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“BUFFER MEMORY” TECHNIQUE FOR THE COMBINATION OF MICRO-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND INFRARED SPECTROMETRY

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SUMMARY

The buffer memory technique was applied to the combination of micro-high-performance size-exclusion chromatography and Fourier transform infrared spectrometry. A series of polystyrene standards with known molecular weights was studied and the molecular weight calibration was established. Polyethylene glycol of unknown molecular weight was determined from the calibration. The utility of the technique is discussed.

INTRODUCTION

The combination of high-performance liquid chromatography (HPLC) and infrared (IR) spectrometry has so far been accomplished via two approaches, the automated diffuse reflectance-solvent elimination technique¹⁻³ and the direct flow cell technique⁴⁻⁷. With these techniques a number of compounds isolated by HPLC have been successfully detected. However, both techniques have involved some intrinsic problems, as described by Kuehl and Griffiths² and Vidrine⁸.

Recently, we proposed the novel potassium bromide buffer memory technique^{9,10} which has been used to couple conventional dispersive IR with micro-HPLC (MHPLC). The technique was applied to the detection of sample components separated in the normal-phase mode. In this technique, the effluent from the MHPLC column is deposited via a UV flow-cell on a potassium bromide plate as a narrow, continuous band (about 1.5 mm wide) with instantaneous elimination of the carrier solvent. After collecting all the solutes, the “memorized” plate, the “buffer memory”, is automatically brought into the optical path of the IR spectrometer and IR chromatograms are measured at fixed wavenumbers.

This technique offers several advantages over conventional HPLC-IR techniques; most important is that completely continuous chromatograms can be obtained without interference from the mobile phase.

In this paper, the applicability of the buffer memory technique to size-exclusion chromatography (SEC) is demonstrated, and the analytical potential of this technique is discussed.

Previous work from this laboratory¹¹ has generally shown that a miniaturized SEC column can be used for the determination of the components of polymer materials.

EXPERIMENTAL

Spectrometer

The IR instrument employed was a JEOL JIR-40X Fourier transform (FT) spectrometer with a $3 \times$ beam condenser. The system used a TGS pyroelectric bolometer, a potassium bromide-germanium beam splitter and a water-cooled Globar source. All spectra were measured at 8 cm^{-1} resolution in the double-precision mode. The operating software was that normally supplied by JEOL.

Chromatographic system

The micro-high-performance liquid chromatograph was a JASCO Familic 100N system equipped with a Uvidec-100 II UV detector. A TSK GEL 3000 H column of ($22 \text{ cm} \times 1 \text{ mm I.D.}$) (excluded molecular weight $6 \cdot 10^4$) was used for the SEC separations. Tetrahydrofuran was used as the mobile phase at a flow-rate of $8 \mu\text{l}/\text{min}$.

Procedure

The interfacing device developed¹⁰ was simply placed in the sample chamber of the FT-IR spectrometer. With the device, the effluent from the MHPLC column is deposited on to a potassium bromide plate as a narrow, continuous band; the carrier solvent is instantaneously evaporated with a gently warmed stream of dry nitrogen. After collecting all the solutes, the "memorized" plate is automatically brought into the IR beam of the FT-IR spectrometer, and interferograms are sequentially measured and stored in the data system. At the end of the chromatogram, each stored interferogram is recalled and transformed. Then, absorbances in the selected regions are integrated and plotted in a recorder. Subtraction of the absorption bands due to water retained on the plate was performed by using the initial file in which no solutes were memorized. A potassium bromide plate, on which only the mobile phase is memorized, is placed in the reference beam. The transfer line between a column and a plate is designated not to cause the extra-column broadening. More details have been described elsewhere¹².

Chemicals

Three polystyrenes of molecular weight 37,000, 10,200 and 2800 were purchased from Toyo Soda (Tokyo, Japan). Polystyrene with a wider molecular weight distribution (average molecular weight about 500) and Carbowax 6000 were kindly supplied by Dr. Hirata (Toyohashi University of Technology). The chromatographic solvent used was tetrahydrofuran of HPLC grade (Kanto Chemicals). A large single crystal of potassium bromide ($35 \times 35 \times 3 \text{ mm}$) was obtained from JASCO. The crystal was cut into four equal pieces and used as a buffer memory substrate.

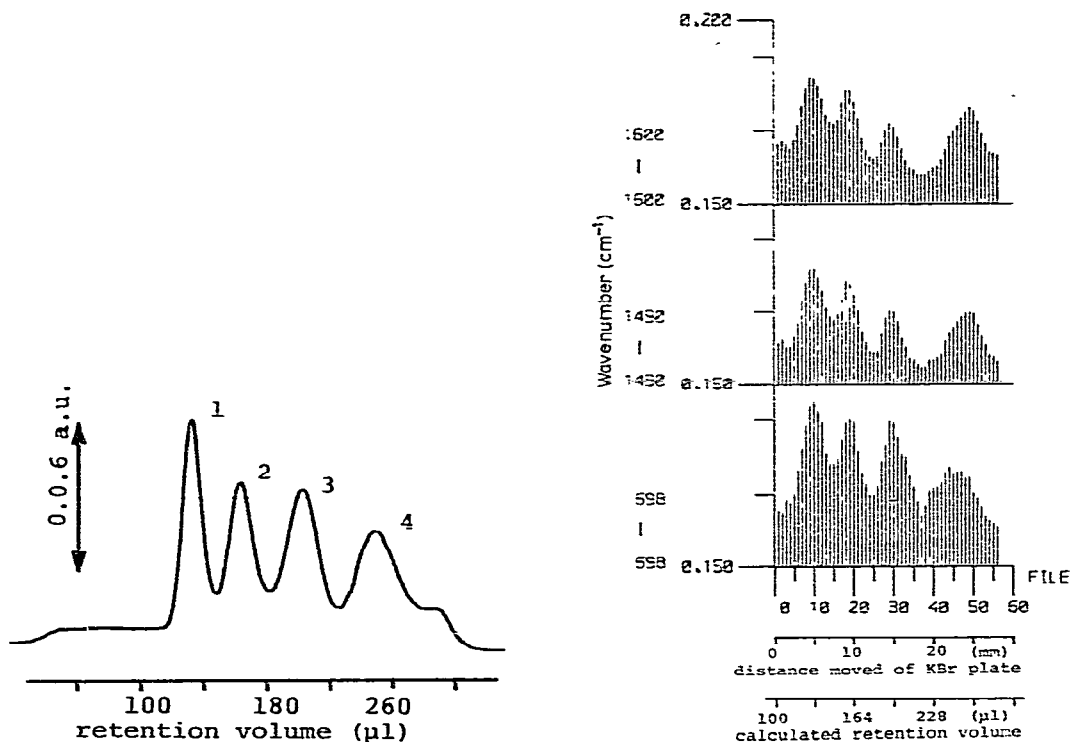


Fig. 1. SEC chromatogram of polystyrene standards measured with a UV detector at 254 nm. MHPLC conditions: column, 22 cm \times 0.1 cm I.D. PTFE tube; packing, TSK GEL G-3000 H; mobile phase, tetrahydrofuran, 8 μ /min. Peaks: 1 = mol.wt 37,000 (8.8 μ g); 2 = mol.wt. 10,200 (7.2 μ g); 3 = mol.wt. 2800 (7.7 μ g); 4 = mol.wt. 500 (28.7 μ g).

Fig. 2. FT-IR chromatograms at various wavenumbers. Spectrometer: JEOL JIR-40X. Mirror rate: 1.6 mm/sec. Detector: TGS. Accumulation: 64 \times times. Resolution: 8 cm^{-1} . Peak maxima of PS 37,000, 10,200, 2800 and 500 appear at 130, 159, 192 and 254 μ l, respectively.

RESULTS

SEC has a unique separation mechanism based on differences in the molecular size of the components of a sample. Its chromatograms are usually evaluated by determining the molecular weights corresponding to a given retention volume, with help of pre-determined calibration graphs. For this purpose, a mixture of polystyrene standards was eluted through a micro-SEC column. The resulting UV chromatogram and FT-IR chromatograms at characteristic absorption bands of the solutes are shown in Figs. 1 and 2, respectively. It can be seen that the FT-IR chromatograms obtained by the buffer memory technique reflect the original chromatographic separation without a decrease in resolution. In the present work, interferometric data are collected every 0.5 mm on the plate. The flow-rate of the mobile phase is 8 μ l/min and the deposition rate of the effluent is 1.25 mm/min. Hence the elution volume between each file corresponds to 3.2 μ l. The calculated elution volumes at each peak maximum are indicated in the figures, with file numbers and distances on the plate.

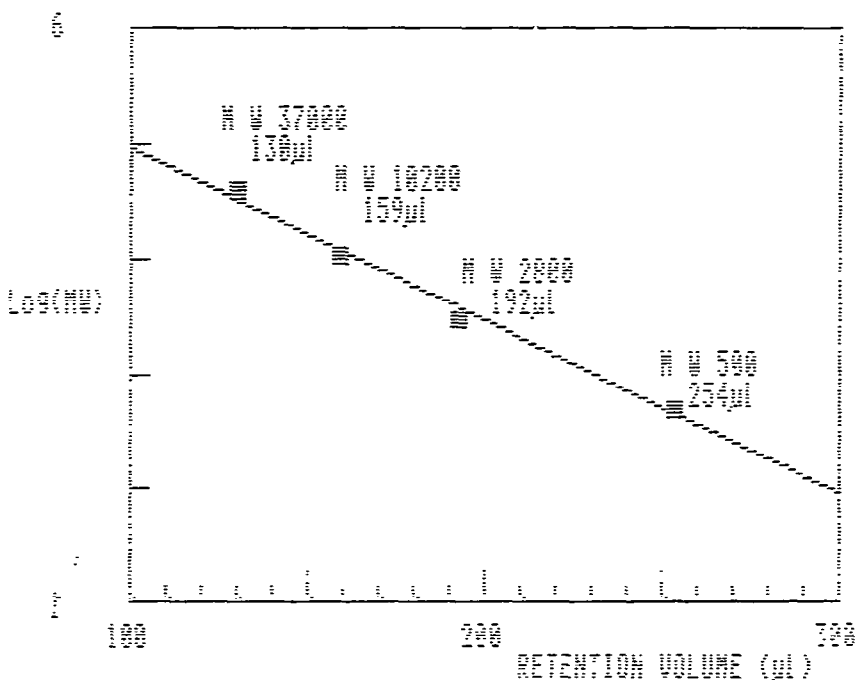


Fig. 3. Peak position calibration graph for a series of polystyrene standards obtained by the potassium bromide buffer memory technique. MW = Molecular weight.

Fig. 3 shows the peak position calibration graph obtained by plotting the retention volumes for each peak against the logarithms of the corresponding molecular weights.

Carbowax 6000 was analysed under the same conditions (the molecular weight was expected to be about 6000). This sample is of interest because it is a high-molecular-weight polymer of polyethylene glycol that has been widely used as a stationary phase in gas chromatography. Naturally, it cannot be analysed by gas chromatography. In addition, it has no absorption in the UV region so that it is impossible to detect it on a UV detector.

The FT-IR chromatograms of the compound are shown in Fig. 4. Its peak maximum appears at a retention volume of 161 μl , which corresponds to a molecular weight of 10,800 on the calibration graph established above. However, this value is greater than expected value. This is due to the fact that, in general, different types of molecules give different calibration graphs because of their characteristic molecular configurations. According to Mori and Yamamoto¹³, the reduced molecular weight of polyethylene glycol is given by

$$M = 1.21 \cdot M_s^{0.916} \quad (1)$$

for $M < 10000$, where M_s is the molecular weight of polyethylene glycol obtained from the peak position on the polystyrene calibration graph. Utilizing this equation, the molecular weight of Carbowax 6000 is calculated to be 5990. The transmittance

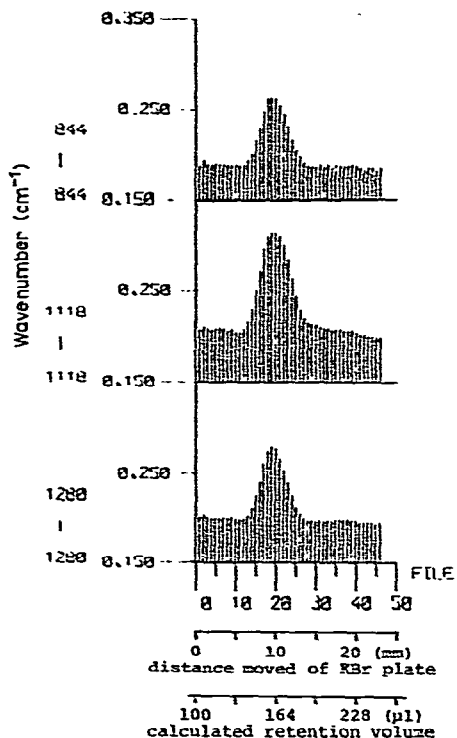


Fig. 4. FT-IR chromatograms of Carbowax 6000 at various wavenumbers. Sample weight: 15.7 μg . Other conditions as in Fig. 2.

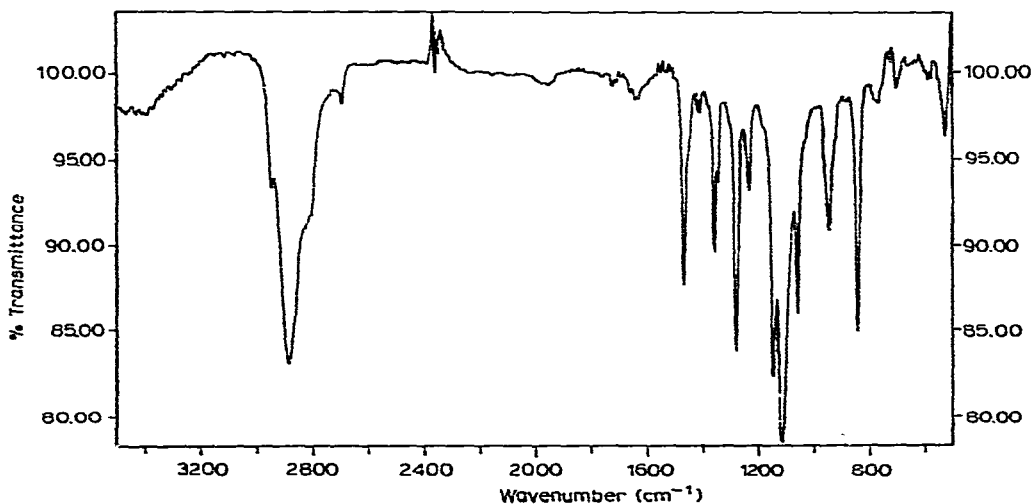


Fig. 5. IR spectrum of Carbowax 6000 obtained from file no. 19 in Fig. 4.

spectrum of file No. 19 in Fig. 4 is shown in Fig. 5. This is just spectrum of polyethylene glycol.

DISCUSSION

This work was intended to obtain good chromatograms, rather than a high sensitivity of the technique. We regard these experiments as preliminary in terms of sensitivity and FT-IR system parameters. In fact, the width of the IR beam focus of the FT-IR spectrometer equipped with the $3 \times$ beam condenser was about 2.5 mm, while that of the solute-deposited band was about 1.5 mm. In order to improve the signal-to-noise ratio, a small aperture (4×1 mm) was placed in front of the memorized plate in this experiment. The use of this aperture resulted in a significant reduction in the measurement efficiency. Moreover, an MCT detector would have been desirable but was not used here. We plan to optimize these parameters in the future.

The buffer memory technique investigated here has several advantageous features, outlined below.

Use of MHPLC

For the separation, this technique utilizes MHPLC, developed by Ishii and co-workers¹⁴⁻¹⁷. Its separation ability is comparable to that of conventional HPLC. One of the principal advantages of using MHPLC is that the flow-rate of the mobile phase is extremely low, compared with that of conventional HPLC. Hence it is easy to eliminate solvents and interference from the mobile phase in the spectrum. In the flow-cell technique, the interference caused by the strong IR absorption of the mobile phase solvent is serious, especially when gradient elution is needed for chromatography. Lack of interference from the mobile phase also enables the total absorbance to be measured in a wide wavenumber range provided that absorption due to the

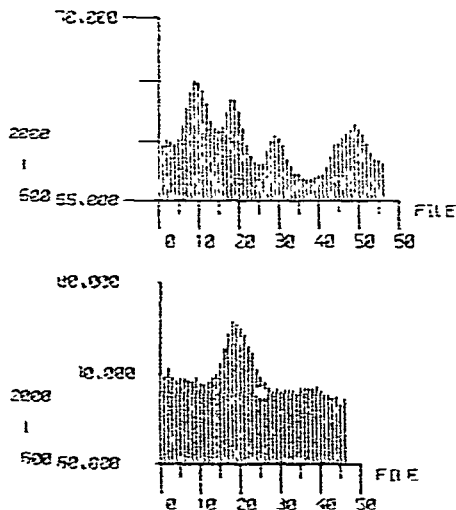


Fig. 6. FT-IR chromatograms of polystyrene standards and Carbowax 6000 for a wide wavenumber range of $600\text{--}2000\text{ cm}^{-1}$. (A) Polystyrene standards; (B) Carbowax 6000. Conditions as in Fig. 2.

background is kept constant in every part of the plate. Fig. 6 shows the chromatograms of polystyrenes and Carbowax 6000 measured at 600–2000 cm^{-1} . It is important to note that the absorbance values obtained in this way are much higher than those in Figs. 2 and 4. The FT-IR chromatograms obtained with a wide wavenumber range seem to be similar to those at fixed wavenumbers. This result gives us the important information that IR can be used as an MHPLC detector by measuring the total absorbance over a wide wavenumber range even if the characteristic absorption wavenumbers of the sample are unknown. Actually, this is not the best way to obtain highly sensitive detection with a low signal-to-noise ratio, but IR can be useful as a universal detector in LC based on this non-dispersive¹⁸ concept using the potassium bromide buffer memory technique.

Perfectly continuous chromatogram

Because the effluent is deposited continuously on the potassium bromide plate, a perfectly continuous chromatogram is obtained, whereas chromatograms obtained by the automated diffuse reflectance–solvent removal technique are only collections of absorbance values for each fraction.

Further characterization

The sample deposited on the plate remains as the “chromatogram”. This permits subsequent detailed characterization by other instrumental techniques.

Mechanical simplicity

As the buffer memory technique utilizes the well established IR absorption method, there is no need for a sophisticated system and auxiliary instruments. The technique requires only a simple interface and it is very easy to set up and operate the interface for a particular analysis.

However, there is one drawback to this technique. It is not suitable for solutes that are more volatile than the mobile phase used. Although this is a serious problem common to solvent removal techniques, such solutes can be separated by gas chromatography.

In summary, the buffer memory technique enables completely continuous chromatograms to be measured without any interference from the mobile phase solvents.

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