the column was substituted by another capillary with similar flow resistance as column. The base line perturbations were either very small or not observed at all if single liquids were pumped through columns under otherwise identical experimental conditions.

We can conclude that the observed base line perturbations are caused exclusively by the processes taking place within the column and indicate the local changes in the composition of mixed eluent. That is why we call them "eigenzones" (10).

The changes of pressure in a column may also be generated by changing the eluent flow rate. An example of eigenzones produced in this way is shown in Fig. 7. Of course, the shape of eigenzones is in this case more or less influenced also by changing the flow rate within the measuring cell of the detector.

The explanation of the above results is as follows: The binary interactions in the multicomponent systems are usually asymetrical. This results in the well-known phenomena like preferential solvation of solute molecules in mixed solvents or adsorption of solute molecules from dilute solutions and preferential sorption of one componentof mixed liquids on the solid surface. The extent of preferential sorption depends

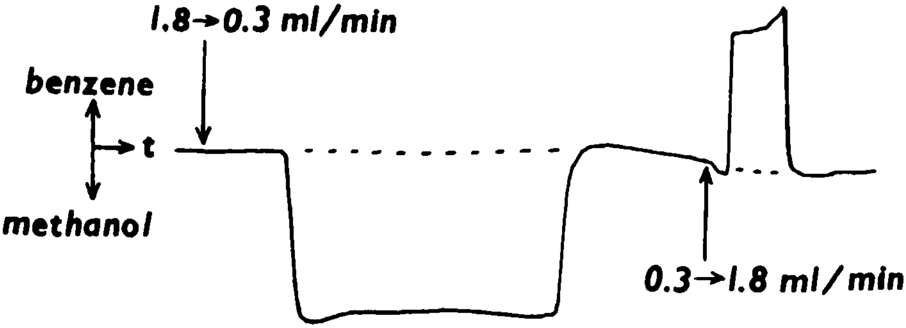

FIGURE 7 Example of the eigenzones generated by sudden change of eluent flow rate.
Column: Separon SI VSK, 8-12 μm, 25x0.6 cm I.D.
Eluent: Benzene/methanol, azeotropic mixture.
Pressure change: 9.3 MPa.
Elution rate: 0.3 ml/min or 1.8 ml/min.
Detector sensitivity: 32x.
Symbols as in Fig. 2.

on temperature and, as has been found recently, also on pressure (9 - 12). Any change of temperature or pressure within GPC column flushed with mixed eluent may thus cause a change of the composition of the liquid sorbed in the domain of column packing, and, consequently, also the change in the composition of the mobile phase in the interstitial volume. The latter is observed in the column effluent by means of differential refractometer as a base line perturbation, eigenzone.

This hypothesis also may be applied to the observations published by Aubert and Tirrell (13,14) and by Basedow et al.(15):

Aubert and Tirrell (13,14) reported the appearance of base line perturbations, when the flow rate of polystyrene (PS) solution in THF pumped through silanized silica gel packings suddenly changed. They observed the base line perturbations with a UV photometer. The sign of perturbations depended on the sign of the elution rate changes: The perturbations were positive if elution rate decreased while the negative detector response was observed if elution rate increased. The height of base line perturbations depended on the pore size of silica gel as well as on the relative molecular mass of PS (M). The width of base line perturbations, i. e. the amount of eluent necessary to reattain the initial base line, was for a given column independent of M and corresponded approximately to the volume of eluent within column.

Basedow et al.(15) found a similar effect as a consequence of stepwise flow rate changes of aqueous solutions of sucrose or dextran ($\overline{M}_w$= 38 000 g/mol) pumped through a column packed either by nonporous glass or by porous glass sorbent CPG. The width of base line perturbations called "storage effect" was roughly equal to the volume of the liquid in column.

In our opinion, the results by both Aubert and Tirrell (13,14) and Basedow et al.(15) can also be explained by the effect of pressure on the extent of preferential sorption in particular system. The data by Aubert and Tirrell indicate that an increase of pressure due to increased flow rate leads to a decrease of the amount of PS adsorbed from THF on silica gel while in the case of the system studied by Basedow et al. the amount of sucrose or dextran adsorbed on the surface of porous glass from water increased with rising pressure. In the system studied by Aubert and Tirrell (13,14), however, the observed effects may be influenced by possible presence of water in eluent (2,3). In the above experiments, the pressure changes may act either directly via their influence on distribution coefficients of solutes or indirectly via induced temperature variations (10).

Elution Volume Changes

It is now generally accepted that in the real GPC systems, the elution volumes of polymers can be affected by various secondary separation mechanism (1). To the most important secondary separation mechanism belong adsorption, partition and incompability controlled by the interactions between solute, eluent and gel. When working with active column packings such as silica gel and with mixed eluents, the retention of solute molecules is fairly influenced by the composition of the quasistationary surface layer of eluent molecules on the inner surface of column packing even if the steric mechanism prevails (4).

Belenkii et al. (16) showed that even small changes in mixed eluent composition may lead to the total change of separation mechanism from size exclusion to adsorption mode. In other words, the extent of preferential sorption of the column packing may govern the retention mechanism.

Recently, Nefedov and Zhmakina (17) found that the change of pressure in the system: porous glass - mixed eluent (CCl_4 plus 4.5 vol. % $CHCl_3$) strongly influenced elution volumes of polystyrenes. The adsorption mechanism of separation prevailed at atmospheric pressure and the elution volumes of polystyrenes increased with their increasing relative molecular mass. On the other hand, separation according to the relative molecular mass was observed in the same column system at pressure 12.5 bar and, finally, typical size exclusion mechanism dominated at 200 bar. Nefedov and Zhmakina (17) reported that the resulting separation mechanism in the above system was extremely sensitive to the composition of the mixed eluent CCl_4 plus $CHCl_3$: When increasing the amount of $CHCl_3$ from 4.5 to 6 vol. %, the separation mechanism changed from adsorption to size exclusion. Unfortunately, this mixed eluent is difficult to study precisely because of poor stability of chloroform and, consequently, of relative high content of impurities. Moreover, $CHCl_3$ is strongly adsorbed at the silica gel surface so that reasonable data can be obtained only when the amount of $CHCl_3$ in eluent is increased and not vice versa (18).

Evidently, chloroform is preferentially sorbed on the silica gel surface from mixed eluent $CHCl_3$ plus CCl_4. Chloroform molecules are able to block active sites on silica gel surface and thus prevent adsorption and extensive retention of polystyrene molecules. This shielding effect is not sufficient at low concetration of $CHCl_3$ in eluent. It seems that the extent of preferential sorption of chloroform increases with increasing pressure (Fig. 6). That is why the exclusion mechanism starts to prevail at certain elevated pressure.

Swenson et al. (19) found the pronounced variations of elution volumes of polystyrenes with elution flow rate in the system silica gel / 2-propanol plus 1,2-dichloroethane. They controlled the flow rate by partly closing the column outlet at constant inlet pressure so that at higher flow rate they had a little lower mean pressure within column. According to our results (Fig. 8), the preferential sorption of 2-propanol on the silica gel surface increases with increasing pressure, consequently, the decreased flow

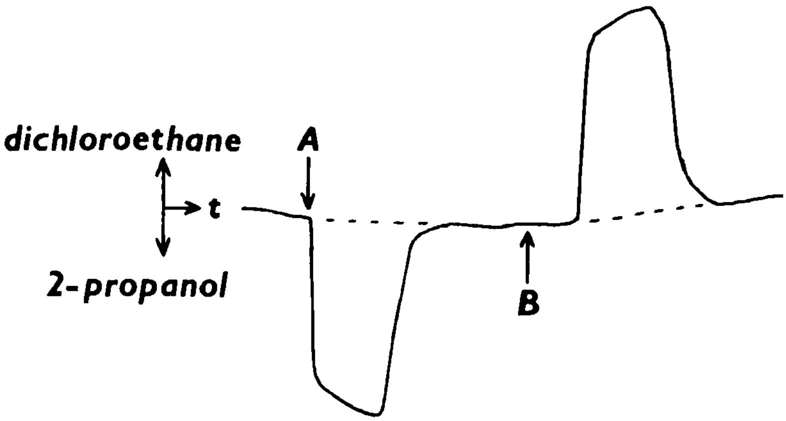

FIGURE 8 Example of the eigenzones.
Column: Silica gel CH-70, 70-200 μm, 25x0.6 cm I.D.
Eluent: 2-propanol/1,2-dichloroethane, 40/60, v/v.
Pressure change: 8.2 MPa.
Elution rate: 1.2 ml/min.
Detector sensitivity: 8x.
Symbols as in Fig. 2.

rate would correspond to decreased surface concentration of 2-propanol. The presence of 2-propanol, however , was reported by Swenson et al. (19) to block the active sites of silica gel and thus to decrease the corresponding elution volumes of polystyrene samples. The flow rate dependence of elution volumes in the system studied by Swenson et al. (19) could also be explained by the effect of pressure on the extent of preferential sorption.

CONCLUSION

Pressure dependence of preferential sorption of mixed eluent components on the surface of GPC column packings may be responsible for the unexpected base line perturbations when the elution rate is changed or outlet of a GPC column is partly blocked. Variations of GPC elution volumes due to varying adsorption of solute within column packing may simultaneously be produced.

The pressure dependence of preferential solvation of solute molecules has not been reported so far but this phenomenon may be expected. The dimensions of macromolecules in mixed solvents would consequently depend on pressure and their GPC elution volumes would be affected. Simultaneously, the size of vacancy peaks caused by displacement and preferential solvation of sample molecules should also vary with pressure within GPC columns. If the column packing does not separate selectively enough in the domain of low molecular substances, the vacancy peaks interfere with the sample peaks (1,20). The extent of this interference may be a function of pressure. Finally, the values of the coefficient of preferential solvation of macromolecules evaluated from GPC vacancy peaks (5) may vary with pressure in the column.

The above mentioned effects of pressure variations will certainly be very small and can be neglected in the majority of practical systems. On the other hand, they should be considered when explaining unexpected and erroneous GPC results if increased pressure sensitivity of the given system is observed. In these cases it is not sufficient to keep flow rate - but not pressure - constant or to correct the GPC elution volumes for flow variations.

REFERENCES

1. Berek, D., Kubín, M., Marcinka, M. and Dressler, M., Gélová chromatografia (Gel Chromatography), Veda Publishing House, Bratislava, 1983, (in Slovak).
2. Spychaj, T., Lath, D. and Berek, D., Polymer, 20, 437, 1979.
3. Spychaj, T. and Berek, D., Polymer, 20, 1108, 1979.
4. Berek, D., Bakoš, D., Bleha, T. and Šoltés, L., Makromol. Chem., 176, 391, 1975.
5. Berek, D., Bleha, T. and Pevná, Z., J. Polym. Sci. Polym. Lett. Ed., 14, 323, 1976.
6. Berek, D., Bleha, T. and Pevná, Z., J. Chromatogr. Sci., 14, 560, 1976.
7. Ioffe, B..V., Refraktometrické metódy v chemii (Refractometric Methods in Chemistry), SNTL, Praha, 1983, (in Czech).
8. Mori, S., J. Liq. Chromatogr., 6, 813, 1983.
9. Berek, D., Chalányová, M. and Macko, T., J. Chromatogr., 286, 185, 1984.
10. Chalányová, M., Macko, T., Kandráč, J. and Berek, D., Chromatographia, 18, 668, 1984.
11. Macko, T., Berek, D. and Chalányová, M., Physical Optics of Dynamic Phenomena and Processes in Macromolecular Systems, Sedláček, B., eds., Walter de Gruyter and Co, Berlin - New York, 1985, p. 521.

12. Ozawa, S., Kawahara, K., Yamabe, M., Unno, H. and Ogino, Y., J. Chem. Soc., Faraday Trans. 1, **80**, 1059, 1984.

13. Aubert, J. H. and Tirrell, M., Rheol. Acta, **19**, 452, 1980.

14. Aubert, J. H. and Tirrell, M., J. Liq. Chromatogr., **6**, 219, 1983.

15. Basedow, A. M., Ebert, K. H., Ederer, H. J. and Fosshag, E., J. Chromatogr., **192**, 259, 1980.

16. Belenkii, B. G., Gankina, E. S., Tennikov, M. B. and Vilenchik, L. Z., J. Chromatogr., **147**, 99, 1978.

17. Nefedov, P. P. and Zhmakina, T. P., Vysokomol. Sojed. A, **23**, 276, 1981.

18. Nefedov, P. P. (personal communication).

19. Swenson, H. A., Kaustinen, H. M. and Almin, K. E., J. Polym. Sci. Polym. Lett. Ed., **9**, 261, 1971.

20. Berek, D., Katuščáková, G. and Novák, I., Brit. Polym. J., **9**, 62, 1977.