Diode-array detection with spectral suppression in size exclusion and adsorption chromatography for rapid evaluation of chemical composition in block copolymers of styrene and methyl methacrylate*

Tatiana M. Zimina†‡, Anthony F. Fell‡§ and John B. Castledine‡
† Institute of Macromolecular Compounds, Russian Academy of Sciences, 31 Bol'shoi pr., St Petersburg 199004, Russian Federation
‡ Department of Pharmaceutical Chemistry, University of Bradford, Bradford BD7 1DP, UK
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Diode-array detection has been applied to evaluate the chemical composition of block copolymers from chromatographic data. Two different modes of chromatography have been used for separation: size exclusion chromatography and adsorption chromatography, based on tertiary mixed eluents, comprising tetrahydrofuran, acetonitrile and dichloromethane on a microcolumn, packed with 5 μm wide-pore silica gel (mean pore size 300 Å). U.v. spectral data acquired on-line were manipulated to generate spectral suppression chromatograms in order to selectively detect each component of a block copolymer chromatographed under both size exclusion and critical thermodynamic conditions. The content of polystyrene and poly(methyl methacrylate) residues in a series of model block copolymers of nominally constant composition ($\sim$1:1, w/w), but differing in molecular weight, could be rapidly determined by this novel combination of chromatographic selectivity enhanced by digital discrimination. Thus, diode-array detection has significant potential for fast on-line monitoring of polymer processing.

(Keywords: block copolymers; liquid adsorption chromatography; diode-array detection; microcolumn; size exclusion chromatography; polystyrene; poly(methyl methacrylate))

INTRODUCTION

Evaluation of the chemical composition of block copolymers is an important problem in polymer chemistry[1]. Size exclusion and gradient adsorption liquid chromatography are often used for this purpose together with multichannel detection, including, for example, u.v.-i.r., i.r.-LALLS, i.r.-viscometry and other detection modes[2,3]. The authors have found no reports on the application of u.v. diode-array detection for the analysis of chemical composition in block copolymers. However, this technology is widely used for determining the composition of overlapping chromatographic peaks in other areas[4,5].

The aim of the present work was to evaluate the compositional distribution of block copolymers with respect to the chromatographic elution profiles by diode-array u.v. detection coupled with either size exclusion chromatography (s.e.c) or 'liquid adsorption critical chromatography' (l.a.c.c.)[6,7]. Under s.e.c. conditions block copolymers are eluted according to their size. In the case where a defined adsorption energy (the critical adsorption energy) is involved, block copolymers elute by a mechanism described as chromatography under critical conditions, as predicted theoretically elsewhere[6] and confirmed experimentally in our previous work[7]. The phenomenon of 'critical chromatography' is observed when the adsorption and exclusion processes involved in the retention of macromolecules are equally balanced. This leads to the suppression (in s.e.c. terms) of the retention volume of that component in a block copolymer for which the critical adsorption energy conditions are met—in the case under study this means that the more polar component, poly(methyl methacrylate) (PMMA), is suppressed by l.a.c.c. This technique enables the block copolymer to be chromatographed as if it were a single, adsorptively inert component. Thus its size can be determined directly as an apparently single component under s.e.c. conditions. Full details of l.a.c.c. have been described in earlier publications[6,7].

In the present work, the technique of spectral suppression is utilized to enhance the selectivity of detection of block copolymers separated under s.e.c. and l.a.c.c. conditions.

THEORY

Spectral suppression

If the Beer–Lambert law is obeyed, then for a given solution of a pure compound, X, the absorbance, $A_X(\lambda_1)$, at wavelength λ_1 is directly proportional to that at any other wavelength λ_2:

$$A_X(\lambda_1)=K_X(\lambda_1,\lambda_2)A_X(\lambda_2) \qquad (1)$$

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§ To whom correspondence should be addressed

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where the constant $K_X(\lambda_1, \lambda_2)$ is independent of the solute concentration and thus characterizes the pure compound X at the two wavelengths chosen. Equation (1) may be rearranged, to represent the conditions for suppressing the signal for pure compound X:

$$A_X(\lambda_1) - K_X(\lambda_1, \lambda_2) A_X(\lambda_2) = 0 \quad (2)$$

Hence, if the chromatographic signal detected at λ_2 is multiplied by the constant $K_X(\lambda_1, \lambda_2)$ and then subtracted from that detected at λ_1, the chromatographic peak of the pure compound X is completely suppressed[4,5]. Thus, the baseline of the spectral suppression plot will be flat before, during and after the elution of the pure compound X. Any positive or negative deflection from this baseline would indicate the presence of another compound, Y. Indeed, in general, Y could represent a group of two or more compounds whose spectral properties at λ_1 and λ_2 differ from those of X. The sensitivity of this technique[5] depends, in part, upon the spectral differences between X and Y at λ_1 and λ_2.

The deviation, S, of the spectral suppression plot from zero, due to the presence of compound Y, which elutes simultaneously with X, can be expressed at any given point in the elution profile as follows:

$$S = [A_X(\lambda_1) + A_Y(\lambda_1)] - K_X(\lambda_1, \lambda_2)[A_X(\lambda_2) + A_Y(\lambda_2)] \quad (3)$$

Substituting equation (1) appropriately, yields an expression for the residual signal, S, observed for component Y:

$$S = A_Y(\lambda_1) - K_X(\lambda_1, \lambda_2) A_Y(\lambda_2) \quad (4)$$

Hence, provided that λ_1 and λ_2 are chosen such that $A_Y(\lambda_2) = 0$ and $A_X(\lambda_1)$, $A_X(\lambda_2)$ and $A_Y(\lambda_1)$ represent non-zero absorbance values, then the profile observed after spectral suppression of component X is identical to the chromatographic detector response observed for compound Y at λ_1, i.e.:

$$S = A_Y(\lambda_1) \quad (5)$$

According to the Beer–Lambert law, since Y does not absorb at λ_2:

$$A_X(\lambda_2) = K_X(\lambda_2) C_X \quad (6)$$

and for compound Y at λ_1, incorporating equation (5) into the Beer–Lambert law yields:

$$A_Y(\lambda_1) = K_Y(\lambda_1) C_Y = S \quad (7)$$

where C_X and C_Y are the concentrations of solutes X and Y, and $K_X(\lambda_2)$ and $K_Y(\lambda_1)$ are the proportionality coefficients, respectively.

Clearly, the concentration ratio of Y to X at any point in the chromatographic elution profile is given in general terms by:

$$C_Y/C_X = k[A_Y(\lambda_1)/A_X(\lambda_2)] \quad (8)$$

where $k = K_Y(\lambda_1)/K_X(\lambda_2)$.

However, in this particular case, when $A_Y(\lambda_2) = 0$, then combining equations (6) and (7) gives:

$$C_Y/C_X = k[S/A_X(\lambda_2)] \quad (9)$$

Thus, the ratio of the concentrations of Y to X is directly proportional to the ratio of the spectral suppression plot (at λ_1, λ_2) to that of the chromatographic signal at λ_2. If a plot of this ratio is made, a square-wave graph (i.e. a constant concentration ratio throughout the chromatographic profile) is indicative of a homogeneous distribution of Y to X. Deviations from this square-wave form would show the distribution of Y and X to be heterogeneous, with respect to the chromatographic separation.

The constant, k, can be determined experimentally as the slope of the plot of C_Y/C_X *versus* $S/A_X(\lambda_2)$ for standard mixtures of Y and X. Since, in general, components of the mixtures may have dissimilar elution profiles, then peak areas (rather than peak heights) should be used in this calculation, because such measurements are independent of the chromatographic resolution between Y and X.

EXPERIMENTAL

Samples

Block copolymers of styrene and methyl methacrylate, P(S-MMA), were obtained from Polymer Laboratories (Church Stretton, UK) and their characteristics, based on n.m.r. data from the manufacturer, are presented in *Table 1*. Size exclusion chromatograms of the samples are shown in *Figure 1*. Chromatographic standards of polystyrene (PS) and PMMA were used to examine the pure components of the block copolymers for evaluation of the spectral ratios involved.

Table 1 Characteristics of the samples of poly(styrene-*block*-methyl methacrylate)[a]

Sample no.	Molecular weight	PS fraction (%)
1	14 000	50
2	35 000	50
3	58 500	50
4	107 800	50

[a] According to the manufacturer, based on n.m.r. data

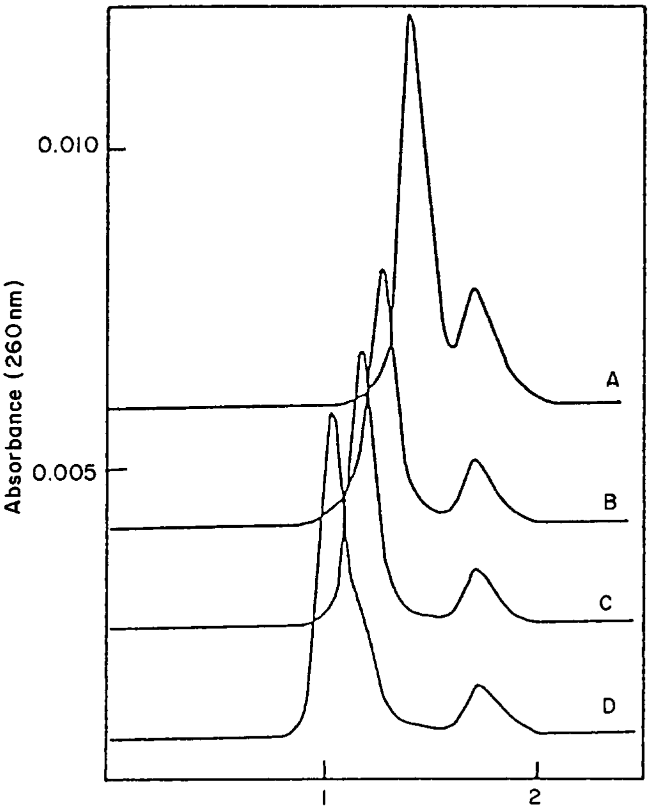

Figure 1 S.e.c. chromatograms of the samples of P(S-MMA): (A) 14000; (B) 35000; (C) 58500; (D) 107800

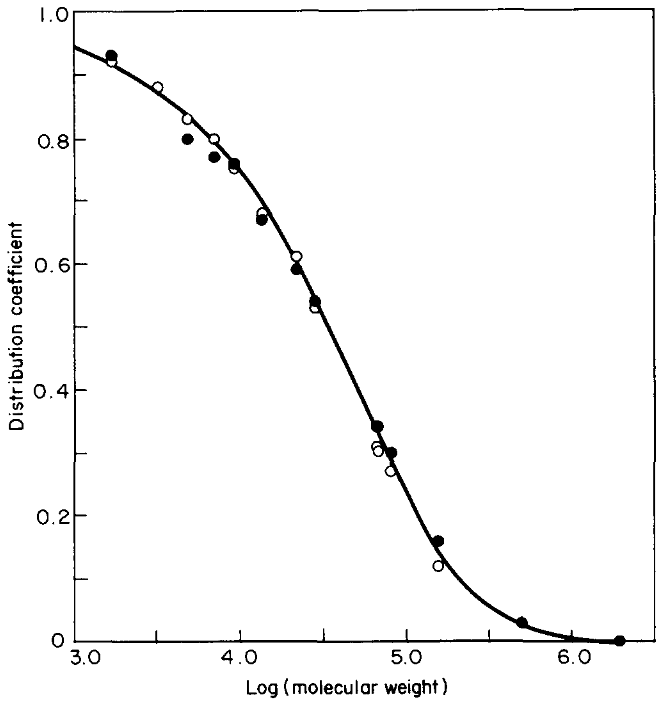

Figure 2 Calibration plot of the distribution coefficient *versus* the logarithm of the molecular weight of the polystyrene standards in (●) THF and binary mixed eluent comprising (○) ACN-DCM (40:60, v/v)

Column

A S5X silica gel microcolumn (250 × 2 mm) was provided by Phase Separations Ltd (Clwyd, UK). The efficiency of the column was 26 000 theoretical plates m^{-1} with respect to benzene. The mean pore diameter of the silica gel was 300 Å, with the mean particle size being 5 μm. The calibration relationship based on PS standards is shown in *Figure 2*.

Eluents

Mixed mobile phases comprising tetrahydrofuran (THF), dichloromethane (DCM) and acetonitrile (ACN) were used. S.e.c. was carried out in a mobile phase with the composition: THF-DCM-ACN (10:45:45, v/v/v). Isocratic adsorption chromatography or chromatography under near critical conditions (l.a.c.c.) was carried out using an eluent with the composition: THF-DCM-ACN (4:48:48, v/v/v).

Chromatographic conditions

The flow rate was 200 μl min^{-1}, and the temperature was 27°C. Concentrations of the block copolymer samples were in the range of 0.5–1 mg ml^{-1}, and the mass of sample applied to the column was 0.5–1 μg. The concentrations of pure components used to determine the absorbance ratios were 11.0 and 12.0 mg ml^{-1} for PMMA and PS, respectively.

Equipment

Two LKB HPLC pumps model 2150 (LKB Bromma, Uppsala, Sweden) and a PU 4046 high-pressure stirred-solvent mixer (Philips Analytical, Cambridge, UK) were used to deliver a defined composition of mixed mobile phases. A Rheodyne 7410 injection valve with a 1 μl loop volume (Rheodyne, Cotati, USA) was used for injecting the samples. The temperature was maintained using a TC 931 Column Heater (Applied Chromatography Systems Ltd, Macclesfield, UK). A diode-array detector, model HP 1040A with 4.5 μl cell volume and 6 mm optical path length (Hewlett-Packard, Waldbronn, Germany), coupled with a HP85B computer and HP Think Jet plotter, were used for data collection and printing. The HPLC ChemStation with standard Data Editor software (Hewlett-Packard) was employed for data processing.

Chromatographic data were collected as seven chromatograms at the following wavelengths: 225, 230, 235, 245, 255, 265 and 275 nm, with a band width of 20 nm. An eighth channel at 500 nm (bandwidth 100 nm) was used to compensate for detector baseline drift. Spectral data were collected in the range of 225–400 nm in 2 nm steps. The analysis time was 5 min.

RESULTS AND DISCUSSION

Application of multidimensional analysis for evaluating the composition of complex macromolecular compounds

The application of spectral methods, such as n.m.r. and i.r., for analysing the chemical composition of macromolecules with complex architecture, and of block copolymers in particular, often raises the question of data reliability. This is because of the possible presence in these products of macromolecular impurities with similar chemical characteristics which can superpose systematic interferences and distort the true data. This problem has also been pointed out by other authors[8,9]. So, for a more authentic evaluation of the chemical composition of block copolymers, it is desirable to separate a product from its macromolecular by-products.

Some examples of the distortion of the data obtained for block copolymers in bulk solution without separation of the components, are shown in *Figure 3*. Three-dimensional plots of chromatographic data using the coordinates retention time, wavelength and absorbance, show the chromatography of two samples of block copolymers (numbers 2 and 4 in *Table 1*) under both s.e.c. and l.a.c.c. conditions. In *Figure 3a* the unimodal s.e.c. chromatogram of block copolymer 2 is presented, where no resolution of the component peaks is observed. In *Figure 3c* the application of adsorption forces under l.a.c.c. conditions enables the methacrylic components to be resolved, so that the unimodal chromatogram splits into three zones: PS homopolymer, block copolymer and PMMA homopolymer, in ascending order of retention time. *Figure 3c* illustrates the l.a.c.c. mode, where the block copolymer should elute as if it were a pure PS component. Thus it may be concluded that this block copolymer (sample 2) contains a PS homopolymer impurity of the same size as that of the block copolymer. As regards the methacrylic component of the block copolymer, this method does not enable its size to be evaluated.

In *Figure 3b* the chromatogram of sample 4 under s.e.c. conditions is presented. In this case the PS homopolymer impurity is resolved from the block copolymer under s.e.c. conditions, indicating that the product contains PS precursor. In *Figure 3d* the application of adsorption forces under l.a.c.c. conditions yields a different chromatographic profile with a peak representing overlapping P(S-MMA) and PS homopolymer, followed by a PMMA homopolymer peak. The peak overlap of the block copolymer and homopolymer is to be expected when the l.a.c.c. mode is used; this confirms that the sample contains PS precursor.

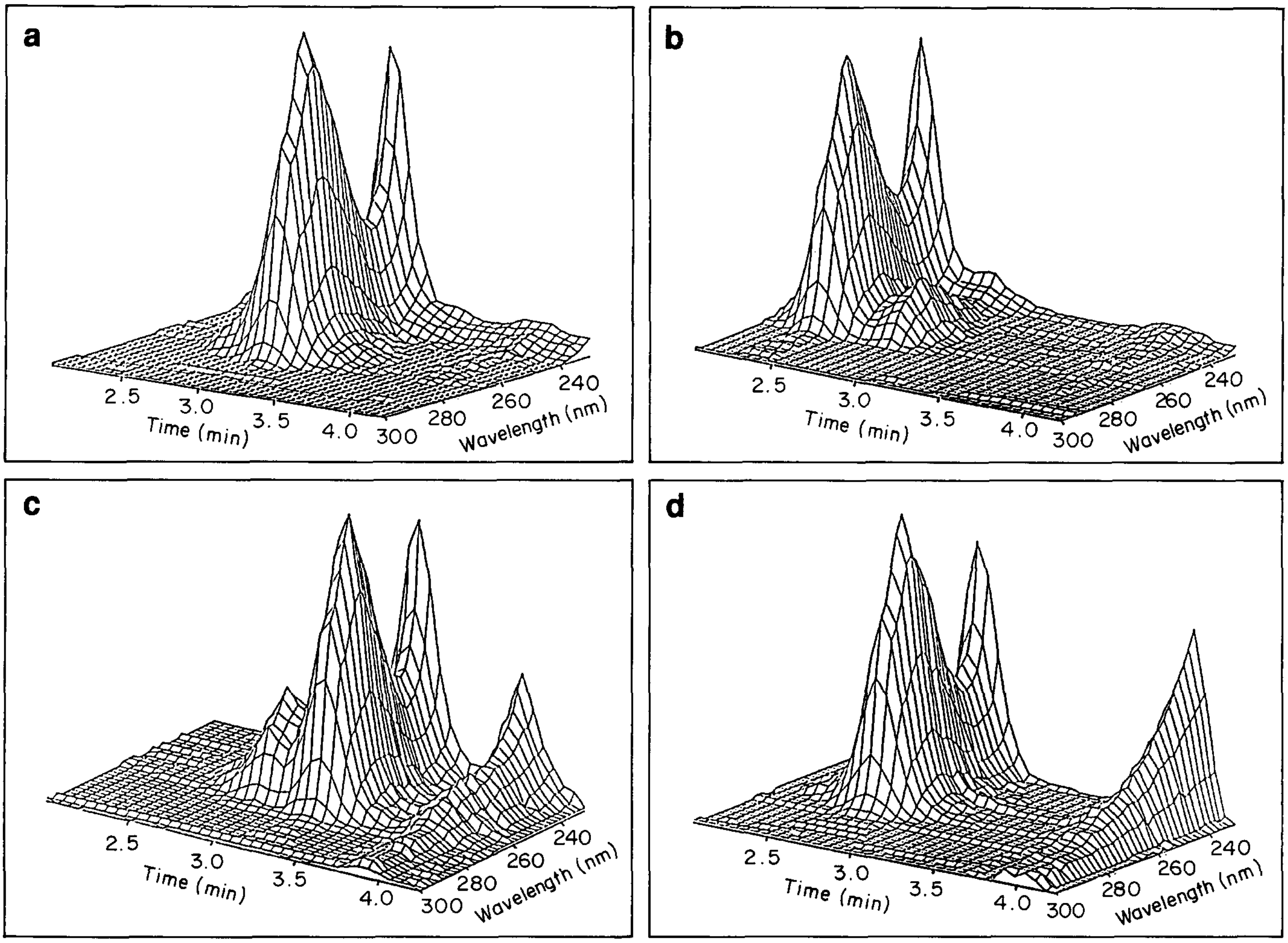

Figure 3 Three-dimensional plots (retention time, wavelength, absorbance) of the chromatograms of P(S-MMA) (samples 2 and 4 in *Table 1*) in s.e.c. and l.a.c.c. conditions. Flow rate, 200 μl min^{-1}; S5X silica gel column, 250 × 2 mm; mixed mobile phase, THF-ACN-DCM. Sample 2 in (a) s.e.c. and (c) l.a.c.c. conditions. Sample 4 in (b) s.e.c. and (d) l.a.c.c. conditions

These two examples clearly show the necessity of separating the macromolecular components in the products of copolymerization for the correct analysis of chemical composition in block copolymers.

Application of the spectral suppression technique for evaluating the composition of block copolymers

The application of diode-array detection makes it possible to calculate the composition of two known polymeric components in a block copolymer, where the spectra are sufficiently different, at every point in a chromatogram. This can be compared with the distribution of macromolecules separated according to their sizes in s.e.c., or according to composition or other parameters, as determined by the mechanism of separation.

The spectral suppression method, described above, was applied for evaluating the composition of PS-PMMA block copolymers. In the following discussion, the PS component corresponds to X and the PMMA component to Y, their weight concentrations in the block copolymer being C_X and C_Y, respectively. The measured value of C_Y/C_X, described by equation (8), is the ratio of the weight fractions of each component in the block copolymer.

The appropriate wavelengths selected for this procedure were: $\lambda_1 = 235$ nm and $\lambda_2 = 265$ nm. These two wavelengths are convenient for the application of the spectral suppression method because the PMMA component (Y) does not show any detectable absorbance at 265 nm; thus, it is possible to record at this wavelength the pure response of the PS component (X) in the block copolymer. On the other hand, at 235 nm both components show comparable absorbance, while the background absorbance of the eluent is small.

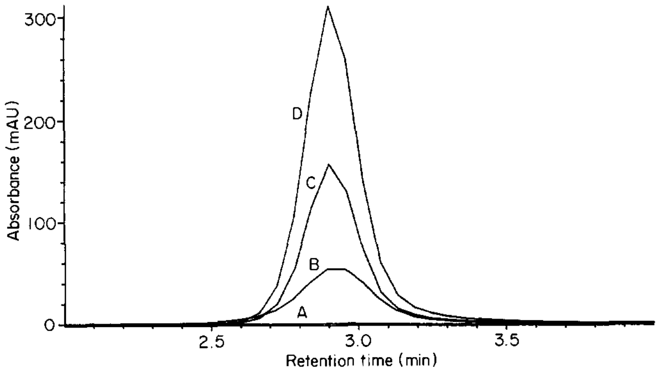

Figure 4 Chromatograms of PS (80 000) and PMMA (82 000) recorded at 235 and 265 nm. PMMA at (A) 265 and (B) 235 nm and PS at (C) 235 and (D) 265 nm, respectively

In *Figure 4* the s.e.c. chromatograms of the individual homopolymers corresponding to those comprising the block copolymers, i.e. PS (80000) and PMMA (82000), were recorded at these characteristic wavelengths. The mean value of the proportionality coefficient for compound X, $K_X(\lambda_1, \lambda_2)$, was determined from these chromatograms and found to be: $K_X(\lambda_1, \lambda_2) = 0.55 \pm 0.005$. The accuracy of the $K_X(\lambda_1, \lambda_2)$ value is of great importance for the final results. However, it could be limited by the noise level due to the background absorption of the eluent in the spectral range below 240 nm. With high sensitivity detection, differences in the absorbance of chromatographic standards with different molecular weights could be observed. The plots of the absorbance ratio data at 225, 230, 235, 245, 255, 265 and 275 nm obtained for 0.1% solutions of PS standards (28000, 60000, 156000) with respect to 265 nm (*Figure 5*) show that the error depends on the wavelength. At wavelengths lower than 240 nm it exceeds 5%. For 1% solutions of PS and PMMA, however, the fluctuations in the baseline were negligible and the error did not exceed 1%. Thus, the lower absorbance limit for reliable determination of absorbance ratio data from chromatograms using the diode-array spectrophotometer may be estimated as 10 mAU. In the case of PS and PMMA, this corresponds to 0.5–1.0 μg of the substances applied to the column.

On inserting the experimental value of 0.55 for $K_X(\lambda_1, \lambda_2)$ into equation (3) and taking into account the zero absorbance of PMMA at 265 nm, the contribution of the PS signal may be suppressed in chromatograms obtained at 235 and 265 nm. The residual plot, S, represents the pure response of the PMMA component in the block copolymer (or mixture of homopolymers). This novel possibility for obtaining chromatographic profiles of the pure components in block copolymers could be very useful for the rapid on-line qualitative estimation of a polymer product. Thus the presence of homopolymers can be detected and the type of compositional distribution can be characterized, enabling the parameters of the polymerization process to be corrected on-line.

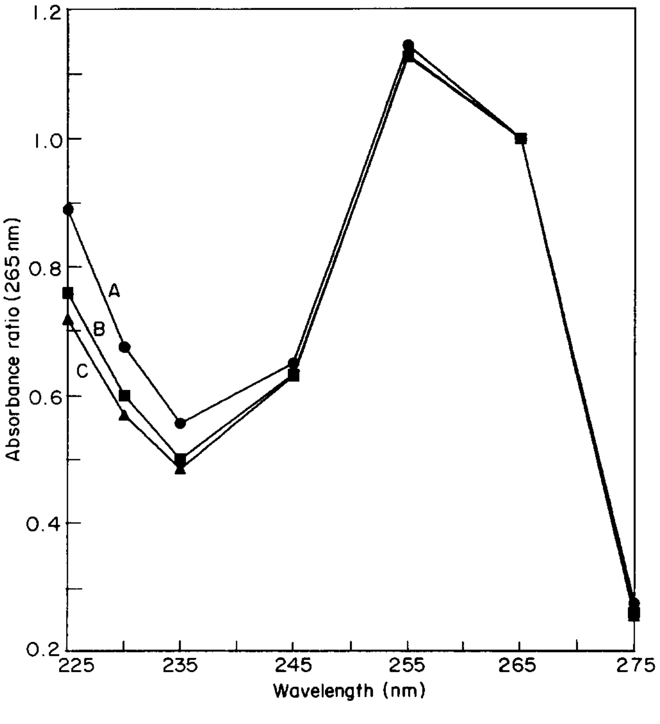

Figure 5 Absorbance ratios for PS standards. Molecular weights: (A) 156000; (B) 60000 and (C) 28000 at 225, 230, 235, 245, 255 and 275 nm, obtained with respect to 265 nm as the reference wavelength

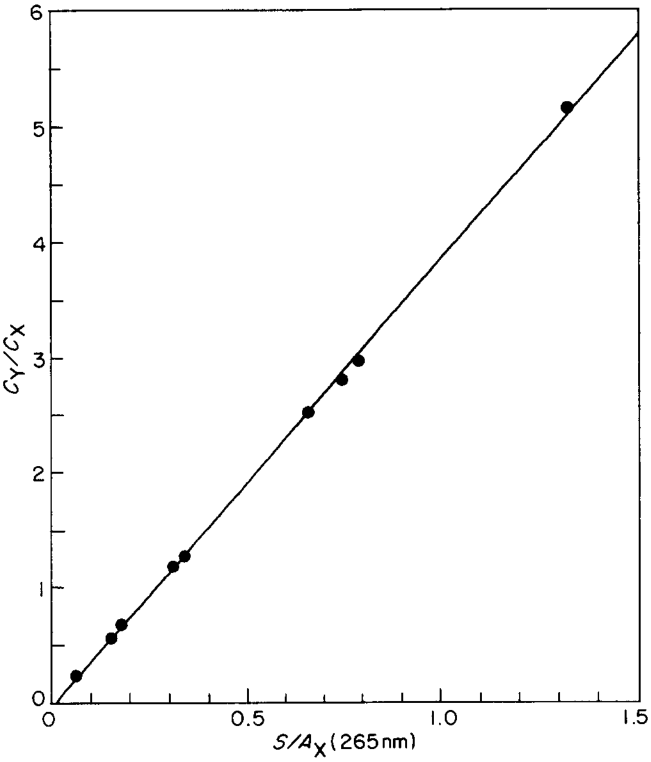

Figure 6 Calibration relationship of the weight ratio of the components (C_Y/C_X) *versus* S/A_X(265 nm) [equation (9)] in mixtures of PS and PMMA: (●) experimental points (based on peak areas)

For evaluating the raw data (taking into account that raw data were collected at the limits of sensitivity of the diode-array detector), the procedure based on equation (9) and the experimental calibration relationship were used. These data were obtained for mixtures comprising polymer standards: PS (66000) and PMMA (60000) by weight. The shift of the chromatographic profiles recorded at different wavelengths, observed by the spectral suppression technique (which enables the components to be recorded as the pure substances), caused by the differences in molecular weight, makes it incorrect to use the ordinates of the s.e.c. chromatogram for calculating the composition at every point of the chromatogram.

For a more accurate interpretation of the dual wavelength detection data, the areas of the chromatographic profiles for the spectral suppression technique can be used, utilizing the integration procedure of the Data Editor software in the Hewlett-Packard ChemStation. Thus, for the calibration plot the values of C_Y/C_X (by weight) for the standard mixtures were used as the ordinate, against S/A_X(265 nm) as the abscissa.

The calibration plot presented in *Figure 6* could be approximated by a linear plot with the regression coefficient, $r^2 = 0.999$:

$$y = 3.9x - 0.04 \quad (10)$$

The negative intercept value, being in the range of experimental error, was not significant. The calibration slope, $k = 3.9 \pm 0.1$ was used for evaluating the value of $f = (C_Y/C_X)$, according to equation (9). In this case, peak heights were used for calculation, utilizing the calibration

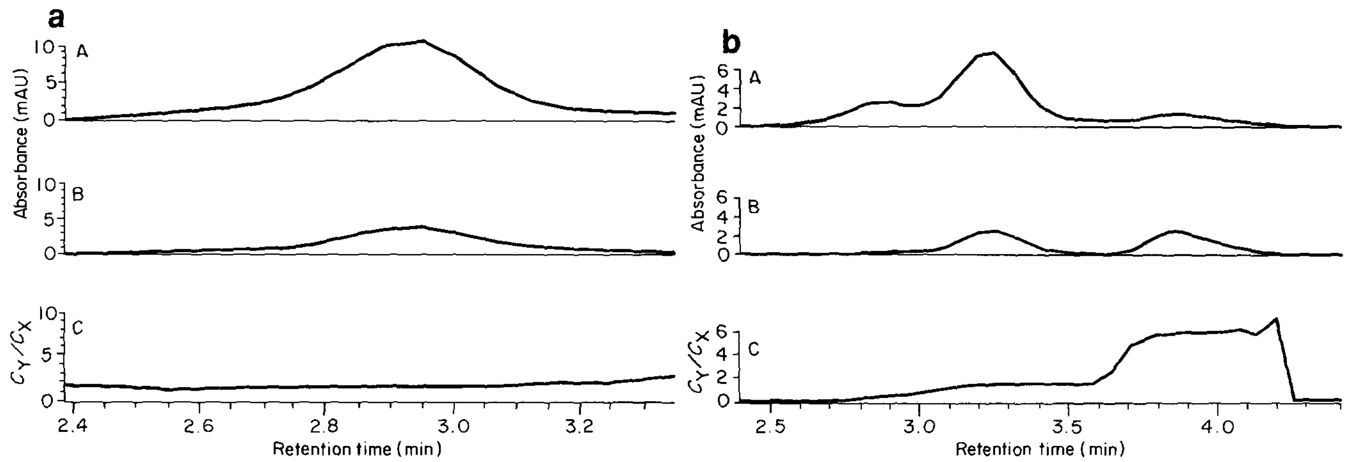

Figure 7 Chromatograms of P(S-MMA) (35000; sample 3 in *Table 1*) under (a) s.e.c. and (b) l.a.c.c. conditions compared with C_Y/C_X data [equation (9)]: (A) signal at 265 nm; (B) suppressed signal at 235 nm; (C) C_Y/C_X

Table 2 Chemical composition of the samples of poly(styrene-*block*-methyl methacrylate) obtained by diode-array detection utilizing spectral suppression

	S.e.c. conditions		L.a.c.c. conditions	
Sample no.	PMMA/PS ratio	PS fraction	PMMA/PS ratio	PS fraction
1	1.6	38	1.6	38
	1.7	37	1.6	38
	1.8	36		
2	1.4	42	1.3	43
	1.6	38	1.4	42
	1.5	40	1.2	45
3	1.5	40	1.7	37
	1.4	42		
	1.25	44		
4	1.8	36	1.5	40
	1.75	36	1.2	45
	1.8	36	1.2	45

parameters obtained from area data. Thus the accuracy of the procedure and the systematic error, both for peak heights and peak areas, could be assessed. Data on the composition of the block copolymers, based both on areas and on peak heights, could then be compared. These data were in good agreement, confirming the validity of the calibration graph.

The data obtained using the calibration plot are presented in *Table 2*. These data correspond to the chromatographic peak height. The distribution data are shown in *Figure 7* both for s.e.c. and for l.a.c.c. chromatographic conditions.

The corresponding values of the weight fraction of PS in block copolymer, *W*, may be calculated from the formula:

$$W = 1/(f+1) \qquad (11)$$

Comparison of the data presented in *Tables 1* and *2* shows that the values of the PS weight fraction in block copolymers obtained utilizing diode-array detection are lower then those quoted by the manufacturer using n.m.r. spectroscopy. However, taking into account the general difficulties of assessing data on chemical composition in complex macromolecular compounds, they are within 10% and are in reasonably good agreement. It should be pointed out, however, that the proposed method enables the composition of the block copolymer to be evaluated very rapidly. The spectral suppression technique enables the distribution of these particular pure components in a chromatographic zone to be visualized for the first time. Moreover, the possibility of obtaining spectra at any point in the chromatogram enables the individual zones to be identified in a polymodal chromatogram.

The advantages of computer-aided diode-array detection coupled with s.e.c. and l.a.c.c., make this a very valuable combination of techniques with significant potential in polymer chemistry as well as in pharmaceutical chemistry, ecology and molecular biology, where polymer analysis is becoming increasingly of interest.

However, the disadvantages of the method should be noted. These include the problems of spectral selectivity. The characteristic u.v. absorption bands in macromolecular compounds are rather broad. In the present case, additional chromophoric groups could not be detected in the spectra of the block copolymers. This lack of specificity can lead to some errors in evaluating absorbance ratio data. The background absorption of eluents in the u.v. range can also introduce problems, but these problems are, to some extent, characteristic of most spectral methods.

It can be concluded that u.v. dual wavelength detection, utilizing the diode-array detector offers a valuable technique for the rapid evaluation of chemical composition in complex macromolecules separated by s.e.c. or l.a.c.c.

CONCLUSIONS

The diode-array detector can be successfully deployed for analysing the chemical composition of macromolecular solutes by liquid chromatography and has particular value for rapidly evaluating the distribution of the chemical composition.

In particular, the spectral suppression technique enables the individual components of a block copolymer to be visualized, thus enabling the progress of a polymerization reaction to be monitored on-line. Moreover, the quality of the products can also be evaluated.

The limit of spectral sensitivity with state-of-the-art equipment was found to be ~10 mAU. Below this limit the signal-to-noise ratio is too low for quantitative analysis to be practicable.

Isocratic adsorption chromatography under l.a.c.c. conditions offers an additional approach to that of s.e.c. These methods permit resolution of the products of polymerization into zones representing their chemical composition, separation from the primary product of any homopolymers and components with low conversion value, and evaluation of their weight fraction—all in a single isocratic run. Moreover, coupling these chromatographic procedures with diode-array detection and digital data processing yields a facile method for evaluation of the distribution of the chemical composition.

The principal advantage of the proposed detection technique is the fast processing of data, thus permitting the characteristics of polymeric products to be assessed very rapidly. This is further enhanced by coupling with high-speed microcolumn s.e.c., thus enabling the chromatographic data to be obtained in ~3 min.

The combination of s.e.c. or l.a.c.c. with rapid multichannel detection utilizing the u.v. diode-array detector permits a number of digital techniques to be exploited for deconvoluting spectrochromatographic data. These include principal components analysis[10], iterative target testing factor analysis[11,12], and partial least squares analysis[13]. These hybrid technologies would be expected to facilitate the analysis of polymer systems, even where the spectra are very similar, i.e. strongly correlated. These novel strategies in polymer analysis combined with diode-array detection can yield additional information on the characteristics of complex macromolecular systems.

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