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NEW UNIVERSAL DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRY: THE DENSITY DETECTOR

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SUMMARY

A new universal detector measuring density (mass per unit volume) according to the mechanical oscillator method is presented. It achieves high baseline stability without temperature compensation by the use of a reference cell even at a resolution in density of $3 \cdot 10^{-7}$ g/cm^3. It is shown that the new detector, which with be commercially available in the near future, may be an alternative to the differential refractometer, which until now has been the only usual universal detector for high-performance liquid chromatography.

INTRODUCTION

In high-performance liquid chromatography (HPLC), the only universal detector of widespread use is the differential refractometer. However, this does not yield satisfactory results in some cases: *e.g.* if there is not a sufficiently large difference in the refractive indices of eluent and eluted substance, the response of such an instrument may be very poor.

A real alternative may be a density detector, as we have already shown in several communications[1–5]. There is, however, no such instrument on the market as yet; the commercially available density meters have been designed for absolute measurements and thus are not well suited for the use as HPLC detectors. Nevertheless several authors have tried to apply these instruments to gel permeation chromatography of polymers[8–11]. Encouraged by the promising results of our latest investigations we have now developed a new instrument, which has been especially designed to meet the demonds of HPLC.

PRINCIPLE OF DENSITY MEASUREMENT

The desired high precision in measurement of density can be achieved only by using a density measuring device based on the mechanical oscillator method, which has been developed by Kratky, Leopold and Stabinger[12–13]. As the measuring principle has already been described, it will be outlined here only briefly.

The measuring cell of such an instrument is an oscillating capillary tube (usu-

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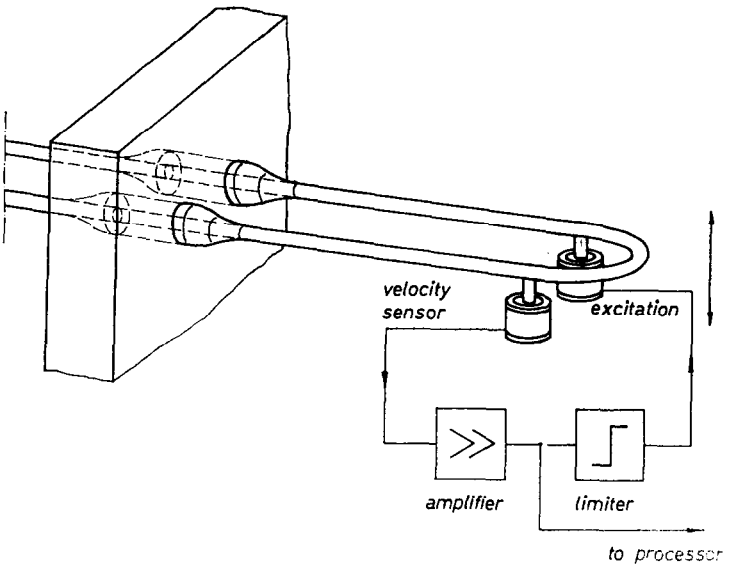

Fig. 1. Measuring cell of a sensity meter according to the mechanical oscillator method.

ally made of glass), as is shown in Fig. 1: the density d of a sample can be calculated from the period T of the cell using the equation

$$d = A \cdot T^2 - B \tag{1}$$

where A and B are constants for each individual cell at a given temperature. A density change Δd causes a change ΔT of the period. If the change in density (and thus in the period) is small, one may write

$$T_1 \approx T_2 \approx T$$

and

$$\Delta d = 2A \cdot T \cdot \Delta T \tag{2}$$

which is a good approximation for the very small density changes occurring in HPLC due to the low concentrations of eluted substances.

The period T_M of the measuring cell can be determined by counting the number N_B of periods T_B of a time base (usually an oven-controlled quartz oscillator) during a predetermined number N_M of periods T_M of the measuring cell:

$$N_M \cdot T_M = N_B \cdot T_B \tag{3}$$

Hence, as the signal of a density detector is N_M instead of T_M, the density change is better represented by

$$\Delta d = 2A \cdot N_B \cdot (T_B/N_M)^2 \cdot \Delta N_B \tag{4}$$

RESPONSE OF A DENSITY DETECTOR

The density change Δd caused by a concentration c_1 of eluted substance is given by

$$\Delta d = (1 - d_0 \cdot v_i^*) \cdot c_i = m_i/V_i \cdot a_i \quad (5)$$

where d_0 is the density of eluent, v_i^* is the apparent specific volume of eluted substance, c_i is the concentration of eluted substance (w/v), m_i is the mass of eluted substance in the volume V_i, and a_i is the response factor.

For a given flow-rate F the volume V_i passing through the measuring cell during N_M periods of the cell is

$$V_i = F \cdot N_M \cdot T_M = F \cdot N_B \cdot T_B \quad (6)$$

Combination of eqns. 5 and 6 yields

$$\Delta d = (a_i \cdot m_i) / (F \cdot N_B \cdot T_B) \quad (7)$$

and with eqn. 4 one obtains

$$m_i = 2A \cdot F \cdot N_{B2}/N_{M2} \cdot T_{B3} / a_i \cdot \Delta N_B \quad (8)$$

or for a given solvent (causing a given N_B) at a given flow-rate:

$$m_i = k / a_i \cdot \Delta N_B \quad (9)$$

This means that under defined chromatographic conditions the response of a density detector represents the mass of eluted substance passing through the measuring cell during one measuring interval (independent of its length). The detector integrates each slice of a peak and its response is inherently digital, which eliminates any problems arising from digitizing and integrating an analog chromatographic trace. Moreover, it has to be pointed out that the response factor of a density detector is much less dependent on the molecular weight of the sample than it is for the differential refractometer detector[2,14,15], which is very important for its use in gel permeation chromatography (GPC).

CONSTRUCTION OF THE DENSITY DETECTOR

The most important problem in the design of a density meter is the temperature dependence of density. In most organic solvents one would have to keep temperature constant to 0.001°C to measure density with an accuracy of $1 \cdot 10^{-6}$ g/cm^3 or to use a reference cell to compensate for temperature changes. The DMA 60 (from A. Paar, Graz, Austria) uses this concept: the frequency of the reference cell (filled with the same solvent as the measuring cell) is multiplied using a phase-locked loop to yield a time-base of *ca.* 100 kHz. This is, of course too low for chromatographic applications, as the desired high resolution is achieved only at measuring intervals of more

than 100 s! Our first approach[7] to a density detector also used a reference cell, but the periods of both cells were determined separately by comparison with an oven-controlled 5 Mc quartz oscillator; compensation for temperature fluctuations was achieved by calculation.

In this way a satisfactory baseline stability at measuring intervals of several seconds was achieved, but the division procedure lead to increased noise.

The best way to overcome these difficulties seemed to be to use only one measuring cell and to make temperature changes very slow compared with the time required for an average chromatogram. Fortunately, the group of Stabinger had developed a new micro-cell for other applications, which is also now produced by the Paar company. Up to two of these cells, which proved to be very suitable, can be placed together with the columns in a thermostatted box, the dimensions of which allow the use of several (even 60 cm long) columns. The inner wall of the box (made of stainless steel) is covered with a copper tube connected to a good thermostat (in this case a Haake F3C); it is separated from the outer wall by 5 cm of polyurethane foam. The measuring cell is placed in a block of brass, which is mechanically, electrically and thermally isolated from the bottom of the box; hence the cell is thermally coupled to the box only by the air inside. In this way, any temperature fluctuations of the thermostat are eliminated, which results in a very stable baseline.

The cells are connected to an intelligent interface, which also provides up to two analog inputs for dual detection (*e.g.* density–UV or density–RI), memory capability for the storage of chromatographic raw data, two analog outputs (for a strip-chart recorder) and a serial interface for the transfer of data to a personal computer (the software for data acquisition and data reduction has been written for the IBM-PC or compatible computers)[14].

A typical chromatographic system using the density detector is shown schematically in Fig. 2.

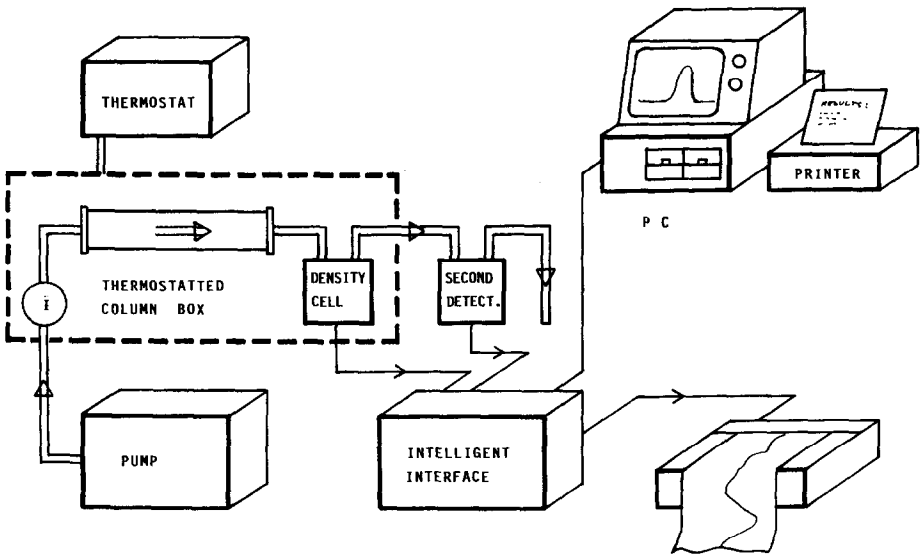

Fig. 2. Chromatographic system using the density detector (schematic).

RESULTS AND DISCUSSION

The performance of the new detector was tested at 25.00°C and a flow-rate of 1 ml/min. To make this test more rigorous, chloroform was chosen as a solvent because of its high thermal expansion coefficient. The recorder traces obtained during a period of 1 h at different sensitivities showed that the drift of the baseline during the time required for an average chromatogram is sufficiently small (typically less than ± 3 digits per hour) and can be considered as linear, which makes compensation rather easy. The noise level over a short period (1–2 min) is typically ± 1 digit.

For practical reasons, the attainable resolution in density (the density change equivalent to a change in the detector signal of 1 digit) was determined by injection of various amounts of a sample of well known specific volume (and response factor) instead of a two-point calibration (as is done for absolute density measurement). This procedure was chosen in order to ensure real chromatographic consitions.

From a series of chromatograms using different columns were determined (1) the reproducibility of peak areas, (2) the linear range, and (3) the sensitivity of the detector.

Chloroform (Baker Analyzed HPLC Reagent) was used eluent (at a flow-rate of 1 ml/min). Polystyrene 50000 (Pressure Chem. Comp., Pittsburgh, PA, U.S.A.) was injected from a 50-μl loop on two different column sets: (a) one column of Microgel M (60 cm), from Polymer Laboratories (b) two columns of LiChroSpher 100CH8 (25 cm) from Merck. The concentrations of the samples covered a range from $7.5 \cdot 10^{-6}$ g/cm^3 to $150 \cdot 10^{-6}$ g/cm^3. Chromatograms were taken using N_M = 4000 periods of the measuring cell, corresponding to a measuring time of 4.66 s (in chloroform). Raw data were transferred to an IBM-PC and integrated by the pro-

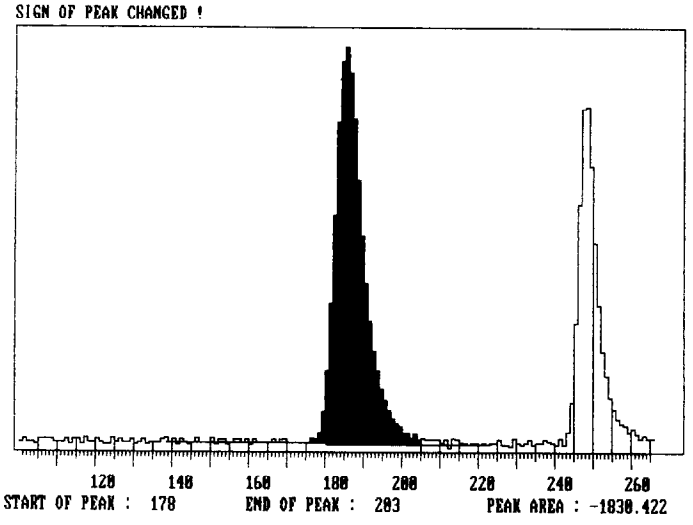

Fig. 3. Integration of a chromatogram by the GPC program. Injected, Polystyrene 50000 (50 μl 0.300% w/v) in chloroform column packing, Microgel M (60 cm); flow-rate, 1 ml/min; internal standard, diethyleneglycol–dimethyl ether (V_e = 19.84 ml). Baseline before peak, 23310090.69 ± 0.83; baseline after peak, 23310090.91 ± 1.09; peak area, 1830.42 (26 slices).

TABLE I

REPRODUCIBILITY OF PEAK AREAS FROM THE DENSITY DETECTOR

Repeated injections of Polystyrene 50000 (50 μl 0.15%) in chloroform at a flow-rate of 1.00 ml/min using different columns. Temperature, 25.00°C; measuring interval, 4000 periods (4.66 s).

Run No.	*Column set*	*Peak area (digits)*
1	PL Microgel M (60 cm)	901.16
2	PL Microgel M (60 cm)	909.54
3	PL Microgel M (60 cm)	905.27
4	LiChroSpher 100CH8 (2 × 25 cm)	905.66
5	LiChroSpher 100CH8 (2 × 25 cm)	907.27
Average		905.77
Standard deviation		3.08 (=0.34%)

gram described in a previous communication[14] (Fig. 3). The reproducibility of peak areas is shown in Table I.

The linear range of the detector was tested (in addition to previous investigations[4] on the concentration dependence of the apparent specific volumes) by injecting different concentrations and plotting peak areas *vs.* the sample size, as can be

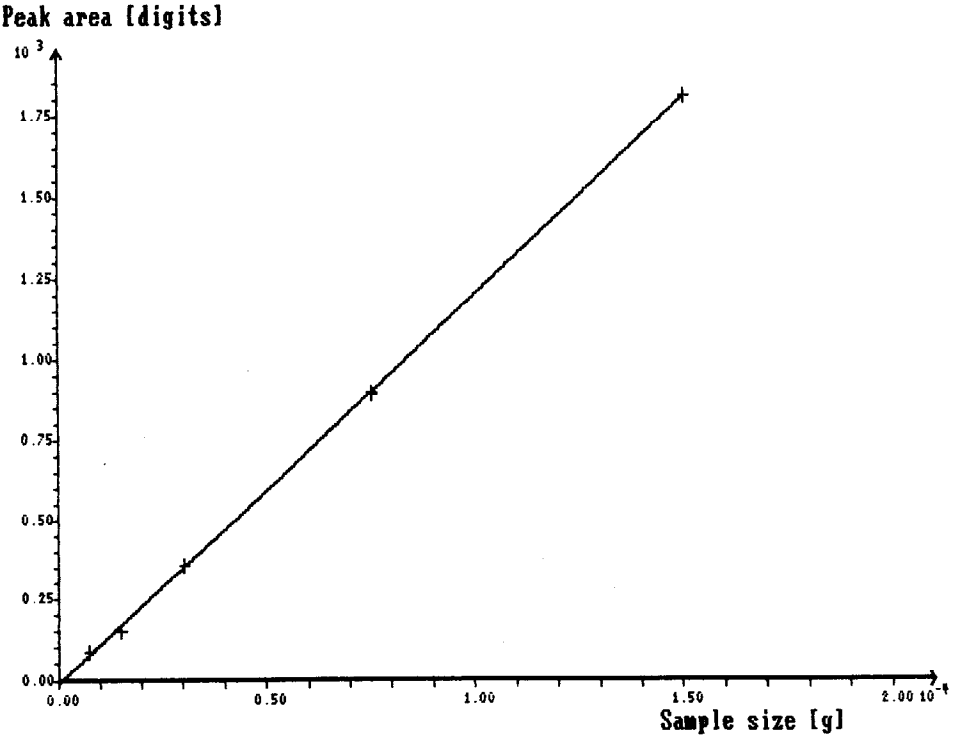

Fig. 4. Linearity of the density detector. Injected, Polystyrene 50000 in chloroform; flow-rate, 1.0 ml/min; injected volume, 50 μl; column packing, PL Microgel M (60 cm); measuring interval, 4000 periods (4.66 s).

seen from Fig. 4. The slope of this plot was used (together with the previously determined apparent specific volume of polystyrene in chloroform at the measuring temperature of 25.00°C) to calculate the constant k, which represents the sensitivity of the detector. At the measuring time of 4.66 s a resolution in density (which is direct proportional to the number of periods per measuring interval) of $3.5 \cdot 10^{-7}$ g/cm^3 is achieved.

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