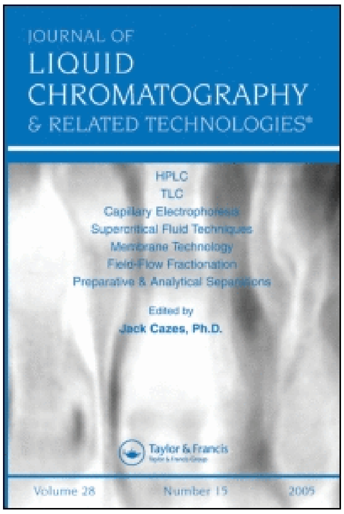

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DIFFERENTIAL GEL PERMEATION CHROMATOGRAPHY

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DIFFERENTIAL GEL PERMEATION CHROMATOGRAPHY

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ABSTRACT

A new technique called Differential Gel Permeation Chromatography (DGPC), in which a reference solution is used as eluent and the solution to be analyzed as the injected solution, is proposed. It can detect minute differences between the reference solution and the analyzed solution. A null signal indicates that the reference solution and the analyzed solution are identical and a nonzero signal reflects the detailed differences between the reference and the analyzed solution.

Two examples, concentration identification of five ethanol solutions and brand identification of three lubricant oils, are given to show the application of the DGPC method. The potential application of the DGPC method to the identification of counterfeit products is also discussed.

INTRODUCTION

In both routine analysis and research work we are often required to answer such questions as: are the composition and concentration of a sample that has been

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analyzed exactly the same as those of a reference sample? If not, how can we describe the differences? Although there are a lot of analytical techniques available, only a few of them are suitable for any particular task. Gel Permeation Chromatography (GPC) may be a good choice when the sample consists of components of differential sizes.

The GPC technique uses a solvent or solvent mixture as the eluent and a solution prepared by dissolving the sample in a volume of solvent drawn from the eluent reservoir as the injected solution. The GPC column can separate components by size. If a refractometer is installed as the signal detector, the GPC curve of a sample is a graph of the difference in refractive index between the injected solution (sample + solvent) and the eluent (solvent) as a function of the retention volume after separation.

It is often necessary to compare two different samples, say an unknown sample and a reference sample, as would be the case in detecting of counterfeit versions of a product. In such cases, each sample solution is compared to the eluent solution and then the two GPC curves are compared to each other. When the analyzed sample and the reference sample have the same composition and concentration they should have the same GPC curves under fixed experimental conditions; otherwise there must be some differences between their GPC curves.

In practice, however, performing GPC experiments is not without problems and it is difficult, or even impossible, to keep all of the experimental parameters exactly the same during the period of measurement. Even minor fluctuations of any parameter can produce changes in the GPC curves. Small differences in two GPC curves due to the different composition of the two solutions can be swamped by differences due to uncontrolled changes in experimental conditions. Many experiments in our laboratory have shown that when the differences between two samples are small, conventional GPC is not a reliable method, due to the unavoidable small variations in the experimental parameters.

To overcome the limitations of conventional GPC a new technique, called differential gel permeation chromatography (DGPC), is proposed. In DGPC a reference sample solution is used as the eluent and the sample solution to be analyzed is the injected solution. If the analyzed sample and the reference sample are identical, the DGPC curve of the analyzed sample will be a null signal, i.e., a horizontal straight line on the refractive index difference versus retention volume graph.

It is to be noted, that this null signal phenomenon will not be affected by changes in the experimental parameters during the experiment, such as injected volume and flow rate of eluent, because, in this case, they make no difference to the measurement. However, when the analyzed sample and the reference sample are not the same, the differences in composition between the two samples will yield a DGPC curve from which one can see, directly, the detailed differences between the analyzed sample and the reference sample.

The DGPC technique was developed on the basis of eluent gel permeation chromatography (EGPC), which has found practical applications in micellar systems (1–3). In this paper, two examples are given to show the application of the DGPC method. The first is the identification of the concentration of five aqueous solutions of ethanol, and the second involves distinguishing between three brands of lubricant oils.

EXPERIMENTAL

Concentration Identification of Five Aqueous Solutions of Ethanol

The aqueous GPC system comprised two columns, each 30 cm long packed with TSK-PW (G2000 and G1000), and a model R410 differential refractometer. All measurements were carried out at a temperature of 35°C, controlled by means of a block thermostat. The ethanol used was analytical grade purity. Five aqueous solutions of ethanol were prepared with concentrations (w/w) of 5.0%, 4.9%, 4.8%, 4.5%, and 4.0%. When doing conventional GPC, the eluent was water and when doing DGPC, the eluent was the 5.0% aqueous solution of ethanol. The flow rate was 1.0 $\text{cm}^3\ \text{min}^{-1}$ and the injected volume was 40 μL.

Brand Identification of Three Brands of Lubricant Oils

Measurements were carried out at room temperature by means of a liquid chromatograph equipped with a model R401 differential refractometer. A single column packed with μ-Styragel (nominal porosity 500 Å, length 30 cm) was used. Analytical grade purity THF was used as the solvent and it was purified and filtered before use. The three brands of lubricant oils were purchased from local shops. The brands were DELVAC 1330 (sample A), DELVAD 1130 (sample B), and ATF 220 (sample C), all MOBIL products.

When doing conventional GPC, the eluent was THF and, when doing DGPC, the eluent was the THF solution of one of the three oils. A 2% (w/w) THF solution for the eluent or the injected sample was prepared. As before, the flow rate was 1.0 $\text{cm}^3\ \text{min}^{-1}$ and the injected volume was 40 μL.

RESULTS AND DISCUSSION

Concentration Identification of Five Aqueous Solutions of Ethanol

In principle, given five aqueous solutions of ethanol with concentrations (w/w) of 5.0%, 4.9%, 4.8%, 4.5%, and 4.0%, we can identify which is which

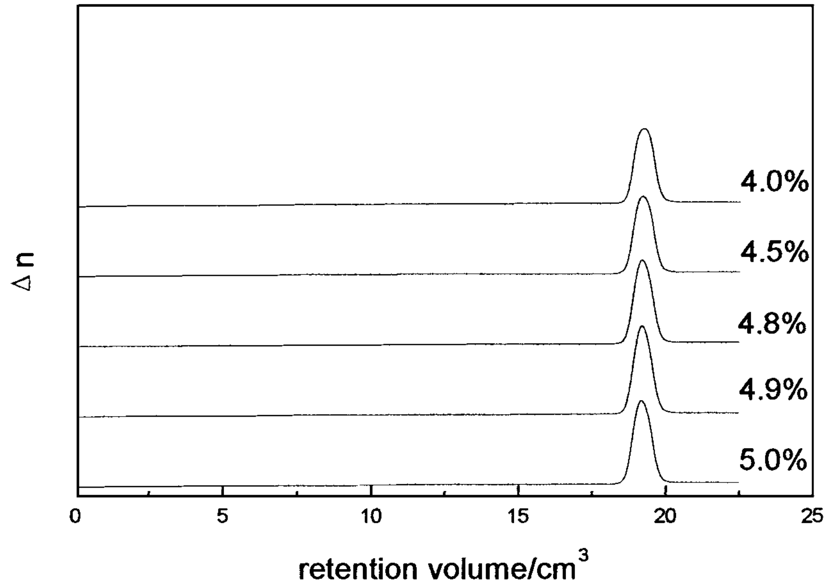

Figure 1. Conventional GPC curves of ethanol solutions, using water as eluent. The concentrations of each sample are indicated.

using conventional GPC. According to the relationship (4)

$$A = k\frac{dn}{dc}W, \tag{1}$$

where A is area under the peak of the GPC curve, k is the instrumental response constant, dn/dc is the refractive index increment, and W is the mass of solute in the injected solution; the higher the concentration, the larger the GPC peak is.

However, this is true only if the experimental conditions and injected volume are kept unchanged as the experiment is repeated for each sample. The experimental

Table 1. Areas of the Conventional GPC Peaks from Figure 1

Concentration of Ethanol in Injected Solution (w/w)	Peak Area (cm^2)
4.0%	0.73
4.5%	0.80
4.8%	0.88
4.9%	0.91
5.0%	0.88

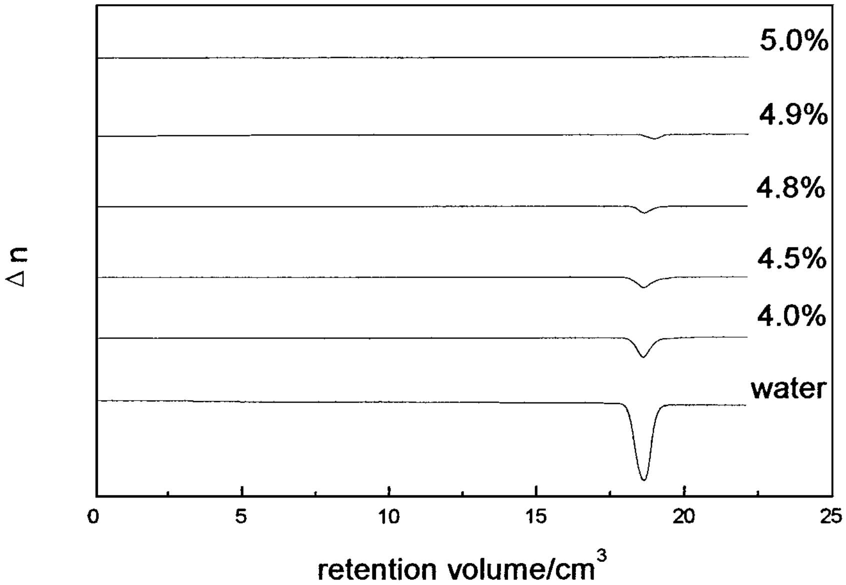

Figure 2. DGPC curves of ethanol solutions and water, using 5.0% ethanol solution as eluent. The concentrations of each sample are indicated.

chromatograms obtained by using conventional GPC are shown in Fig. 1 and the areas of the five peaks are listed in Table 1. Table 1 shows that the ordering of the areas is different from that of concentrations, e.g. the area of the 4.9% solution peak is larger than that of the 5.0% solution. This seemingly abnormal situation is frequently encountered in our daily work, especially when the difference of concentration between samples is small. The failure of conventional GPC in this exercise of concentration identification may be partly due to the fact that the error in the injected volume was more than 2% without considering the fluctuations of other experimental parameters.

When DGPC was employed to analyze the same samples, a 5.0% ethanol solution was used as the eluent. The injected samples were ethanol solutions of 5.0%, 4.9%, 4.8%, 4.5%, 4.0%, and water, respectively. The DGPC curves were shown in Fig. 2. The DGPC curve of 5.0% ethanol solution is a horizontal line because there is no difference between the eluent and the injected sample.

The DGPC curve of 4.9% ethanol solution has a small negative peak indicating the lower concentration of ethanol. As the concentration of the injected sample decreases, i.e. the difference between the injected sample and the reference solution increases, the size of the negative peak increases. An extreme situation is reached when pure water is injected. The DGPC curve of water is the mirror image of the conventional GPC curve when a 5.0% ethanol solution is injected as the sample solution, as was demonstrated in so-called vacancy GPC (5,6).

As a matter of fact, conventional GPC and vacancy GPC are two special forms of DGPC. DGPC will turn into conventional GPC when the reference is pure solvent and the injected solution is the sample to be analyzed, and into vacancy GPC when the reference is the analyzed sample and the injected solution is pure solvent or solvent mixture.

Even a cursory comparison of Fig. 1 and Fig. 2 shows that DGPC has a higher sensitivity than conventional GPC for detecting the differences between samples.

Brand Identification of Three Brands of Lubricant Oils

As a practical application, three brands of lubricant oils, DEVLAC 1330, DEVLAD 1130, and ATF 220, which have similar GPC curves, as shown in Fig. 3, were identified by DGPC. The differences in their DGPC curves are obvious, as shown in Fig. 4, when DELVAC 1330 solution was used as the eluent.

Figure 4a gives the differential results for DELVAC 1330 to DELVAC 1330, Fig. 4b those of DELVAD 1130 to DELVAC 1330, and Fig. 4c those of ATF 220 to DELVAC 1330. Figure 4a is a straight line because the difference between the reference (eluent) and the analyzed sample (injected solution) is Zero. Figure 4b indicates that at retention volume of 7.0 cm^3 there is a small positive peak and at

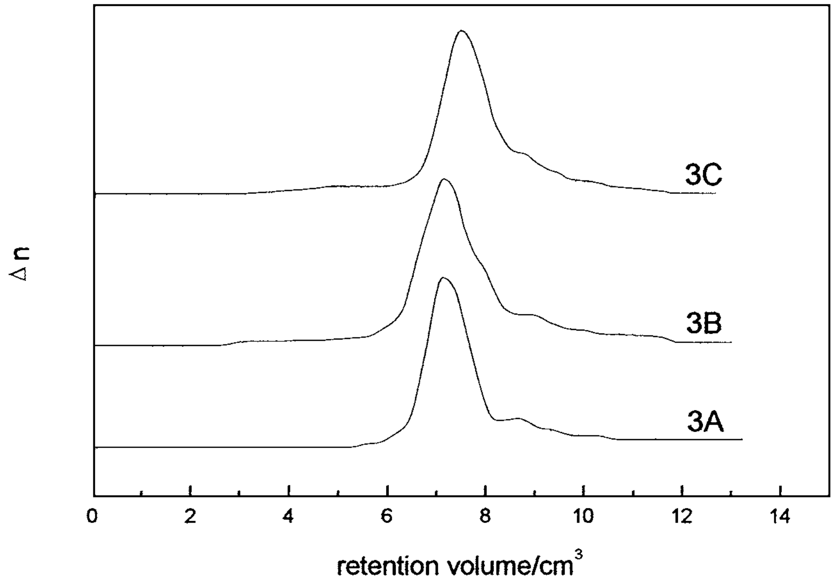

Figure 3. Conventional GPC curves of the three oil samples, using THF as the eluent. DELVAC 1330, b) DELVAD 1130, and c) ATF 220.

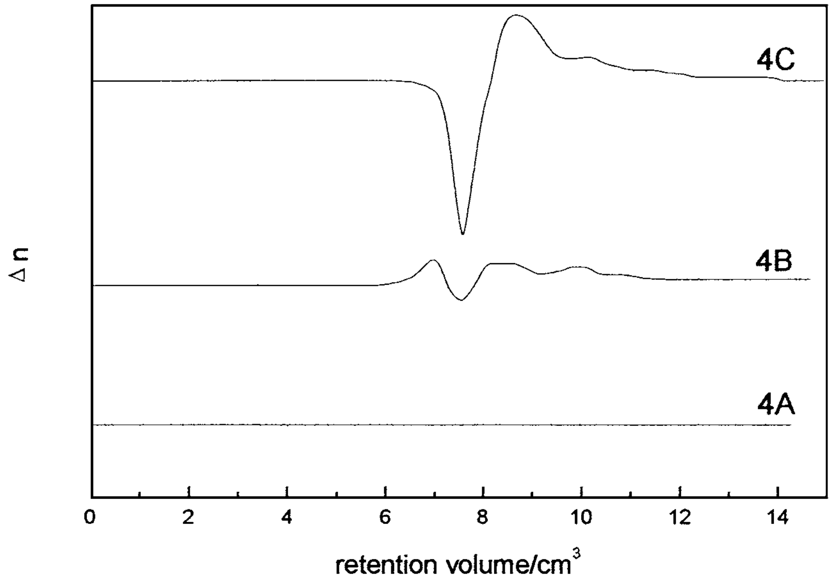

Figure 4. DGPC curves of the three oil samples, using THF solution of DELVAC 1330 as eluent. a) DELVAC 1330, b) DELVAD 1130, and c) ATF 220.

retention volume of 7.5 cm^3 there is a small negative peak. Figure 4c indicates that at the retention volume of 7.5 cm^3 there is a large negative peak and at the retention volume of 8.6 cm^3 there is a small positive peak. Figure 5 and Fig. 6 are the DGPC curves of the three oil solutions when DELVAD 1130 solution and ATF 220 are used as the eluents. The high sensitivity and validity of DGPC gain further support from the two straight lines as shown in Fig. 5b and Fig. 6c, and three pairs of roughly symmetric curves, i.e. Fig. 5a–Fig. 4b, Fig. 6a–Fig. 4c, and Fig. 6b–Fig. 5c.

The above consistent experimental results embody the basic mathematical principle that A minus B equals B minus A in magnitude, but opposite in sign. The high sensitivity of DGPC, as shown by the results in Figs. 4–6, makes it particularly useful for identifying counterfeit products when one is sure of having access to the genuine article for use as the reference sample. This sensitivity results, firstly, from the elimination of a need to study the difference of two difference curves, as is the case in conventional GPC.

This not only gets rid of a mathematical process that can introduce large errors into the calculations, but also means that errors in the measurements due to fluctuations in experimental parameters are no longer important. Secondly, the choice of a suitable reference sample, i.e. one as close as possible to the sample to be analyzed, reduces the size of the maximum signal received by the detector.

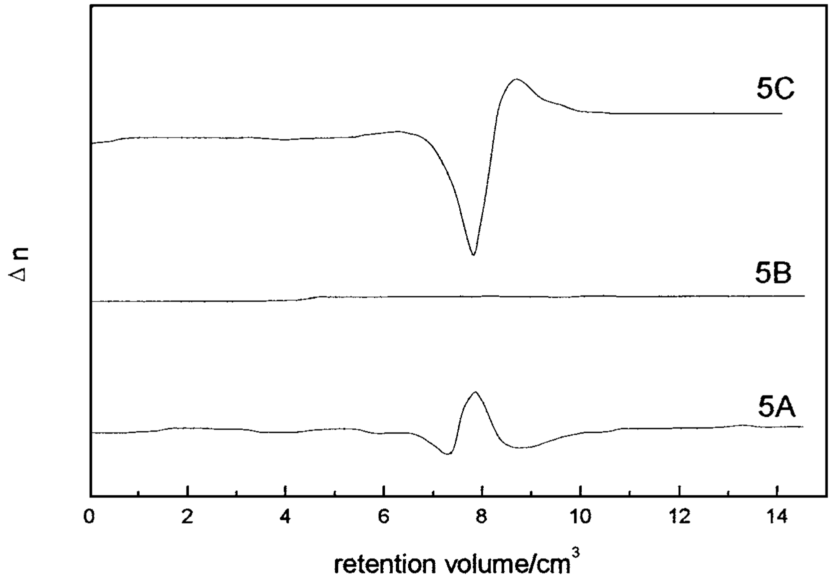

Figure 5. DGPC curves of the three oil samples, using THF solution of DELVAD 1130 as eluent. a) DELVAC 1330, b) DELVAD 1130, and c) ATF 220.

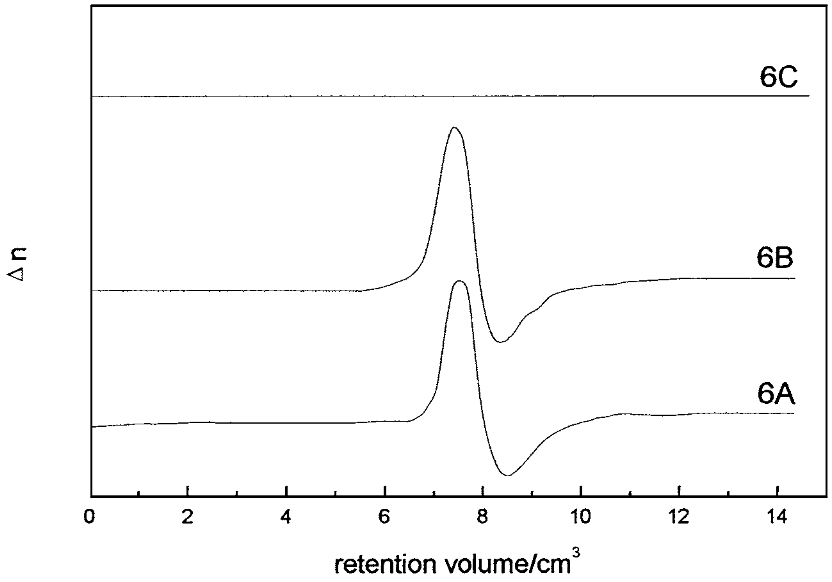

Figure 6. DGPC curves of the three oil samples, using THF solution of ATF 220 as eluent. a) DELVAC 1330, b) DELVAD 1130, and c) ATF 220.

This allows one to turn up the gain of the detector, thereby further increasing the signal to noise ratio.

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