

Application of an Oligomer as an Internal Standard in Gel Permeation Chromatography

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Synopsis

The usefulness of an oligomer as an internal standard in gel permeation chromatography (GPC) has been investigated under deliberately varied experimental conditions—temperatures of the oven and the siphon, and flow rate. It was demonstrated that the effects of variations in the flow rate and temperature of the siphon on the elution volumes can be eliminated, while the effects of variations in temperature of the oven on the elution volumes can be significantly minimized, even if not completely eliminated. Use of an oligomer, possibly having a chemical composition similar to that of the samples, is recommended as an internal standard in GPC experiments for better reproducibility and precision.

INTRODUCTION

In a previous note,¹ it was shown that the use of an oligomer as an internal standard reduces the uncertainty in molecular weight measurements from the usual 5% to better than 1% and avoids the labor of frequent calibration of the columns. To summarize briefly, any effects of unavoidable variations in the experimental conditions on elution volume (EV) of a specimen are easily discernible from the peak position of the internal standard and can be eliminated using the equation

$$V_{\text{corr}} = V_{\text{ob}} \times \frac{S_{\text{calib}}}{S_{\text{ob}}} \quad (1)$$

where V_{corr} = corrected elution volume of a sample, V_{ob} = observed elution volume of the sample, S_{calib} = elution volume of the internal standard used while preparing the calibration curve, and S_{ob} = observed elution volume of the internal standard.

In the present paper, we have investigated the usefulness of an internal standard under deliberately varied experimental conditions. They included variations in the temperature of the siphon and the oven, and variations in flow rate. Such variations are normally caused by changes in ambient temperature which may affect the temperature of the siphon and the oven, particularly when operated at elevated temperatures. The flow rate can

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TABLE I
Observed and Corrected Elution Volume of Fractions as a Function of Flow Rate^a

| Mol. wt | Observed <i>EV</i> | | | | | | Corrected <i>EV</i> for fraction, ml | | |
|----------|--------------------------|------------------|------------|------------------|-------------|------------------|--------------------------------------|------------|-------------|
| | 0.49 ml/min ^b | | 1.0 ml/min | | 1.82 ml/min | | 0.49 ml/min | 1.0 ml/min | 1.82 ml/min |
| | Fraction | <i>n</i> -Decane | Fraction | <i>n</i> -Decane | Fraction | <i>n</i> -Decane | | | |
| 2100 | 30.91 | 35.000 | 31.38 | 35.51 | 31.14 | 35.27 | 31.35 | 31.38 | 31.34 |
| 10,300 | 29.22 | 34.93 | 28.60 | 35.38 | 28.56 | 35.31 | 28.68 | 28.69 | 28.71 |
| 19,850 | 27.25 | 35.00 | 27.51 | 35.38 | 27.55 | 35.40 | 27.64 | 27.60 | 27.62 |
| 51,000 | 25.59 | 35.00 | 25.86 | 35.40 | 25.80 | 35.34 | 25.96 | 25.93 | 25.92 |
| 98,200 | 24.50 | 34.98 | 24.90 | 35.48 | 24.80 | 35.37 | 24.87 | 24.91 | 24.89 |
| 173,000 | 23.82 | 34.94 | 24.15 | 35.43 | 24.05 | 35.27 | 24.20 | 24.20 | 24.20 |
| 411,000 | 22.69 | 34.89 | 23.05 | 35.40 | 22.97 | 35.29 | 23.09 | 23.12 | 23.11 |
| 867,000 | 22.02 | 35.00 | 22.30 | 35.41 | 22.19 | 35.30 | 22.34 | 22.35 | 22.32 |
| 2145,000 | 21.38 | 34.96 | 21.68 | 35.38 | 21.62 | 35.29 | 21.72 | 21.75 | 21.75 |

^a Temperature of the oven = 130°C; temperature of the siphon = 130°C.

^b Flow rate.

be affected under normal operating conditions by the partial blockage of the filters at the ends of the columns. The effects of the flow rate and the temperature of the oven in themselves have been studied in detail by many investigators² and hence need no separate comments.

EXPERIMENTAL

Polystyrene fractions obtained from Waters Associates were used as samples, and *n*-decane was used as an internal standard. A stock solution containing 0.02% (w/v) of *n*-decane in *o*-dichlorobenzene was prepared. A polystyrene fraction, 5 mg, was dissolved in 5 g stock solution of *n*-decane, and 2 ml was injected into the gel permeation chromatograph (GPC).

A GPC manufactured by Water Associates was used with Styragel columns of upper porosity rating of 5×10^4 – 1.5×10^5 Å, 1×10^4 Å, 700–2000 Å, and 100–300 Å. *o*-Dichlorobenzene was used as a solvent. An additional piece of tubing was used in the siphon box to enable the solvent to reach the temperature of the siphon, especially when the temperatures of the oven and the siphon were different. The siphon had a capacity of 5 ml.

RESULTS

The results are divided into three parts: (1) change in flow rate, (2) change in temperature of the siphon, and (3) change in temperature of the oven.

Change in Flow Rate

Keeping the temperatures of the siphon and the oven constant at 130°C, the fractions were injected at three different flow rates. The results are shown in Table I. It can be seen that the results show a variation in the observed elution volumes of the fractions. The elution volumes exhibit a slight maximum with increasing flow rate (columns 2, 4, and 6 of Table I). A similar trend in the elution volume with flow rate has been observed by Meyerhoff³ and Boni et al.⁴ However, when the observed elution volumes are corrected with the aid of the internal standard, the elution volumes of the fractions (columns 8, 9 and 10 of Table I) show virtually no change in their elution volumes for the range of flow rates covered by the table.

Change in Temperature of the Siphon

It is observed that at any given flow rate and temperature of the oven, there was a decrease in elution volume on decreasing the temperature of the siphon. An example is shown in Table II. Though there is a considerable decrease in the observed elution volumes of the fractions (columns 2 and 4 of Table II), on decreasing the temperature of the siphon, there is no change in their elution volumes when corrected with the aid of the internal standard (columns 6 and 7 of Table II). Changes due to variations in the temperature of the siphon are not real changes in elution volume but are

TABLE II
Observed and Corrected Elution Volume of Fractions
as a Function of Temperature of the Siphon

| Mol. wt | Observed <i>EV</i> , ml | | | | Corrected <i>EV</i> for fraction, ml | |
|---------|-------------------------|------------------|----------|------------------|--------------------------------------|-------|
| | 130°C ^a | | 60°C | | 130°C | 60°C |
| | Fraction | <i>n</i> -Decane | Fraction | <i>n</i> -Decane | | |
| 2100 | 33.16 | 37.68 | 31.22 | 35.47 | 31.25 | 31.25 |
| 10300 | 30.35 | 37.75 | 28.61 | 35.57 | 28.54 | 28.56 |
| 19850 | 29.16 | 37.70 | 27.42 | 35.42 | 27.46 | 27.48 |
| 51000 | 27.28 | 37.67 | 25.72 | 35.55 | 25.71 | 25.68 |
| 98200 | 26.16 | 37.62 | 24.63 | 35.44 | 24.69 | 24.67 |
| 173000 | 25.44 | 37.60 | 24.06 | 35.55 | 24.02 | 24.03 |
| 411000 | 24.21 | 37.25 | 22.78 | 35.48 | 22.78 | 22.80 |
| 867000 | 23.53 | 37.76 | 22.17 | 35.55 | 22.13 | 22.14 |
| 2145000 | 22.83 | 37.68 | 21.54 | 35.52 | 21.51 | 21.52 |

^a Temperature of the oven = 60°C; flow rate = 1 ml/min.

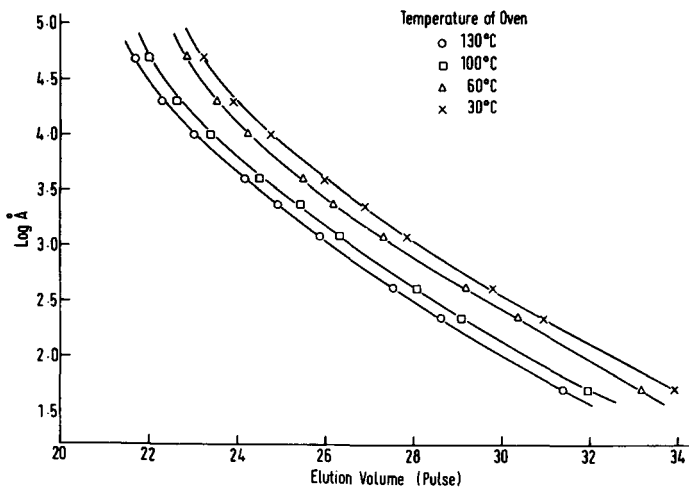
^b Temperature of siphon.

the result of variations of the siphon marker due to altered volume of the solvent dropping in the siphon and that of siphon itself. This error can be eliminated by virtue of eq. (1).

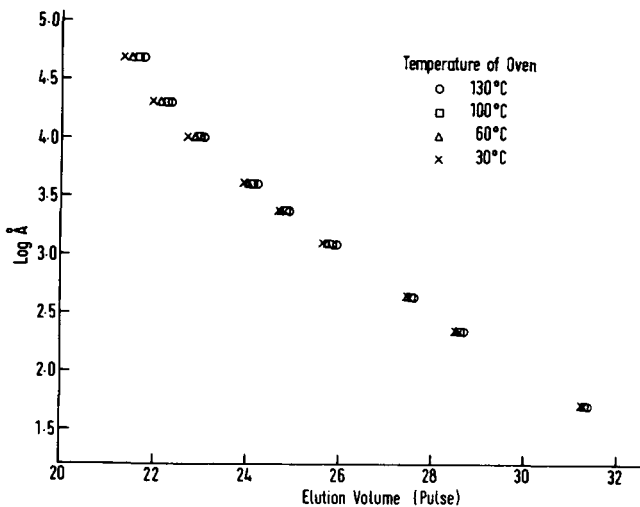
Change in Temperature of the Oven

Keeping the flow rate and the temperature of the siphon constant, the temperature of the oven was lowered from 130°C to 30°C. The results are shown in Figures 1a and 1b. The observed elution volumes of the fractions and those of *n*-decane, and the corrected elution volumes for the fractions for the oven temperatures of 130°C and 60°C are given in columns 4, 5, and 9 of Table I and columns 2, 3, and 6 of Table II, respectively. There is a large increase in elution volumes when the temperature of the oven is lowered from 130°C to 30°C. This increase in elution volumes increases as the molecular weight of the fractions decreases. It can be seen from Figure 1b that by the use of the internal standard, the change in elution volumes of the fractions can be reduced, if not entirely eliminated. It should also be noted from Figure 1b that the change in the corrected elution volumes due to change in temperature of the oven decreases with decrease in molecular weight.

Assuming a linear increase in elution volume with decrease in temperature of the oven, for the fraction corresponding to a molecular weight of 2,145,000, the decrease in elution volume is 1.5/100, or 0.015 pulse/°C, before correction and 0.42/100, or 0.0042 pulse/°C, after correction. Thus, the uncertainty in elution volume for that fraction has been reduced by a factor of 3.5. The usual experimental uncertainty in our experiment is ± 0.02 pulse; hence, even if there is a change in temperature of the oven of about 5°C (0.02/0.0042), we are still within the range of our experimental accuracy, if the elution volume is corrected with the help of the internal



(a)



(b)

Fig. 1. Plots of log of chain lengths (in Å) of polystyrene fractions vs. their observe elution volumes (Fig. 1a) and corrected elution volumes (Fig. 1b) at different temperatures of the oven. Flow rate was 1 ml/min and temperature of the siphon was 130°C.

standard. This is an extreme case; even so, it can be seen from Figure 1b that the difference in the corrected elution volumes decreases with molecular weight.

CONCLUSIONS

The usefulness of a suitable internal standard for precision GPC work has been demonstrated and supported by experimental data. It was demonstrated that any change in the elution volume due to change in the

flow rate and the temperature of the siphon can be eliminated using an internal standard. It is shown further that the effects of changes in temperature of the oven on the elution volume can be very significantly minimized, even if not completely eliminated. However, for 5°C variations in oven temperature, the residual changes in the corrected elution volumes are still within the experimental uncertainty. Even further reduction of these variations should be achievable if the molecular weight and the chemical composition of the sample and the internal standard are not widely different.

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