CHROM. 15,018

DESIGN OF A HIGHLY SENSITIVE INFRARED DETECTOR AND APPLICATION TO HIGH-PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY FOR COPOLYMER ANALYSIS

SADEO MORI*

Department of Industrial Chemistry, Faculty of Engineering, Mie University, Tsu, Mie 514 (Japan)

and

AKIO WADA, FUMIKO KANEUCHI, ATSUO IKEDA, MITSUO WATANABE and KOICHI MOCHIZUKI

Japan Spectroscopic Co., Ltd., Ishikawa-cho, Hachioji, Tokyo 192 (Japan)

(First received March 9th, 1982; revised manuscript received May 5th, 1982)

SUMMARY

An infrared spectrophotometer designed to increase the sensitivity of absorption spectrometry in high-performance size exclusion chromatography is described. It employs an optical comb attenuator which has one tooth and is effective only in the range of 0–10 % reduction of the reference beam. The sensitivity of the system is at least 40 times greater than that of a conventional instrument. Solvents which have around 5 % transmittance at specified wavelength settings can be used as the mobile phase. The compositional heterogeneity of acrylonitrile–styrene and styrene–methyl methacrylate copolymers have been determined using this detector.

INTRODUCTION

The physical properties of copolymers are dependent on their composition and molecular weight. Several studies have been made of the determination of the copolymer composition as a function of molecular weight, using size exclusion chromatography (SEC, or GPC) in combination with ultraviolet (UV) and differential refractive index (RI) detectors for copolymers such as styrene–butadiene[1,2], acrylonitrile–styrene[3] and styrene–methyl methacrylate[4,5]. The use of a UV detector in series with an RI detector is limited by the condition that one component should absorb UV radiation and the other should not. This reduces the number of copolymers that can be examined by this dual-detector system. In addition, several problems arise such as the difficulty in assigning the precise elution volume of a specific solute on both UV and RI chromatograms due to the different peak broadening effects[4].

Infrared (IR) absorption as a measure of solute concentration in the effluent of SEC has the advantage of being able to monitor functional groups. Appropriate selection of the wavelength setting in an IR detector may significantly expand its range of applicability to copolymer analysis. A small number of studies employing a

conventional IR spectrophotometer as an SEC detector have been reported; both stop-flow and on-line procedures have been utilized[2,6-13] for styrene–butadiene[2], styrene–methyl methacrylate[7], styrene–tert.-butyl methacrylate[8], styrene–vinyl stearate[9,10], styrene–acrylonitrile[12] and vinyl chloride–vinyl acetate[11] copolymers, polyethylene[6,8] and coal products[13].

Conventional IR spectrophotometers and detectors. however. have limited usefulness as SEC detectors in several respects. such as poor sensitivity for most polymers except those having intense absorption bands. and are restricted to SEC solvents having reasonable IR transmittance windows and good solubility for the polymer involved. Since high-performance (HP) SEC is performed at lower solute concentrations than is conventional SEC. good detector sensitivity is especially required. SEC–Fourier transform IR spectroscopy (FTIR) has been applied in the separation of silicone polymers[14] and solvent-refined coal[15]. but its cost precludes its use as a universal detector in SEC. A limited use of a conventional IR detector for high-performance liquid chromatography (HPLC) has been made in the analysis of glycerides[16,17] and detector performance and sensitivity have been discussed[17].

In this paper. a highly sensitive IR spectrophotometer exclusively designed for use as a detector in HPSEC and HPLC is described. Any solvent which has 5 % transmittance at a specified wavelength setting can be used as a mobile phase in SEC. Application of this detector to the compositional heterogeneity of poly(acrylonitrile–styrene) (AS) and poly(styrene–methyl methacrylate) (SM) copolymers as a function of elution volume is demonstrated.   ·

INSTRUMENTATION

The IR detector (Model HPIR-100) is a double-beam spectrophotometer based on the optical null-balance system and the output obtained is absorbance instead of conventional transmittance. It consists of an IR source. sample and reference cells. a photometer and a monochromator. a detector. an electrical system and an optical comb attenuator (OCA). IR radiation from the light source (a coil of Nichrome wire of diameter 0.6 mm on 16 × 4.2 mm I.D. ceramics: 900–1000°C) is split into two symmetrical beams, one of which passes through a sample cell and the other through a reference cell and OCA. These beams are chopped by a rotating sector mirror (chopping speed. 13 Hz) before entering the entrance slit of the monochromator which is of the Littrow type equipped with a set of gratings. The incident. chopped radiation dispersed in the monochromator passes successively the exit slit and is then focused onto a detector (a highly sensitive vacuum thermocouple). Since the sample and reference beams enter the detector alternately at an interval of 13 Hz. the difference in energy between sample and reference chopped beams results in an alternating current (a.c.) which is amplified by a preamplifier and a main amplifier. The a.c. signal drives a servo motor through a servo amplifier to control the position of the OCA. which in turn adjusts the energy of the reference beam to be equal to that of the sample beam and reduces the a.c. signal to zero. A shift in the setting of the OCA is converted into absorbance output by a potentiometer. A block diagram of the IR detector is shown in Fig. 1.

Two main modifications to the IR detector are made in order to increase sensitivity. First is the use of optical scale expansion of transmittance rather than the
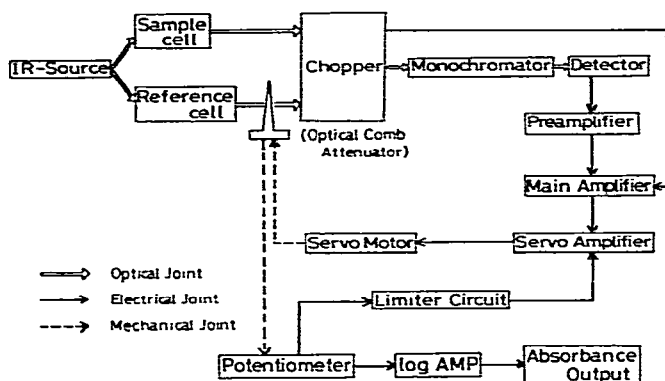
Fig. 1. Block diagram of the IR detector.

conventional mechanical expansion by means of a gear ratio. For this purpose, the OCA has one tooth and is designed to be effective only in the range of 0–10% reduction of the reference beam. Nevertheless, the pen on the recorder can be moved over the full scale, corresponding to 0–100% reduction of the reference beam or 100–0% transmittance for a conventional IR spectrophotometer. This means that the dynamic range is extended ten-fold in the range of 90–100% transmittance, in other words the sensitivity increases to ten-fold. The second modification is electrical amplification of the absorbance output. The range of the output can be selected out of 0.04, 0.02 and 0.01 a.u.f.s., which corresponds to an increase in sensitivity of about 11-, 22- and 44-fold, respectively compared with the transmittance scale of a conventional IR spectrophotometer. The optical scale expansion enables this amplification because of the low baseline noise even at high scale expansion.

Several minor modifications are also made. One is to equip the detector with a limite circuit in order to protect the optical comb system from overloading when the energy difference between the sample and the reference beams exceeds 10%. To minimize the noise increase accompanying scale expansion, the drive speed of the OCA is reduced to one-half of the conventional one, resulting in high signal-to-noise ratios. Three different time constants, 0.5, 1 and 4 sec, can be selected and the noise level at 4 sec is below $1 \cdot 10^{-4}$ a.u. The IR source is sealed to protect it against solvent vapour and the outside of its case is cooled by an electric fan. The CaF_2 or NaCl window is set at the exit of the IR radiation. This seal can also reduce the fluctuations in temperature of the IR source accompanying variations in room temperature. The slit width is variable in the range of 0–4 mm. This detector employs a grating (120 lines per millimetre) to select wavenumbers between 4000 and 1200 cm^{-1} (CaF_2 window) and 650 cm^{-1} (NaCl window) with a resolution of 24.5 cm^{-1} at 3000 cm^{-1} and 7.5 cm^{-1} at 1700 cm^{-1} at a slit width of 1 mm.

The flow-through cell consists of a pair of CaF_2 or NaCl windows (each 3 mm wide) separated by a PTFE spacer of width 0.5 mm, the whole held together as a sandwich by metal plates. Two holes are drilled through the metal plate and one window plate. The whole cell volume is 32.5 μl and the effective cell volume through which IR radiation passes is 20 μl. The flow-through cell is shown in Fig. 2.
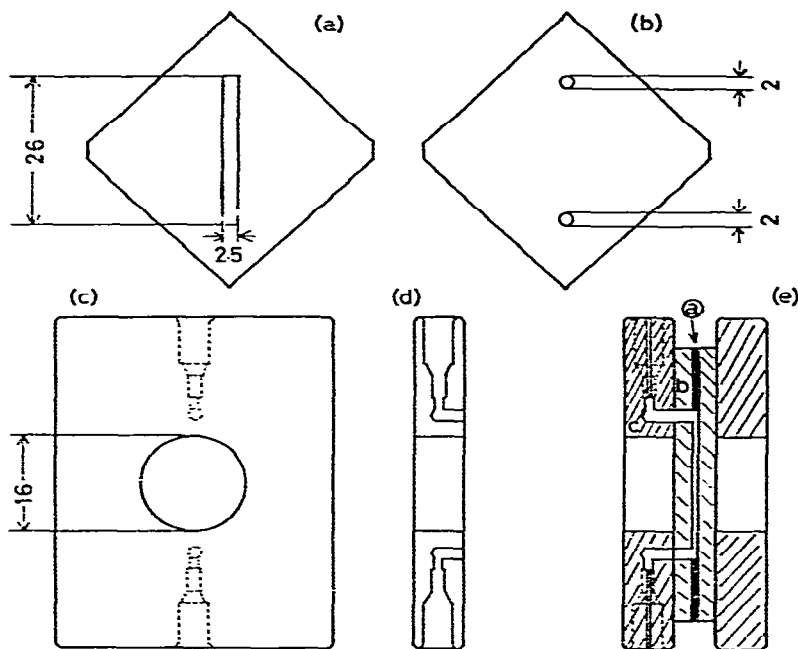
Fig. 2. Flow-through cell. (a) PTFE spacer; (b) front window; (c) front metal plate; (d) side cross-section of the front metal plate; (e) side cross-section of the flow-though cell (dimensions in mm).

EXPERIMENTAL

A JASCO (Japan Spectroscopic, Tokyo, Japan) TRIROTAR high-perform-ance liquid chromatograph was used with a Model HPIR-100 IR detector. A JASCO Model UVIDEC-100 variable-wavelength UV detector and a Shodex Model SE-11 RI detector were also used for comparison purposes. Two Shodex A80M HPSEC columns (50 cm × 8 mm I.D.) packed with a mixture of polystyrene gels of nominal porosity 10^3, 10^4, 10^5 and 10^6 Å were used. Sample solutions were injected using a variable loop injector Model VL-611. A JASCO Model IR-G IR spectrophotometer was used for the measurement of solvent transmission windows.

AS and SM copolymers were prepared in our laboratory and the styrene con-tents were 59.4 and 48.6% (w/w) respectively. Polystyrene (PS) standards of narrow molecular weight distribution for a calibration curve were obtained from Pressure Chem. (Pittsburgh, PA, U.S.A.). Dibutylphthalate (DBP) was used for the measure-ment of detector linearity and detection limit. Several solvents used for the measure-ment of solvent transmission window were commercially available and were used after purification.

The mobile phase for SEC was chloroform. The sample concentration was 0.1% for PS standards and 0.2% for copolymers. The sample injection volume was 0.25 ml and the flow-rate was 1.0 ml/min. Detector noise and drift was measured using toluene as eluent in an actual SEC system.

RESULTS AND DISCUSSION

Performance of IR detector

Noise, drift and sensitivity are in general the most important features to be considered when evaluating whether an IR detector with function adequately as an SEC detector. High short-term noise degrades the signal-to-noise (S/N) ratio of an IR spectrum and detector drift tends to camouflage both noise and small peaks. As both the noise and the drift make difficult the setting up of an accurate baseline, low noise and small drift are desired in order to calculate accurate molecular weight averages from size exclusion chromatograms. Reduction of noise facilitates the mechanical and electrical amplification and enables the absolute sensitivity of the detector to be raised and the detection limit to be lowered. These considerations are very important in HPSEC where the sample load should be less than about one-tenth the load in conventional SEC.

The IR detector described in this paper is at least two orders of magnitude more sensitive than a conventional IR spectrophotometer so that the noise level and the baseline fluctuation must be discussed first. Fig. 3 shows the noise level and the
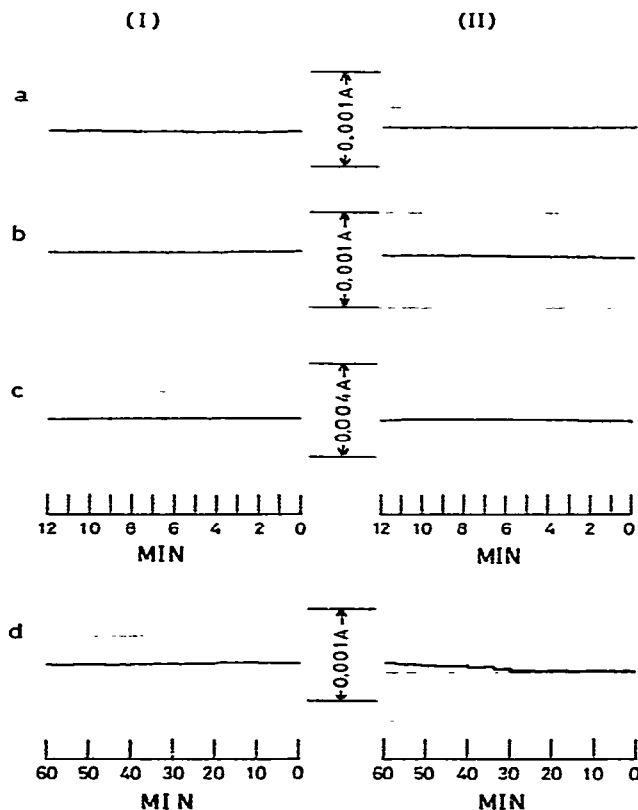
Fig. 3. Noise level and baseline drift of the IR detector at two different transmittances of incident radiation. (I) 70% Transmittance at 2070 cm^{-1}; (II) 5% transmittance at 1580 cm^{-1}. (a) Attenuator 0.01 a.u.f.s., pump flow-rate 0; (b) attenuator 0.01 a.u.f.s., pump flow-rate 1 ml/min; (c) attenuator 0.04 a.u.f.s., pump flow-rate 1 ml/min; (d) as in (a). A = Absorbance units, a.u.

baseline drift at 5% and 70% transmittance of the incident radiation at gain 2, slit width 1 mm and time constant 0.5 sec. The baseline is very smooth and the noise level is less than $2 \cdot 10^{-5}$ a.u. (absorbance), which is over twenty times better than that obtained by Parris[17]. The baseline drift is $1 \cdot 10^{-4}$ a.u./h at 5% transmittance and $2 \cdot 10^{-5}$ a.u./h at 70% transmittance.

The detector should have a wide linear dynamic range so that major and trace components can be determined in a single analysis over a wide concentration range. A linear relationship between the detector output (absorbance) and the solute concentration was obtained from low (1 μg/ml) to high concentrations (100 μg/ml) of DBP at 1725 cm^{-1} in chloroform, using a flow-through cell of path length 0.5 mm under the conditions of slit width 2 mm, gain setting 2 and time constant 4 sec. The plot passed through the origin, its equation with relative standard deviation 2.9% being:

$$\text{Absorbance} = 3.08 \cdot 10^{-4} \cdot \text{concentration} \ (\mu\text{g/ml})$$

The detection limit, at a signal-to-noise ratio of 2, for DBP was estimated to be 0.33 ppm or 0.33 μg/ml using this equation and a noise level of $5 \cdot 10^{-5}$ a.u. The absolute sensitivity for this system is of the order of 10 ng.

In order to minimize the detector noise level, the solvent used as the mobile phase should have a low absorbance at the wavelength employed for measurement of the eluting solute. As a rule of thumb, a solvent having over 30% transmittance at the wavelength employed is preferable and a chart showing the infrared transmission regions for a number of solvents, *i.e.*, the so-called solvent transmission windows, is based on this concept in most cases[8]. However, the IR detector, HPIR-100, used in this work is designed so as to be very stable even at low incident energy such as 5% transmittance. Fig. 4 shows solvent transmission windows for several solvents used for SEC: solvents having over 5% transmittance are designated as transparent. This chart shows that our IR detector enables the group of solvents used for SEC and the region of wavelength employed to be extended.

Solute peaks may become broadened during passage through a detector cell. In order to avoid peak broadening due to mixing within the cell, it should possess a small volume. The cell volume of our IR detector is about 30 μl, while the volumes of conventional microcells employed in UV and RI detectors in HPLC are 5–10 μl. To evaluate the amount of peak broadening in our IR detector system, two PS standards of narrow molecular weight distribution were injected into the SEC system in which were placed UV and IR detectors in series in this order. The dead volume between the two detectors was 0.06 ml. The cell volume of the UV detector was 8 μl. The IR absorption profile was determined from the variation of the absorbance of the C–H stretching mode (2950 cm^{-1}) and was then compared with the UV absorption profile. Fig. 5 shows the normalized UV and IR absorption profiles of the size exclusion chromatograms. For comparison purposes, the peak position in the UV profile was made to coincide with that in the IR profile. The peak width at half-height and that at 1% of the peak height of the IR profiles were 0.84 and 1.76 ml for PS of molecular weight (MW) 37,000 and 1.24 and 3.20 ml for MW 2200, while the corresponding values from the UV profiles were 1.0 and 2.04 ml and 1.40 and 3.48 ml, respectively. In every case, the width from the UV profiles was 1.1–1.2 times that from the IR profiles. The UV detector was encountered first but nevertheless yielded higher peak
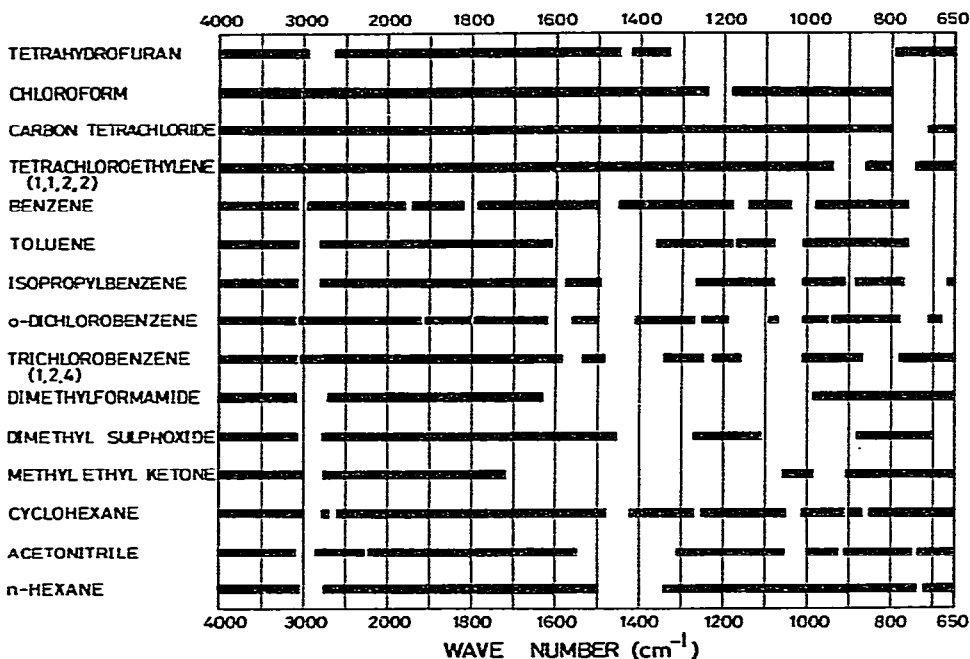
Fig. 4. Solvent transmission windows. The horizontal bars designate regions in which transmission exceeds 5% in a 0.5-mm pathlength NaCl cell.

widths than those from the IR detector. Consequently, peak broadening in the IR cell can be said to be smaller than in the UV cell, despite the larger cell volume of the former.

Several factors may be responsible for this result, including the difference in the shapes of the cells and in the detector time constants. The cell cavity of the IR detector is shown in Fig. 2; the incident radiation passes through the cavity per-
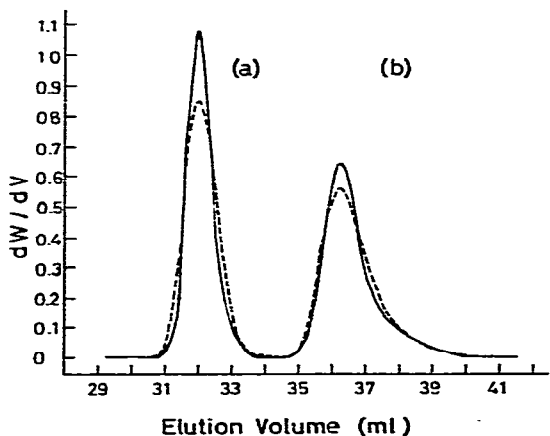

Fig. 5. Comparison of IR absorption profile (——) and UV absorption profile (– – – –) of size exclusion chromatograms for PS copolymers: (a) MW 37000; (b) MW 2200.

pendicular to the direction of solvent flow. On the other hand, in the cell of the UV detector (10 × 1 mm I.D.) the incident radiation passes through the cell in the same direction as the solvent flow. The influence of cell shape on the peak broadening is not clear. The effect of the detector time constant on peak retention times has been discussed by Low and Haddad[18]. An increase in detector time constant results in a reduction of peak height, an increase in peak skew and a shift in the peak maximum toward longer retention. The time constant used in our work was 0.5 sec for the IR detector, 1.2 sec for the UV detector. These effects are clearly illustrated in Fig. 5, although direct comparison of the time constants may not be strictly appropriate. A similar comparison of an IR detector and a UV detector was made previously, where the former (cell volume 30 μl) had a higher column efficiency than the latter (cell volume 8 μl)[17].

Application to copolymer analysis

AS copolymer in the effluent from SEC columns can be monitored at 2222 cm^{-1} for the nitrile stretching vibration of acrylonitrile and at 1497 cm^{-1} for the phenyl group of styrene. Chloroform used as the mobile phase is transparent at 2222 cm^{-1} and has 30% transmittance at 1497 cm^{-1}. Chromatograms of acrylonitrile and styrene units of an AS copolymer are shown in Fig. 6. The concentration of each component can be determined by repeated injection of the same sample solution into the SEC system and using different wavelength settings to monitor separately the specific functional groups.
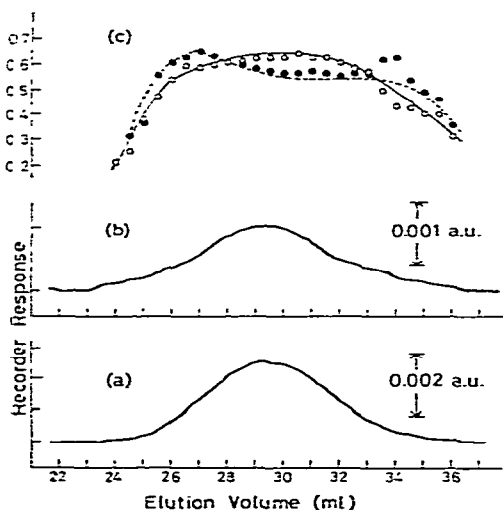

Fig. 6. Chromatograms and styrene weight fraction for acrylonitrile-styrene copolymer. (a) Styrene unit (1497 cm^{-1} at 0.02 a.u.f.s.); (b) acrylonitrile unit (2222 cm^{-1} at 0.01 a.u.f.s.); (c) styrene weight fraction distribution obtained by IR detector (O—O) and by UV–RI detectors (●– – –●).

In order to calculate the copolymer composition as a function of elution volume, calibration curves for absorbance *vs.* concentration of each component at the respective wavelength settings must be constructed. The acrylonitrile content was determined from a calibration curve constructed for polyacrylonitrile homopolymer

in dimethylformamide in the concentration range 0.1–0.5 mg/ml. The absorbance of the solution at each concentration was measured by injecting the solution into the SEC system, with the SEC columns disconnected, using a 2-ml loop injector. The equation for the calibration curve was:

$$\text{Absorbance} = 2.92 \cdot 10^{-6} \cdot \text{Concentration } (\mu g/ml)$$

Similarly, the styrene content was determined from a calibration curve constructed for PS homopolymer in chloroform. The equation obtained for the calibration curve was:

$$\text{Absorbance} = 4.40 \cdot 10^{-6} \cdot \text{Concentration } (\mu g/ml)$$

The styrene weight fraction at each elution volume was calculated from both contents at the same elution volume and the result is shown in Fig. 6c. The reproducibility of the pumping system used in this work is excellent and the use of any internal standard to check the elution volume[8] is not required. For comparison purposes, the styrene weight fraction distribution obtained in the UV–RI detector system is also shown in Fig. 6c. These values were determined according to the method of Adams[1]. As the effect of peak broadening is different in the UV and RI detectors, corrections were made to all measurements of the distribution[4]. The response factor of the RI detector for acrylonitrile was assumed to be 0.796 times that for styrene. This value was obtained by extrapolation of a linear plot of RI response factors of several AS copolymers and of polystyrene vs. the styrene contents of the copolymers, assuming the RI response factors of the copolymers to be the sum of the products of the RI response factor and weight fraction for styrene and for acrylonitrile, respectively. The average styrene weight fraction for the whole copolymer as calculated from the styrene content at each increment and the sum of weight per cent at the same increment of two IR chromatograms was 0.590. This is in good agreement with the value for the unfractionated sample of 0.594. The average styrene weight fraction obtained from the UV–RI detector system was 0.585.

Although the two distributions in Fig. 6c are not in agreement, their trends are similar. It can be concluded that the result obtained from the IR detector is more reliable than that from the UV–RI detectors because it is hard to get accurate assignments of the elution volume of a specific solute from UV and RI chromatograms. The molecular weight of the AS copolymer can be calculated from the weight fraction obtained with two IR chromatograms. Weight- and number-average molecular weights of the copolymer were $2.55 \cdot 10^5$ and $5.46 \cdot 10^4$ PS equivalents.

The methyl methacrylate (MMA) content in the SM copolymer can be monitored at 1730 cm^{-1} for the C=O stretching vibration. The absorption at 1497 cm^{-1} of the phenyl group cannot be applied to the analysis of the styrene content because this band is overlapped by that assigned to the MMA unit. Instead, a wavenumber of 2950 cm^{-1} might be employed to monitor the copolymer concentration. The equation of the calibration curve constructed for MMA homopolymer in chloroform at 1730 cm^{-1} is

$$\text{Absorbance} (= D'') = 7.87 \cdot 10^{-5} (= K'_M) \cdot \text{Concentration } (\mu g/ml)$$

and that at 2950 cm^{-1} is:

$$\text{Absorbance } (= D') = 2.02 \cdot 10^{-5} \, (= K_M) \cdot \text{Concentration } (\mu g/ml)$$

The equation of the calibration curve for PS homopolymer at 2950 cm^{-1} is

$$\text{Absorbance } (= D - D') = 2.60 \cdot 10^{-5} \, (= K_S) \cdot \text{Concentration } (\mu g/ml)$$

where D is the absorbance of the copolymer at 2950 cm^{-1}.

The last two equations imply that monitoring the absorbance at 2950 cm^{-1} cannot provide accurate data on the copolymer concentration. However, if the absorptivity of the homopolymer at a specified wavelength is identical to that of the copolymer, the styrene content at each elution volume i can be determined from the data at 2950 cm^{-1} by obtaining the MMA content from the data at 1730 cm^{-1}:

$$\text{Styrene concentration } (\mu g/ml) = (K'_M D_i - K_M D''_i)/K'_M K_S$$

Similarly, the styrene weight fraction, W_S, can be calculated as follows

$$(W_S)_i = \frac{K'_M - K_M R_i}{K'_M - (K_M - K_S) R_i}$$

where

$$R_i = D'_i/D_i$$

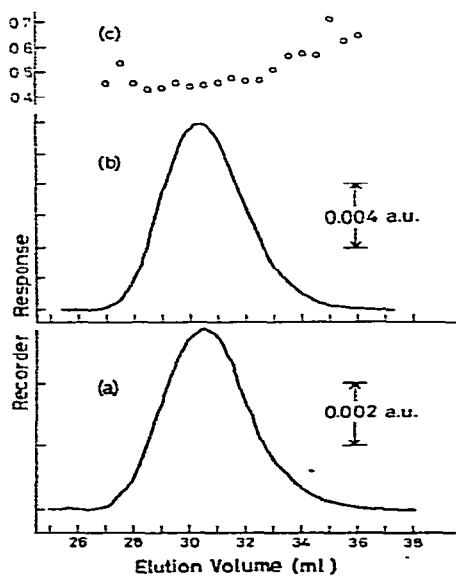The results are shown in Fig. 7. A detailed discussion on these copolymer analyses will be published elsewhere.

Fig. 7. Chromatograms and styrene weight fraction for styrene–methyl methacrylate copolymer. (a) Copolymer trace (2950 cm^{-1} at 0.02 a.u.f.s.); (b) MMA unit (1730 cm^{-1} at 0.04 a.u.f.s.); (c) styrene weight fraction distribution.

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