

Short Communication

Reversed-phase separations of high-molecular-weight polystyrenes in a dichloromethane–acetonitrile solvent system

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ABSTRACT

High-resolution separation of polystyrenes ranging from monomer and oligomers to the high-molecular-weight polymers (> 900 000 dalton) has been achieved in a single gradient separation on a variety of small-pore-size reversed-phase columns. Separation of the high-molecular-weight polymers in the acetonitrile–dichloromethane solvent system appeared to be based on retentive interactions with the stationary phase, despite the fact that the polymers were nominally too large to enter the pores of the small pore size column.

INTRODUCTION

In a previous publication [1] we examined the retention behaviour of polystyrenes in a methanol–dichloromethane solvent system on C_{18} reversed-phase columns of various pore sizes. Elution of the polystyrenes generally obeyed the principles of adsorption chromatography except when the polymer was excluded from the pores, at which point the elution was complicated by additional mechanisms which resulted in the polymer eluting at the polymer solubility composition according to the precipitation redissolution process, or prior to the polymer solubility composition by a process we have previously described as pre-elution. The process of pre-elution contributed to band broadening and the formation of multiple peaks of the higher-molecular-weight monodisperse polystyrenes on a 120-Å column and a 300-Å column. These effects resulted in virtually zero selectivity and limited the separation of molecular weights. In this methanol–dichloromethane solvent system, elution of the polystyrenes was also dependent upon the mass load and the flow-rate pre-elution being dominant at low mass loads and high flow-rates.

In the present study we report the separations of polystyrenes of various molecular weights in a solvent system of acetonitrile and dichloromethane. By selection of gradient shape it is possible to achieve single-column separation of polystyrenes from monomer through to molecular weights of 10^6 .

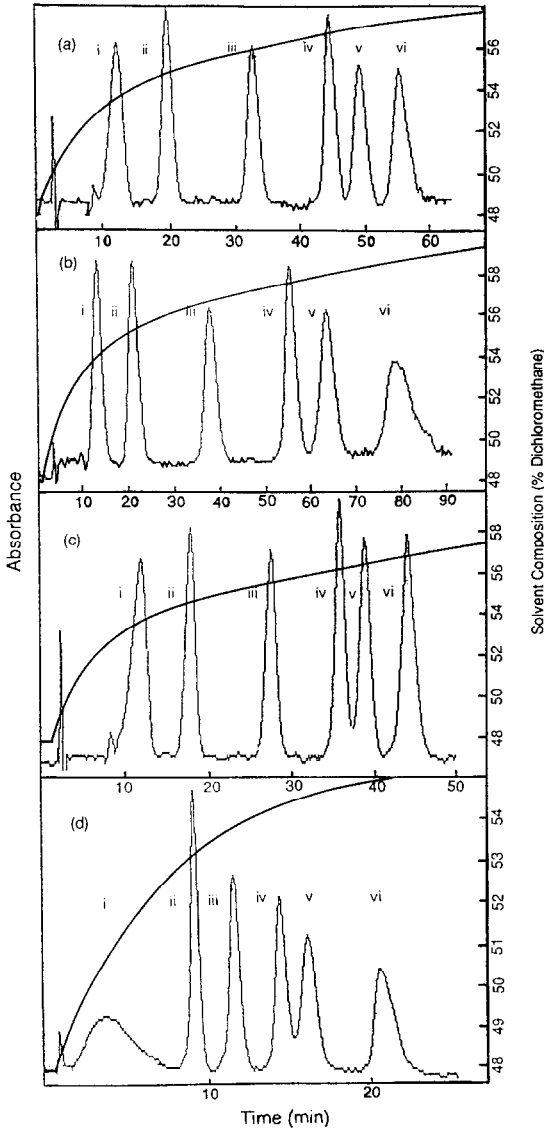


Fig. 1. Separation of polystyrenes on various columns. (a) μ Bondapak, (b) Serva, (c) Hibar 25 cm, (d) Hibar 12.5 cm. Molecular weights: i = 17 500; ii = 50 000; iii = 110 000; iv = 200 000; v = 410 000; vi = 929 000 dalton. Elution conditions: dichloromethane-acetonitrile (48:52); dichloromethane-acetonitrile (64:36); gradient run time 5 h on curve 3; flow-rate 1 ml/min.

EXPERIMENTAL

Equipment

All chromatographic experiments were performed using two M6000A pumps, a 660 solvent programmer and U6K injector (Waters Assoc., Milford, MA, USA). The detector used was variable-wavelength UV-VIS 200 set at 262 nm (Activon Scientific Products, Thornleigh, Australia). Data acquisition and analysis was done with a laboratory-built system. The columns used were a μ Bondapak C₁₈, 30 cm \times 3.9 mm I.D., nominal pore size 120 Å, carbon load 10% (Waters Assoc), a Serva 300 Å C₁₈, 5 μ m particle size, 25 cm \times 4.6 mm I.D., nominal pore size 300 Å, carbon load 11.6% (Serva, Heidelberg, Germany), and two Hibar C₁₈ cartridge columns, of 25 cm and 12.5 cm column length, 4.0 mm I.D., 10 μ m particle size, nominal pore size 60 Å (Merck). Column temperature was maintained at 25.0°C in a thermostatted water-jacket. The solvent composition at the peak maxima was calculated using the equation representing the gradient profile [2] and these values corresponded to the values obtained from gradient profiles.

Chemicals and reagents

Acetonitrile and dichloromethane (HPCL grade) were obtained from Mallinckrodt Australia. The monodisperse polystyrene standards used were molecular weights 3600, 110 000, 200 000, 410 000 and 929 000 dalton (Waters Assoc.), and 17 500 and 50 000 dalton (Polysciences, Warrington, PA, USA).

RESULTS AND DISCUSSION

Gradient elution of high-molecular-weight polystyrenes on small-pore-size columns in a methanol-dichloromethane solvent system generally produced peaks which had severe tailing or formed into multiple peaks [1]. In contrast, similar experiments in a dichloromethane-acetonitrile solvent system produced gaussian like peaks. The initial separations were obtained on the same columns which were used for the previous work [1], and Hibar cartridge columns gave similar results. Non-linear gradients gave best separations, beginning with an initial mobile phase composition of dichloromethane-acetonitrile (48:52) followed by convex gradient elution (NO. 3 on the Waters 660 solvent programmer) to a final mobile phase composition of dichloromethane-acetonitrile (64:36) using a gradient run time of 5 h. Elution of the highest-molecular-weight polystyrene occurred well within this time. Fig. 1 illustrates the separations on the various columns. It is obvious that these separations compare very favourably to size-exclusion chromatography [3-6] and field-flow fractionation [7,8]. Separation of broad-range polystyrenes also compared favourably with size-exclusion methods.

The optimal flow-rate for separations was found to be within range of 1-2 ml/min, although this may be an effect of the gradient function. High flow-rates (5 ml/min) had little effect on the resolution and elution of the highest-molecular-weight polystyrene was obtained within 16 min (Fig. 2).

The mobile phase elution composition φ_e , of all the polystyrenes was greater than that of the polymer solubility composition, φ_s (Table I). This is in strong contrast with the methanol-dichloromethane solvent system used in the previous paper

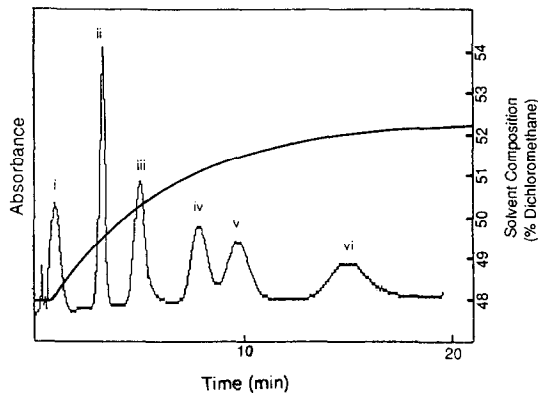


Fig. 2. Separation of polystyrenes on a Hibar 12.5 cm columns at a flow-rate of 5.0 ml/min. Elution order and conditions as in Fig. 1.

[1]. With the current solvent system it appears that polymer-stationary phase interactions were likely to be occurring even for the highest molecular weights. As the higher-molecular-weight polystyrenes would be excluded from the pores in all four columns in a good solvent such as dichloromethane, such interactions would only be expected if (a) the surface area outside the pores was sufficient for adsorption interactions to retain the polymer, (b) the polymer underwent solvent induced configurational changes, or (c) disentanglement of polymer chains allowed retention by "snaking" into the pores. The latter mechanism was proposed in detail by Larman *et al.* [9] and was referred to as the "softball" model. Lochmüller and McGranaghan [10] have demonstrated that adsorption of the higher-molecular-weight polystyrenes is possible on small-pore-size columns as illustrated by the isocratic elution of these polystyrenes.

TABLE I

ELUTION COMPOSITION, φ_e (% DICHLOROMETHANE), OF THE POLYSTERENES ON THE μ BONDAPAK, SERVA AND HIBAR 25 cm COLUMNS

φ_i (initial mobile phase composition): dichloromethane-acetonitrile (48:52); φ_f (final mobile phase composition): dichloromethane-acetonitrile (64:36); gradient curve 3, run time = 5 h; flow-rate = 1.0 ml/min. Polymer solubility composition φ_s , is also tabled.

Molecular weight	φ_e (%)			φ_s (%)
	μ Bondapak	Serva	Hibar	
929 000	56.98	58.13	56.17	53.0
410 000	56.62	57.40	55.83	51.0
200 000	56.31	56.96	55.63	48.5
110 000	55.38	55.71	54.90	46.5
50 000	53.88	53.97	53.71	39.5
17 500	52.43	52.47	52.45	28.5

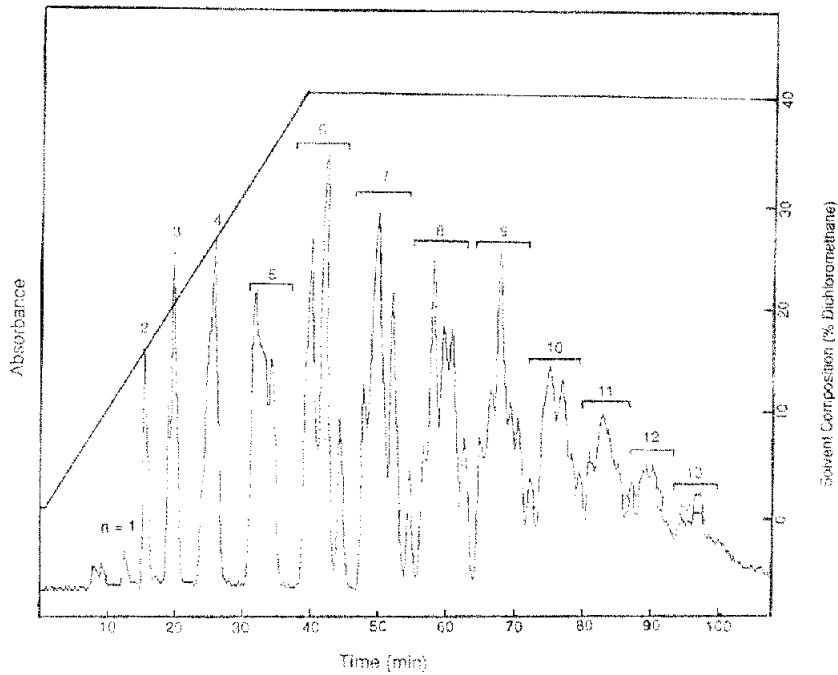


Fig. 3. Separation of oligomers of polystyrene molecular weight 800 on a Hibar 25 cm column. ϕ_1 : 100% acetonitrile. ϕ_2 : dichloromethane-acetonitrile (40:60); linear gradient at 1%/min; flow-rate 1 ml/min

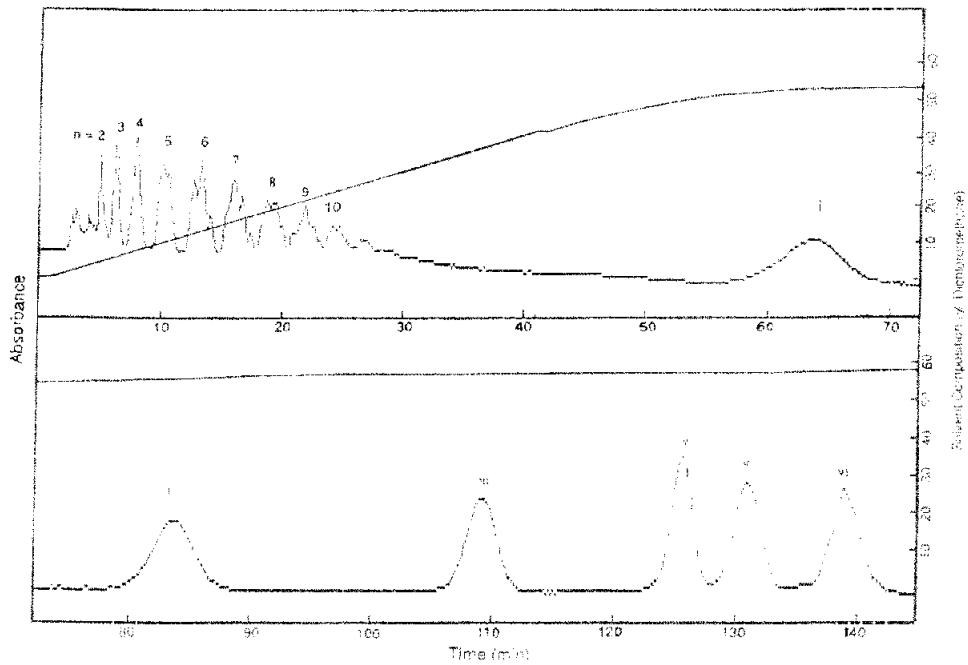


Fig. 4. Separation of polystyrene oligomers through to molecular weight 929 000. ϕ_1 : 100% acetonitrile running to dichloromethane-acetonitrile (40:60) linear gradient at 1%/min followed by a 1-min hold then a convex gradient (curve 3) to a final mobile phase composition of dichloromethane-acetonitrile (66:34) in 6 h. Flow-rate was 1.0 ml/min. Sample concentration was 4 mg/ml for the oligomer 800 and 1 mg/ml for the molecular weights greater than 17 500. Injection volume 8 μ l. Elution order as described in Fig. 1.

Studies showing the oligomeric separations of polystyrenes in various solvent systems were reported in detail by Lewis *et al.* [11]; oligomeric stereoisomers were shown to be separated in an acetonitrile–dichloromethane solvent system but not in a methanol solvent system. Similiar stereoisomer separations have also been achieved in the present study (Fig. 3). The stereoisomers were verified by mass spectroscopic examination of collected fractions. We were then able to combine the separation of the higher-molecular-weight polystyrenes with oligomeric separations in the same analysis. The final separation ranged from styrene monomer up to the 929 000-dalton polystyrene (Fig. 4). The gradient shape is somewhat complicated, although it would still be adaptable to any computer controlled system on a routine basis.

In the dichloromethane–acetonitrile solvent system the mass load of the polymer was not important. No change in the retention behaviour was observed for sample loads from 0.5 to 50 μg , in complete contrast to results obtained in the dichloromethane–methanol solvent system. This indicates that solvation effects within the polymer are playing an influential role in the dichloromethane–methanol system yet they do not affect the results to the same degree in the current dichloromethane–acetonitrile system.

As expected, injection in large volumes (greater than 40 μl of strong solvent, dichloromethane) gave poor results. Peaks size decreased as the injection volume increased. This was probably due to polymer eluting within the solvent front as described by Glöckner [12] and by Lochmüller and McGranaghan [10]. Studies to determine the mechanism of retention are in progress and will be published at a later date.

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