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QUANTITATIVE GEL-PERMEATION CHROMATOGRAPHY WITHOUT STANDARDS

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SUMMARY

Samples of motor oils are studied using gel-permeation chromatography and refractive index detection. When two eluents of similar properties but different refractive indices are used in succession, two distinct chromatograms are obtained for the same analyte. This information can be used to predict the volume fraction, the mole fraction, and the refractive index of each fraction of the analyte as it elutes from the column. The procedure does not involve complete separation of the components, identification of the components, or knowing the physical properties of the components of the analyte.

INTRODUCTION

In gel-permeation chromatography (GPC), very often one is dealing with samples that have a distribution of components of various sizes. This is particularly true in applications to characterize fossil fuels[1] and polymers[2]. It is unlikely that complete separation of the components in these samples can be achieved in liquid chromatography (LC). Still, it is meaningful to obtain a quantitative distribution curve for these samples, for the purpose of characterization. The difficulty is that since the nature of the components is generally not known, concentration standards are not available to calibrate the response of the detectors. One can, therefore, only obtain chromatograms that show an arbitrary response. The only alternative currently practiced[3] involves the use of preparative-scale columns and the collection of fractions afterwards. By evaporating off the eluent, one can in principle obtain the volume or the weight of sample collected in each fraction. Not only is the procedure tedious and time-consuming, but the results obtained can also be easily influenced by the volatility and the chemical stability of the collected materials. It is thus desirable to develop a procedure to obtain the same information using analytical or micro-scale LC.

Recently[4], we have demonstrated that quantitative analysis is possible without analyte identification using high-performance liquid chromatography (HPLC) and the refractive index (RI) detector when well-resolved peaks can be obtained for the chro-

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matograms. This concept can be extended to the case of unresolved chromatograms. Very briefly, the procedure is based on the relationship between the RI observed for a mixture (the eluent and the solutes), n, and the individual RIs, n_i:

$$\frac{n^2 - 1}{n^2 + 2} = \sum_i V_i \left(\frac{n_i^2 - 1}{n_i^2 + 2}\right) \tag{1}$$

where V_i is the corresponding volume fraction of each component at the detector. For a well-resolved chromatographic peak (single solute), one can see that the concentrations are, respectively, V_x and $(1-V_x)$ for the solute and the eluent, while the RIs are, respectively, n_x and n_1. Eqn. 1 then reduces to:

$$F_n - F_{n_1} = V_x(F_{n_x} - F_{n_1}) \tag{2}$$

where F is a defined function such that $F_{n_i} \equiv (n_i^2 - 1) / (n_i^2 + 2)$. For all practical levels of concentration in LC, *i.e.* before column saturation occurs, the left-hand side in eqn. 2 can be simplified[4] for the case of a differential refractometer to give

$$S_1K_1 = V_x (F_{n_x} - F_{n_1}) \tag{3}$$

where S_1 is the integrated response (peak area) for the analyte in eluent 1 and K_1 is a constant for the conditions used with eluent 1, including the eluent flow-rate, the integrating interval, the scale expansion used at the detector, and the RI of the eluent. If the same sample is then eluted with a different eluent, *i.e.* one having a different RI of n_2, V_x and F_{n_x} remain constant while a different peak area is obtained, such that

$$S_2K_2 = V_x(F_{n_x} - F_{n_2}) \tag{4}$$

where 2 indicates parameters relevant to eluent 2. Eqns. 3 and 4 together allow unique values of V_x and F_{n_x} to be obtained. The concentration of the analyte is thus determined without analyte identification.

It can be shown[4] that it is not necessary to know even n_1 or n_2 if only V_x is to be determined. This is done by obtaining the chromatographic peak areas when known concentrations of each eluent is used alternately as samples in the other eluent. This eliminates contributions due to uncertainties in the experimental parameters, as long as those remain fixed throughout. In GPC, however, the eluents are usually of low molecular sizes and may not conveniently elute as samples in each other. In such cases, we can use two additional compounds, with RIs n_3 and n_4, to obtain two more sets of areas in the same eluents. So,

$$S_3K_1 = V_3(F_{n_3} - F_{n_1}) \tag{5}$$

$$S_4K_2 = V_3(F_{n_3} - F_{n_2}) \tag{6}$$

$$S_5K_1 = V_4(F_{n_4} - F_{n_1}) \tag{7}$$

$$S_6K_2 = V_4(F_{n_4} - F_{n_2}) \tag{8}$$

It is more convenient, but not necessary, to use the same concentration V for these two compounds, so that $V = V_3 = V_4$. Doing this, eqns. 5–8 give:

$$\frac{K_2}{K_1} = \frac{S_3 - S_5}{S_4 - S_6} \tag{9}$$

Now, eqns. 5 and 6 give:

$$\frac{F_{n_2} - F_{n_1}}{K_1} = \frac{S_3 - (K_2/K_1)S_4}{V} \tag{10}$$

and eqns. 3 and 4 give:

$$\frac{F_{n_2} - F_{n_1}}{K_1} = \frac{S_1 - (K_2/K_1)S_2}{V_x} \tag{11}$$

Combining eqns. 9–11, the final result is:

$$V_x = V\left[\frac{S_1 - S_2\left(\frac{S_3 - S_5}{S_4 - S_6}\right)}{S_3 - S_4\left(\frac{S_3 - S_5}{S_4 - S_6}\right)}\right] \tag{12}$$

Eqn. 12 implies that quantitative determination is possible without knowing any of the properties of the eluents, the analyte, or the two "calibrating" compounds. The only requirements are that the two RIs n_3 and n_4 are quite different, so that $(S_3 - S_5)$ and $(S_4 - S_6)$ can both be determined with good precision, and that the two RIs n_1 and n_2 are quite different (but not necessarily different from n_3 or n_4), so that the subtractions in the numerator and in the denominator of eqn. 12 can retain significance. We also note that S_3 through S_6 need only be determined once for a given set of eluents 1 and 2.

If now one can independently obtain the values n_1 and n_2, the RI of the analyte, n_x, can be determined. This is because eqns. 3, 4 and 9 give:

$$\frac{F_{n_x} - F_{n_1}}{F_{n_x} - F_{n_2}} = \frac{S_1K_1}{S_2K_2} = \frac{S_1}{S_2}\left(\frac{S_4 - S_6}{S_3 - S_5}\right) \tag{13}$$

The function F_{n_x} can then be solved for in terms of the peak areas and the functions F_{n_1} and F_{n_2}.

When several components, x, y, z, etc. coelute at a given point in the chromatogram, one notes that V_x, V_y, V_z, etc., represent their individual concentrations, and $(1 - V_x - V_y - V_z - \ldots)$ represents the concentration of the eluent. Using the same procedure above, one obtains an expression identical to eqn. 12 except that the left-hand side is replaced by $(V_x + V_y + V_z + \ldots)$. The total concentration is then determined for that point in the chromatogram. Eqn. 13 can still be used, but the calculated refractive index becomes the concentration-weighted RI of all components at that point.

The above procedure requires that the chromatograms in the two different eluents be correlated, so that the correct set of peak areas is used for the calculations. The problem is simplified in GPC, where separation depends on the sizes of the analyte molecules and is relatively independent of the eluent used. Even though the chromatograms are not necessarily totally resolved, the elution order is retained. One can then correlate each slice of the two chromatograms and apply eqns. 12 and 13. So, the tedious "consistency" test reported earlier[4] can be omitted. In what follows, we shall present a study of the distribution of components in motor oils using GPC using this scheme.

EXPERIMENTAL

All reagents and eluents used were reagent grade materials without further purification. The chromatographic system used was conventional, and consisted of a metering pump (Micrometrics, Norcros, GA, U.S.A., Model 750), a 30 cm 4.6 mm I.D., 100 Å, 5-μm PL gel-permeation column (Anspec, Warrensville, IL, U.S.A.), a 1-μl sample loop at a conventional injection valve (Rheodyne, Berkeley, CA, U.S.A., Model 7410), and a commercial RI detector (Waters Assoc., Milford, MA, U.S.A., Model R401) with the reference cell used in the static mode filled with the eluent being used. A flow-rate of 0.67 ml/min was used throughout. Solutions with specified volume fractions were made by pipetting well-defined volumes of the minor component into a volumetric flask, and then filling to mark with the major component.

The output of the RI detector (10 mV full scale) is connected to a digital voltmeter (Keithley, Cleveland, OH, U.S.A., Model 160B), the analog output of which is in turn connected to a computer (Digital Equipment, Maynard, MA, U.S.A., Model PDP 11/10 with LPS-11 laboratory interface). The computer takes readings every 0.05 sec, and averages each set of 10 before storing the information. These numbers then represent the areas (S values) for each 0.5 sec of elution time. All areas are determined using multiple injections (three or more) and are found to be reproducible to ±2.5% (relative standard deviation). Linearity of the detector was independently confirmed by a series of samples at successive dilutions.

RESULTS AND DISCUSSION

It is important first to establish the optimum experimental conditions. The two eluents should be chosen to have significantly different RIs but similar chromatographic properties. A consideration of the solubility parameters, δ, for the eluents recommended by the manufacturer of the column shows that tetrahydrofuran (THF) and benzene are good candidates. The nature of the neutral polystyrene–divinylbenzene particles in the PL gel columns provides a minimum of adsorptive and other interactions in addition to the desired molecular-size selectivity. The similarity in δ for benzene and THF further guarantees closely matching interactions, if any. To test this, we used a mixture of phthalate esters (Supelco, Bellefonte, PA, U.S.A., Kit 606-N) to establish retention volumes, V_R, in each eluent. The molecular weights and the densities of these esters[5] allow us to derive the molar volumes, V_M, for each of them. The results are:

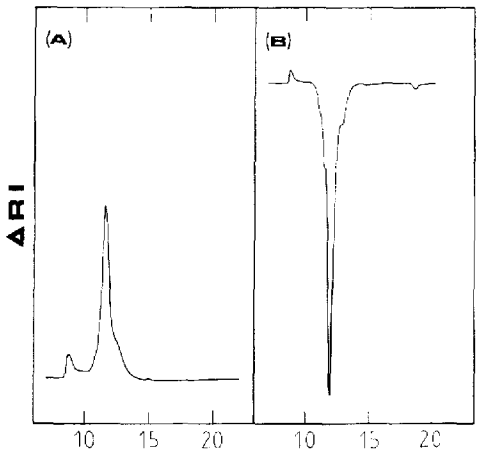
$$\text{benzene: } \log V_M = -0.0894\ V_R + 4.352 \tag{14}$$

THF: $\log V_M = -0.1044\ V_R + 4.582$ (15)

Despite the careful choice of conditions, the retention volumes are not reproduced exactly on changing eluents. The discrepancy can be attributed to slight differences in the degree of swelling of the resins in the two eluents, modifying the effective pore sizes, and probably not the presence of other mechanisms of retention. This is further verified by the close agreement between observed and predicted retention volumes for heptane, bromoethane, and carbon tetrachloride, using eqns. 14 and 15. It is expected that for non-polar substances that form ideal solutions with each of the eluents, the retention volumes will be predicted with reasonable accuracy. Eqns. 14 and 15 thus allow us to correlate particular portions of the two chromatograms and allow application of eqns. 12 and 13. This of course can be checked using the consisitency test reported earlier[4].

In Fig. 1, we show the RI chromatograms obtained for a sample of synthetic motor oil (Amsoil, Superior, WI, U.S.A., synthetic 10W-40) in THF (A) and in benzene (B). The chromatograms show two major features, at 8.7 min and 11.6 min for THF, and at 8.7 min and 11.9 min for benzene. Trials with other natural motor oils (*e.g.* Pennzoil, Oil City, PA, U.S.A., multi-vis 10W-40) give similarly reproducible retention times in the two eluents for the major features. This confirms the feasibility of obtaining correlated chromatograms in two different eluents for these samples. It is interesting to note that the main features in most of these chromatograms appear at *ca.* 10 min, or a retention volume of 6.7 ml. This is the retention volume at which eqns. 14 and 15 give the same V_M. In other words, molecules eluted in this region are expected to show the least variations in retention volumes in the two eluents. Little error is introduced if one assumes that a given component of the sample has identical elution volumes in each of the two eluents.

To provide the values S_3–S_6, samples of known concentrations V_3 and V_4 of heptane and α-chloronaphthalene, respectively, were used. These were chosen based on



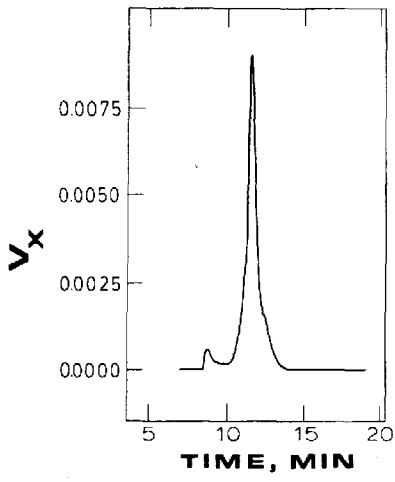


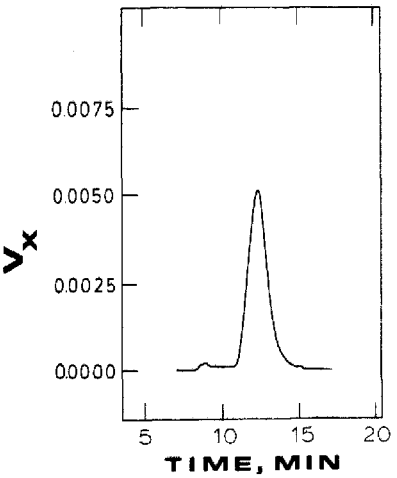
Fig. 1. Refractive index gel-permeation chromatograms of a synthetic motor oil in (A) THF and (B) benzene as eluents. The scale expansion used in B is twice as sensitive as the one used in A.

Fig. 2. Concentration of components as the volume fraction eluted in a 0.5-sec interval (5.6 μl) for a synthetic motor oil.

the large difference in RI for the two compounds. Using eqn. 9, we found that our experimental conditions correspond to a value of $K_2/K_1 = 1.044$. As a check, we used eqn. 12 for samples of known concentrations of carbon tetrachloride, bis-(2-ethylhexyl)-phthalate, butylbenzylphthalate, and dimethylphthalate, and obtained an average accuracy of 3.7%, which can be attributed to uncertainties in the area measurements.

The chromatograms in Fig. 1 are then divided into 0.5 sec intervals. The areas for each interval are successively used as S_1 and S_2 in eqn. 12 to determine the concentrations eluted at each interval. The result is presented in Fig. 2. The ordinate then corresponds to the volume-fraction concentration eluted every 0.5 sec, or every 5.6 μl of eluent. The integration interval was chosen to produce a smooth and continuous display, and not based on the available efficiency of the column. We note that the peak consists of a distribution of components rather than a single component, as judged from the total elution time involved. The total integrated area in Fig. 2 is in good agreement with the 1-μl injection volume. Similar results are obtained with other motor oils, as seen in Fig. 3. Apparently the synthetic motor oil has a sharper distribution of components than the natural motor oil, which is a reasonable result. The utility of this quantitative scheme is now obvious. Fig. 1A or B alone does not provide a correct picture of the distribution of the components, since the RIs of the components are not known. Fig. 2, however, gives the correct amount of materials eluted at any time regardless of the RI of the components, and should be identical with results obtained by the tedious method of collecting fractions.

Using values for $n_{THF} = 1.4050$ and $n_{benzene} = 1.5011$, eqn. 13 can be used to predict the RIs of the components as they elute off the column. The results are shown in Fig. 4. Eqn. 13 naturally becomes meaningless when little or no material is being eluted, *i.e.*, when S_1 and S_2 approach the baseline noise level. We have thus arbitrarily set 1.400 as the "baseline" whenever the volume fraction eluted falls below 3% of the peak volume fraction in Fig. 2. Fig. 4 provides interesting insight into the nature of the



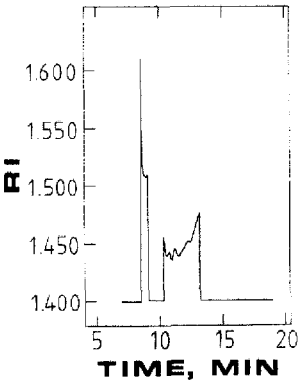


Fig. 3. Concentration of components as the volume fraction eluted in a 0.5-sec interval (5.6 μl) for a natural motor oil.

Fig. 4. Refractive indices of the components as they elute from the column for a synthetic motor oil.

components in the sample even though it represents only the concentration-weighted RIs, and shows information not normally available using other methods. The structure between 10 and 12 min is real, considering the typical efficiencies of this type of column. As expected, the RI distributions obtained are quite different between natural and synthetic motor oils.

One can go one step further and calculate the mole fraction of materials eluted at any point in the chromatograms. This is because the retention volumes are related to the molar volumes according to eqns. 14 and 15. At each integration interval, the total volume V_T is equal to the sum of the volumes of the analytes, $V_T V_x$, and the volume of the eluent, $V_T(1 - V_x)$. The numbers of moles of the analyte and the eluent are, respectively, $V_T V_x / V_M$ and $V_T(1-V_x)/V_E$, where V_M is given by eqns. 14 and 15 and V_E is the molar volume of the eluent. At the concentrations used, $V_x << 1$ and the number of moles of the eluent can be simplified to V_T/V_E. The total number of moles at each interval is also approximately equal to V_T/V_E. The mole fraction eluted at any interval, C_x, is then

$$C_x = V_x V_E / V_M \tag{16}$$

Using eqn. 16, we obtained the concentrations shown in Fig. 5. This is to be compared with Fig. 2. The most obvious difference is the magnification of the feature *ca.* 18 min in Fig. 5. This is a direct result of the weighting according to molar volumes, such that the smaller molecules for the same detector response correspond to a larger number present. The total integrated area for Fig. 5 is in fact the number of moles of sample injected. This is useful information because it reflects the average size of the molecule in the sample. We found that the total C_x area is larger for the natural oil than the synthetic oil, consistent with the distributions in Fig. 2 and 3.

Finally, it should be emphasized that correlated chromatograms in different eluents may not be available for the sample of interest. A very tedious consistency test

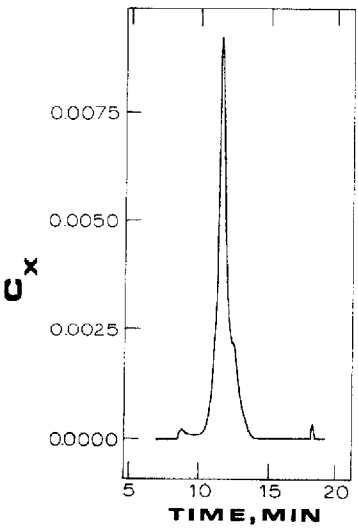


Fig. 5. Concentration of components as the mole fraction eluted in a 0.5-sec interval (5.6 μl) for a synthetic motor oil.

must then be applied[4]. For example, we have performed similar studies using various solvent-refined coals[6]. There, the presence of polar compounds and possible hydrogen bonding with THF contribute to uncertainties in the correlation. It may be possible, however, first to obtain, say, the non-polar aliphatic fraction of these solvent-refined coals before applying this scheme.

In summary, we have devised a method for obtaining quantitative information in GPC that does not require identification of the analytes. The procedure is not only more efficient and more convenient, but the results are also more reliable and more illuminating.

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