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DENSIMETRIC DETECTION IN GEL PERMEATION CHROMATOGRAPHY

VI. AN INTEGRATING DENSIMETRIC DETECTOR

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SUMMARY

A new densimetric detector based on the mechanical oscillator method is presented, which combines high sensitivity with improved resolution in time, and contains variable software. In combination with a printer, peaks can easily be integrated numerically by means of a pocket calculator.

INTRODUCTION

The most frequently used detectors in gel permeation chromatography (GPC) are UV-photometers and differential refractometers. While photometric detection is only possible for light-absorbing substances, refractive index (RI) detection can be applied in most cases. There is, however, one restriction: in the chromatography of sparingly soluble polymers (*e.g.*, as polyamides) severe problems may arise from too small RI differences between solute and solvent, when only one solvent is suitable for the separation. In this case, the measurement of density (mass per unit of volume) can be the method of choice.

Highly sensitive density measurement is possible by determination of the period of a vibrating tube filled with the sample (mechanical oscillator method)[1–3]. This method is also applicable to streaming liquids, which allows the use of such an instrument for detection of eluent zones in chromatography[4–10]. All these instruments compare a predetermined number, n, of periods of the measuring cell, T_m, with a time base, T_b, which is in most cases provided by a quartz-controlled oscillator (frequency 100 kHz). The relative resolution in T of the detector is then

$$R = T_b/nT_m \quad (1)$$

which means that higher sensitivity can be achieved by using longer measuring times.

Another problem is the thermal expansion coefficient of organic solvents, which is larger than that of water by at least one order of magnitude (about $1 \cdot 10^{-3}$ g/cm^3 · °K). As commercially available thermostats cannot maintain temperatures

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to within $\pm 1 \cdot 10^{-3}$ °K, we have shown previously[10] that temperature compensation using a reference cell as time base (via a phase-locked loop) improves baseline stability, but at a sensitivity of 10^{-6} g/cm^3 almost 1 min was required for one measurement, which can be tolerated only in very slow separations.

In this paper a new detector is described, which requires much less time for one measurement at a given resolution (by a factor of 50) and is therefore suitable even for high-speed GPC. Temperature compensation is now achieved by calculation from the separately determined periods of the two cells, the time base being provided by a 5-MHz quartz-controlled oscillator. By variation of the software, the detector can easily be adapted to any individual problem, and has the important advantage that it can be coupled with a printer for easy integration of peaks or calculation of molecular weight distributions.

THEORETICAL

Principle of density measurement

As we have shown previously[6], the relation between the density, ϱ, and the period, T, of an oscillator is

$$\varrho = A\,(T^2 - B) \tag{2}$$

where A and B are constants for an individual cell, which can be evaluated by two-point calibration. A density difference, $\Delta\varrho$, is then

$$\Delta\varrho = A\,(T_1^2 - T_2^2) \tag{3}$$

and, if $\Delta\varrho$ and ΔT are small:

$$\Delta\varrho = A\,(T_1 + T_2)\cdot(T_1 - T_2) \approx 2\,AT_1 \cdot \Delta T \tag{4}$$

The concentration of a solute, c_2, is given by

$$c_2 = \frac{\varrho_{12} - \varrho_1}{1 - \varrho_1 \bar{v}_2^*} = \frac{\Delta\varrho}{1 - \varrho_1 \bar{v}_2^*} \tag{5}$$

where ϱ_{12} is the density of the solution, ϱ_1 is the density of the pure solvent and $\bar{v}_2^*$ is the apparent partial specific volume of the solute.

Temperature compensation

If the cells are thermally coupled, a temperature variation, $\Delta\vartheta$, will occur in both cells simultaneously. The periods of the oscillators will then deviate from their original values:

$$T_1 = T_{1,0} + f_1\,(\Delta\vartheta) \tag{6}$$

$$T_2 = T_{2,0} + f_2\,(\Delta\vartheta) \tag{7}$$

If the thermostat is of good quality, $\Delta\vartheta$ is very small (about $\pm$ 0.01°K). The functions $f_1(\Delta\vartheta)$ and $f_2(\Delta\vartheta)$ are perfectly linear even over a range of 1°K; their slope depends on the content of the cells and is very similar for both cells, as will be shown later.

Combination of eqns. 6 and 7 yields:

$$T_{1,0} = T_{2,0} \cdot T_1/T_2 + f_2(\Delta\vartheta) \cdot T_1/T_2 - f_1(\Delta\vartheta) \tag{8}$$

If the periods of the cells are similar, so that

$$f_2(\Delta\vartheta) \cdot T_1/T_2 \approx f_1(\Delta\vartheta) \tag{9}$$

temperature compensation can be achieved using:

$$T_{1,0} = T_{2,0} \cdot T_1/T_2 \tag{10}$$

MATERIALS AND METHODS

Detector

The density measuring device DMA 60 (A. Paar KG, Graz, Austria) multiplied the frequency of the reference cell by a phase-locked loop[10] to yield a frequency of about 100 kHz, which was used as time base. The new detector compares the periods of both cells (DMA 602 M; A. Paar) separately with a 5-MHz quartz-controlled oscillator and compensates for temperature variations by calculation. The number of periods for one measurement can be freely chosen; in general, 1000 periods were found to be adequate. In this case a sensitivity of $2.5 \cdot 10^{-7}$ g/cm^3 is achieved with a measuring time of about 4 sec, when the cells are filled with a liquid of $\varrho = 1$ g/cm^3. As cells are not in phase, temperature compensation will only work well when the temperature variations are not too rapid. Therefore, it is necessary to place a mixing chamber between the thermostat and the cells to eliminate the heating pulses.

To admit higher flow-rates, a heat exchanger must be placed before the measuring cell to bring the eluate to the temperature of the cell. A steel capillary (100–150 $\times$ 0.05 cm I.D.) is sufficient and does not result in poor separation when a typical GPC column set is used (*e.g.*, three columns of μ-Styragel). The detector has two analogue outputs (each 5 V) for independent registration of compensated and uncompensated signals by means of a x–t recorder, and a digital output which is connected to a matrix printer. This enables ready integration of peaks and calculations of molecular weight distributions.

Integration of peaks

As we have shown previously[9], peaks can be integrated by simple summation if a densimetric detector based on the mechanical oscillator method is used. The total mass of an eluted substance is

$$m_1 = \frac{2A \cdot T_{1,0}^2 \cdot F}{1 - \bar{v}_2^* \varrho_1} \cdot n \cdot \Sigma\Delta T \tag{11}$$

TABLE I

THERMAL COEFFICIENT OF T (IN %/°C) FOR CELLS 1 AND 2 (UNCOMPENSATED) AND FOR 1/2 (WITH TEMPERATURE COMPENSATION) FOR DIFFERENT CONTENTS

Cell	*Air*	*Water*	*THF*
1	+0.00567	+0.00970	+0.02478
2	+0.00607	+0.00980	+0.02338
1/2	−0.000397	−0.000095	+0.00135

where F is the flow-rate and n the number of values. The summation is done by the new detector and the results printed are number of values, elution times, modes (periods of measuring cell, reference cell and compensated signal), actual values of T and ΣT.

Integration can be carried out by the following procedure: (1) subtraction of initial from final value of ΣT; (2) subtraction of $n \cdot T_{1,0}$ to obtain $\Sigma \Delta T$; (3) multiplication by $2A \cdot T_{1,0}^2 \cdot n \cdot F/(1 - \varrho_1 \bar{v}_2^*)$. This can easily be done by a pocket calculator.

RESULTS AND DISCUSSION

Table I shows the temperature coefficient of T for both cells with and without compensation and with different contents [air, water and tetrahydrofuran (THF)].

In the case of water the compensation improves the stability by a factor of 100 (20 in the case of THF), leading to a sufficiently stable baseline. The standard deviation of 50 values is 0.66 at $\bar{T} = 1.047 \cdot 10^8$, and there is practically no drift within the time required for an average chromatogram.

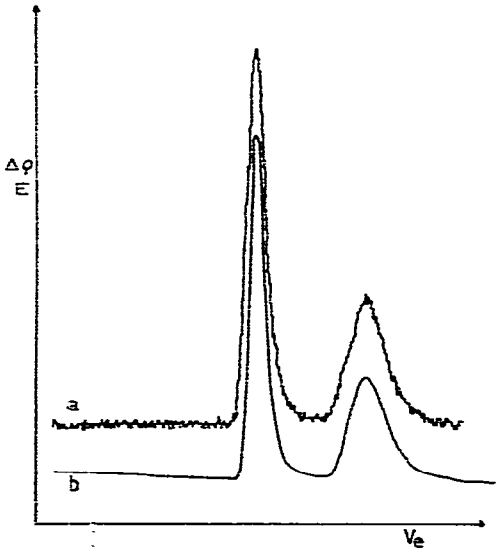

Fig. 1. GPC separation of polystyrenes of average molecular weight of 50000–4000 on PL-Microgel 50-100-500-10^3 Å. Solvent: tetrahydrofuran; flow-rate 1.0 ml/min. Injected volume: 50 µl, each 0.5%. a, Densimetric trace, 200 digits full scale; b, UV trace, 260 nm, E = 1.28 full scale. V_e = Elution volume.

When the cells are filled with a liquid of $\varrho = 1$ g/cm^3, a sensitivity of $2.5 \cdot 10^{-7}$ g/cm^3 can be achieved by measurement over 1000 periods ($\approx$ 4 sec), but higher sensitivities are possible if longer measuring times can be tolerated. In most cases, this resolution will be sufficient, as can be seen from Fig. 1.

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