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CONSTRUCTION AND EVALUATION OF A THERMOSTATTED PERMITTIVITY DETECTOR FOR HIGH-PERFORMANCE COLUMN LIQUID CHROMATOGRAPHY

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SUMMARY

A flow-through permittivity measurement device, suitable as a detector in high-performance liquid chromatography, is described. The bridge method is used, with a planar cell of 7-μl capacity, thermostatted with high precision. The detection limit corresponds to a permittivity change of about $0.5 \cdot 10^{-6}$. The range is linear up to a sample concentration of about 10%. The contribution to the chromatographic peak width is about 3 μl or 0.12 sec, whichever is greater. Sensitivities for a number of compounds were measured and compared with the differences in the dielectric constants of the sample and eluent.

INTRODUCTION

In a previous paper[1], we presented some preliminary results obtained with a detection system based on the measurement of permittivity for high-speed, high-performance liquid chromatography (HPLC). This measurement principle seems attractive in comparison with refractive index measurements, because the relative differences in the permittivities of various compounds are about an order of magnitude larger than the relative differences in refractive indices, as a result of the contribution of the dipole moment of the molecules to the former physical quantity. It seemed worthwhile, therefore, to investigate whether a measurement system capable of a noise level equivalent to a fraction of 10^{-7} of the permittivity (comparable in this respect to the performance of refractometers) can be designed. If this were to be feasible, without affecting other characteristics of the measurement systems[2], an improvement in the detection limit of about a factor of 10 could be attained.

Different principles for the measurement of permittivity were reviewed by Haderka[3,4]. Of the three methods he mentions, *viz.*, use of an oscillator with frequency or beat-frequency counting, use of the resonance principle and use of bridge measurements, we choosed the last technique for the following reasons:

(a) Stable oscillation of a circuit that includes a liquid cell as a condenser is achieved only within relatively narrow ranges of certain parameters of the electronic

circuit which depend on the resistance of the liquid cell. As this resistance can change considerably with the nature of the eluent (*e.g.*, 10 kΩ for methanol, 16 MΩ for chloroform and 70 MΩ for isooctane as the eluent in our cell), adjustment of several circuit parameters would be necessary when changing the eluent.

(b) In order to avoid drift resulting from ambient temperature and humidity, it is advantageous to make use of a reference cell that compensates for these effects. In the frequency methods, this means that interference is caused between two frequencies, one resulting from a circuit incorporating the sample-stream cell and one incorporating the reference cell, and the beat frequency is measured. Apart from difficulties in preventing both circuits from "locking" each other's frequency as a result of parasitic coupling, this method has the drawback that at a perfect balance of the two circuits, which is desirable in order to make drift compensation optimal, the beat frequency is zero. However, passing zero within a chromatogram cannot be tolerated, as the counting of the beat frequency cannot give information on the sign of the signal. As a result, when both positive and negative peaks are expected in a chromatogram, one has to increase one frequency far enough from the other that the highest peak in either a positive or negative direction cannot cause a zero beat frequency.

(c) In the resonance method, the influence of the conductivity will be high and the dynamic range will be small.

(d) A third drawback of the frequency and resonance methods, although perhaps less convincing, seems to be that the effect of noise in various electronic devices cannot be judged by calculation when using these non-linear circuits, whereas they can be readily calculated for the bridge system.

Vespaleč and Hana[5,6] and Erbelding[7] described the use of the frequency method. The cell described by the latter is not suitable for HPLC because of the large volume. Vespaleč and Hana used a cylindrical cell of suitable dimensions and their results will be compared with ours, obtained with flat cells and the bridge method.

The bridge measurement has to be combined with phase-sensitive demodulation of the bridge signal in order to have a d.c. output signal that can have both positive and negative values, and in order to minimize the effect of noise originating from the electronic devices. Commercial phase-sensitive amplifiers are generally not designed to function at frequencies of 1 MHz or higher. These high frequencies are advantageous, however, firstly because at high frequencies the impedance of the cell capacitor is small, resulting in low noise levels, and secondly because of the decreasing influence of the conductivity of the liquid on the output signal. On the other hand, frequencies of 10 MHz or higher are increasingly difficult to handle electronically, even with custom-made equipment. For these reasons, we chose a 1-MHz bridge supply frequency and a built-in phase-sensitive demodulation amplifier unit, constructed from linear integrated circuits designed for R.F. transmission. The cell design is the same in principle as that used previously[1], with the following modifications. Instead of a "stack" of two cells with a common electrode plate which is used at both sides, we mounted the two cells alongside each other on a copper block plated with nickel on one side. This was done in order to allow for proper thermostatting of both cells and of the effluent before entering the cell via the copper block. For thermostatting of the whole cell block, heat pumping by means of thermoelectric devices (Peltier elements) is attractive for the following reasons:

(a) The set temperature of the thermostat can be chosen to be at below or above ambient, as a result of the two-directional action of the heat-pumping device. Normal liquid thermostats need a heat leak to the atmosphere or to tap water, which results in serious temperature fluctuations as a result of draughts. When using tap water as a coolant, precise control is difficult because of the high power dissipation.

(b) This type of thermostat can be built as an integrating part of the detector body and is therefore more easily operated than the usual system with water-circulation tubes connected to a liquid thermostat.

(c) The temperature stability obtained is very good, as was found during the development of this thermostat for application in the very temperature-sensitive microadsorption detector in our laboratory.

We therefore choosed this system for application in this detector as well.

EXPERIMENTAL

Chemicals

Isooctane and ethanol from BDH, Poole, Great Britain (analytical grade), were used as solvents. Sample compounds from different suppliers were used, most of them being of analytical grade or chromatographically pure.

Apparatus

A syringe pump (Labotron 13A) and a diaphragm pump (Orlita DMP 1515) were used as solvent pumps. The syringe injection system according to Huber[2], connected via a zero-dead-volume Swagelock connector (1/16 in.), was used. The dead volume between the syringe needle and the cell cavity was estimated to be 10 μl (3-μl injection piece, 1-μl connector, 6-μl capillary in the block).

Static sensitivity was obtained with a motor-driven microburette feeding a small stream of concentrated solution into the eluent. A Siemens Kompensograph III with a pen travel time of 0.25 sec was used for the dynamic test.

Construction of the detector

Fig. 1 shows the mechanical scheme of the detector and thermostatting device, and Fig. 2 shows the electronic circuitry. The measuring cells were constructed by

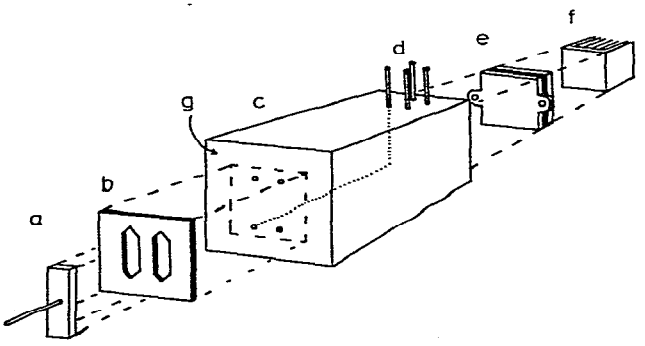

Fig. 1. Mechanical parts of the detector and thermostatting device. a = Electrode and connecting wire; b = PTFE gasket sheet; c = copper block (6 × 6 × 6 cm); d = connecting capillaries (0.25 mm I.D.); e = Peltier element; f = heat sink; g = nickel-plated side of the copper block, showing end of connecting capillaries.

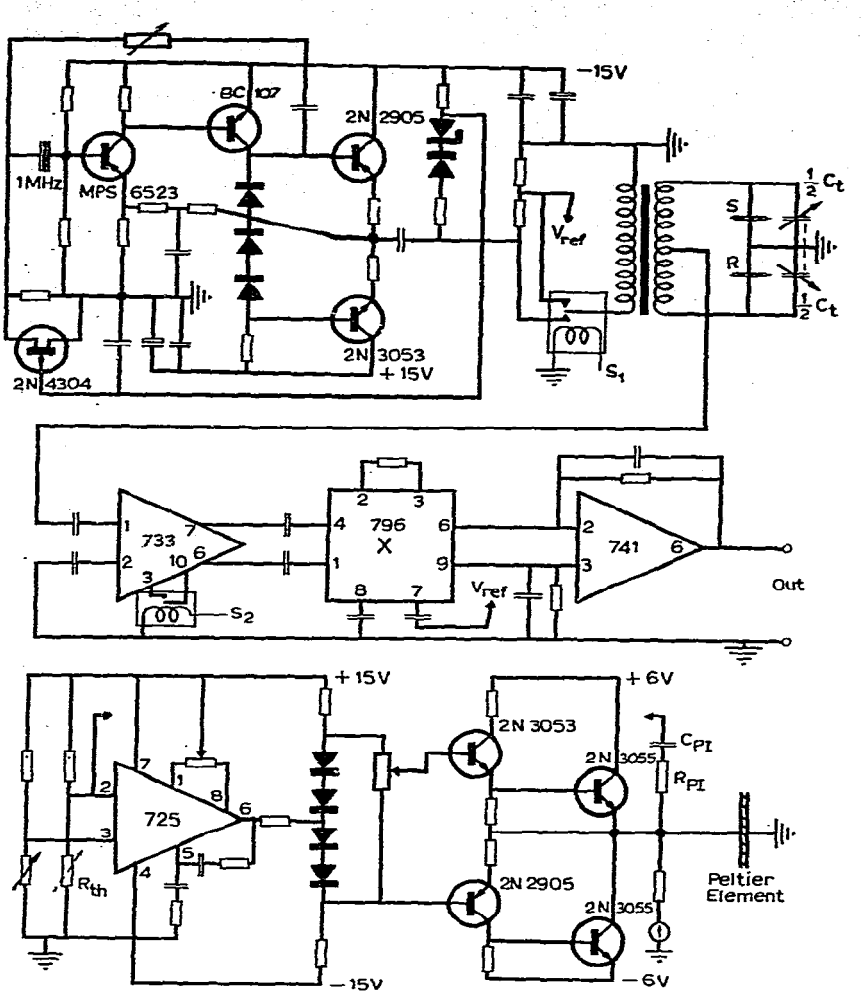

Fig. 2. Detector and thermostatting electronics. Transistor types as indicated. Linear integrated circuits, Fairchild μA series as indicated. "1 MHz"; crystal of that frequency; S and R = sample and reference cell, respectively; C_t = differential quartz trimmer, 12 pF, JFD Type DC 404; S_1 and S_2 = reed switches for attenuation bridge voltage and amplification, respectively; R_{th} = temperature-sensing thermistor. All IC circuits according to manufacturer's data sheets. R_{PI} and C_{PI} were 10 MΩ and 10 μF, respectively, in this unit (see text). Further details of the scheme are available on request.

pressing a PTFE gasket between an electrode and the nickel-plated side of the copper block. The cell volume was 7 μl and the liquid-dependent part of the capacitance was calculated to be 7 pF (with air in the cell).

In preliminary experiments, it was shown that the bridge exciting voltage must be very stable in frequency and amplitude. Therefore, a crystal-based oscillator with amplitude stabilization in the feedback loop was constructed. As the mechanical design of the liquid cells dictated a common earthed electrode (the copper block), the centre tap of the transformer was used as the input of the subsequent amplifier. The transformer was constructed with a ferrite core (Siemens Siferrit). The inductance of

the separate coils was 10 mH. Zero control was obtained by shunting the cell capacitors with a stable differential trimmer.

Amplication of the bridge error signal at maximum gain was 100 in the R.F. amplifier, 4 in the phase-sensitive demodulator and 100 in the final d.c. amplifier, and therefore was 40,000 in total. With the maximum exciting voltage of 2.9 V effective value, deviation of 1 ppm of one capacitor results in a d.c. output voltage change of $\frac{1}{4} \cdot 10^{-6} \cdot 40{,}000 \cdot 2/\pi \cdot 2.9\ \text{V} = 18.5\ \text{mV}$.

The thermostatting device was essentially a PI control system, with the thermistor R_{th} as the temperature sensor. R_{PI} and C_{PI} determined the gain and the integrating action of the controller; they had to be matched with the dynamic properties of temperature fluctuations of the copper block and Peltier element in order to obtain a rapid but non-oscillating control action.

RESULTS AND DISCUSSION

The temperature stability of the copper block was measured by means of an extra thermistor, connected to a Knauer thermistor bridge amplifier. A 14-h run displayed a drift of $5 \cdot 10^{-4}$ °/h, with superimposed short-term fluctuations of about a $2 \cdot 10^{-4}$ °/h maximum deviation from the mean. This temperature stability is better than can be achieved by means of liquid thermostats.

The dynamic behaviour of the capacitance detector is shown in Fig. 3. The values obtained at high flow-rates correspond to a first -ordertime constant of 0.1 sec; in the last stage of the electronic amplifier, the values obtained at low velocities are about constant in the volume units used and amount to a standard deviation of 2.7 μl. As the total content of the detection cell is 7 μl, the value of the standard deviation is relatively low and shows the favourable flow pattern in the cell (perfect mixing in the measuring cell would result in a standard deviation of 7 μl; plug flow, which is the most favourable case for detection, would yield a value of 2.0 μl).

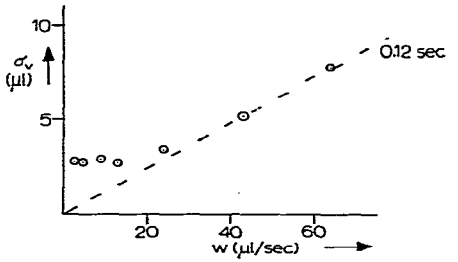

Fig. 3. Contribution to peak broadening of the detector. Syringe injection with the injection port connected directly to the detector; σ_v determined from half-width at 0.6 maximal height. Eluent, isooctane; sample, cyclohexanone. Peak width observed when feeding an electrically generated impulse to the recorder corresponded to 0.04 sec σ_t.

The linearity of the response of the detector with concentration was tested statically with N,N-diethylaniline in isooctane. Stationary concentrations were obtained by delivering a constant flow of a solution of N,N-diethylaniline in the eluent into the main stream of the eluent. By varying the flow-rate and concentration of the sample stream, the concentration in the cell can be varied. Fig. 4 gives the results.

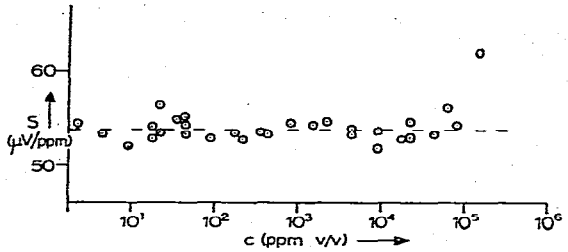

Fig. 4. Linearity of the response of the detector. Static measurement by means of continuous blending of a sample with eluent. Sample, N,N-diethylaniline; eluent, isooctane.

Sensitivities of various compounds in some solvent mixtures were measured dynamically, using a syringe injection on a piece of 1-mm I.D. tubing of length 24 cm and calculated by using the equation $S = AW/q$, where A (V·sec) = area, W (μl/sec) = flow-rate and q (μl) = amount. The results are given in Table I.

TABLE I

SENSITIVITIES MEASURED FOR SOME COMPOUNDS IN ELUENTS OF DIFFERENT POLARITY

Attenuation ×10.

Compound	*Sensitivity S (μV per 1 ppm)*			*ε*	*P × 10²*
	Isooctane ε = 1.93	*Ethanol–isooctane*			
		1:4 (w/w) ε = 4.1	*2:3 (w/w) ε = 8.4*		
Carbon tetrachloride	62.3	—	—	2.24	0
Chloroform	385	835	−399	4.8	1.72
Ethyl butanoate	530	900	−704	5.1	2.29
Ethyl benzoate	690	890	−578	5.94	2.39
Dibutyl phtalate	790	1670	−342	6.55	2.84
Hendecanone-2	765	1050	0	8.4	3.51
Methyl salicylate	1180	1100	166	9.60	4.44
Pyridine	1110	1950	576	11.6	4.76
Benzyl alcohol	668	1820	281	13.1	2.75
n-Hexanol	496	950	846	13.3	2.14
n-Butanol	650	1510	1610	15.1	3.01
Acetone	2150	3250	1240	20.7	10.70
Ethanol	990	2700	2710	24.3	4.95
Methanol	1340	3990	3780	32.6	7.05
Nitrobenzene	4350	3450	1910	34.8	15.72
r (correlation coefficient):					
with $\varepsilon_t - \varepsilon_e$	0.7563	0.9176	0.9072	—	—
with *P*	0.9750	0.7913	0.5239	—	—

The following observations can be made:

(a) In the apolar solvent isooctane, compounds with a high permittivity invariably give high sensitivities. However, a proportional relationship between these quantities cannot be found; alcohols in particular give responses that are relatively low compared to the permittivity of the pure liquid. The same observation was

made by Vespaleč and Hana[5], who considered this phenomenon to be due to dimer formation by alcohols in hydrocarbon solution. This dimer formation, however, is not very probable in these very dilute solutions, and we consider rather that the permittivity of the pure liquid, which is taken as a reference in these comparisons, is affected by intermolecular interactions such as dimer formations.

A better reference is perhaps formed by the contribution to the polarization from the dipole moment of the sample molecules[7]. This contribution is proportional to $\varrho\mu^2/M$ $(= P)$, where ϱ is the density, M the molecular weight and μ the dipole moment. The values of P are given in Table I, and a more constant ratio between this quantity and the sensitivity in isooctane (all values of the ratio between 190 and 288 for the compounds in Table I) can be seen. The correlation coefficient between the sensitivity and P is much better than that between the sensitivity and $\varepsilon_i - \varepsilon_0$, where ε represents permittivity. For the other solvents, the latter quantity gives the better correlation.

(b) On going to more polar solvents, a definite increase in the sensitivity can be observed in some instances. This effect can be explained as a result of the fact that polarization for a mixture is additive[8], not the permittivity. This results in

$$\frac{\varepsilon_m - 1}{\varepsilon_m + 2} = \frac{\varepsilon_i - 1}{\varepsilon_i + 2} \cdot y_i + \frac{\varepsilon_e - 1}{\varepsilon_e + 2} \cdot (1 - y_i) \qquad (1)$$

where ε_m is the permittivity of the liquid, ε_i is the permittivity of the solute, ε_e is the permittivity of the eluent and y_i is the volume fraction of the solute. The sensitivity will be proportional to

$$\left(\frac{\partial \varepsilon_m}{\partial y_i}\right)_{y=0}$$

Differentiation of eqn. 1 yields

$$\left(\frac{\partial \varepsilon_m}{\partial y_i}\right)_{y=0} = \frac{\varepsilon_e + 2}{\varepsilon_i + 2} \cdot (\varepsilon_i - \varepsilon_e) \qquad (2)$$

Fig. 5 shows the dependence of the sensitivity expected on the basis of eqn. 2. The increase in the sensitivity on going to more polar eluents, as observed in Table I, can be explained, at least qualitatively, in this manner.

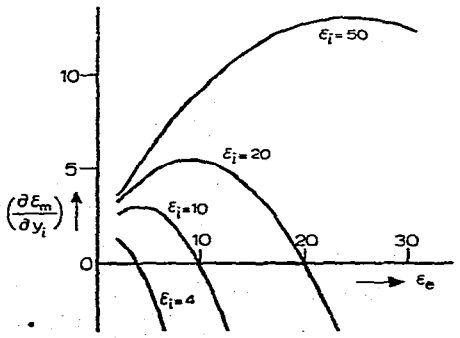

Fig. 5. Sensitivity as a function of the permittivity of the eluent (ε_e) for different permittivities of the solute (ε_i), as expected from eqn. 2.

(c) As expected, with the less polar substances a negative response is obtained with a higher alcohol content in the eluent.

The sensitivities measured are roughly comparable to the theoretical expectation. As mentioned above, a 1 ppm change in the capacity would result in a 18.5-mV d.c. output change. With isooctane as the eluent, the active capacity is about 13 pF, and we measured a total capacity of about 70 pF. This higher value can be explained by the contribution from the PTFE dielectric and the input capacity of the amplifier.

A concentration of 1 ppm of chloroform would give, according to eqn. 2, a change of $1.66 \cdot 10^{-6}$ in the permittivity; or a capacity change of $12.6 \cdot 1.66 \cdot 10^{-6} = 21 \cdot 10^{-6}$ pF. This relative change of 0.30 ppm in one cell capacity (70 pF) would give a d.c. output signal of $0.30 \cdot 18.5$ mV = 5.5 mV at the attenuator × 1 position or 550 μV at the attenuator × 10 position as used in Table I. The experimentally observed value is 385 μV per 1 ppm.

The dependence of the response of the detector on the flow-rate was tested by means of syringe injection and integration with the integrator. The results are shown in Fig. 6 and confirm that the instrument behaves as a concentration detector.

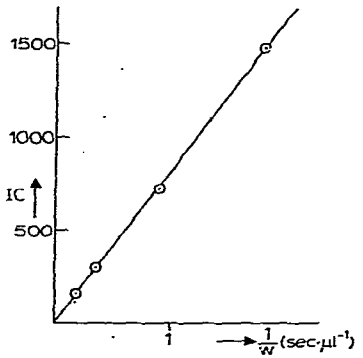

Fig. 6. Dependence of the integrated response on the flow-rate. Eluent, isooctane; sample, acetone; 25-cm open tube, 1 mm I.D., between injector and detector.

The noise was characterized by means of a procedure described elsewhere[9,10], and the results are presented in Table II.

The following observations can be made about the noise level:

(a) The main origin of the noise is outside the electronic system. Calculations, as well as measurement of the contribution of the various electronic parts to the base-line noise, make it clear that these can account for only a few per cent of the total observed noise.

(b) With eluents of higher polarity, a higher noise level is observed. In ethanol–isooctane (2:3, w/w), the increase in sensitivity, mentioned before for a number of compounds, is more than compensated for by the increase in noise level.

(c) Comparison with the results obtained by Vespaleč and Hana[5] is difficult, because their method of noise characterization was not stated. From the actual chromatograms shown, a noise level of about 5 Hz (r.m.s.) can be estimated, which is in rough agreement with 14 Hz (peak to peak?) mentioned in the text. This would correspond to 0.44 ppm of hexanol. Noise levels with both instruments seem to lie

TABLE II

RESULTS FOR NOISE

Integration time (sec)	*Permittivity detector (attenuator × 10)*				*LDC refractometer*	
	Isooctane		*Isooctane–ethanol (2:3, w/w)*		*Isooctane*	
	mV	*ppm hexanol*	*mV*	*ppm hexanol*	*mV*	*ppm hexanol*
1	0.057	0.11	0.62	0.73	0.02	0.5
10	0.075	0.15	2.87	3.40	0.05	1.25
100	0.98	2.0	—	—	0.28	7.0
1000	2.25	4.5	—	—	—	—

at the same order of magnitude. Later, Vespaleč[6] reported a noise level of 3 Hz for the described device.

These results substantiate the gain in detection limit compared with the refractometers, mentioned in the Introduction, for polar compounds in an apolar solvent. The gain is marginal, however, never exceeds a factor of 3 and applies only to apolar eluents.

Fig. 7a and 7b illustrate the possibilities of the detector in practical chromatographic work. Amounts of 4 μg of aliphatic compounds can be measured in the eluate from a short column with a microparticulate packing with a reasonable signal-to-noise ratio and no contribution to the chromatographic peak width.

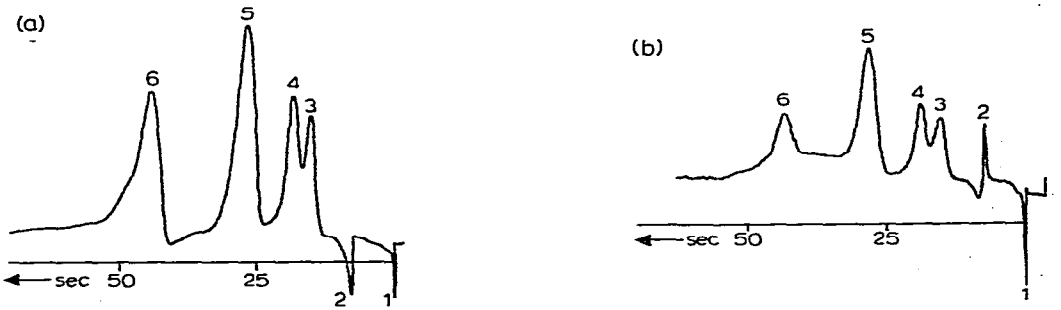

Fig. 7. Separation and detection of triglycerides. Column, 10 cm × 2.7 mm I.D.; Merck silica gel SI 60, 8–9 μm; eluent, isooctane–chloroform (83:7, v/v). a, Injection of 22 μl, 1.5 mg/ml each; b, injection of 22 μl, 0.19 mg/ml each. Peaks: 1 = pressure pulse from injection; 2 = solvent peak; 3 = glycerol tricaprylate; 4 = glycerol tricaproate; 5 = glycerol tributyrate; 6 = glycerol triacetate.

CONCLUSION

In view of the efforts made in this work to stabilize the base-line by thermostatting and the selection of the principle and design of the measurement system, the gain in detection limit of about 3 in comparison with the refractometer is disappointing. It should be noted that this gain is achieved only for a polar (dipole) molecule in a non-polar solvent, and that the detector is usable only within a limited range of solvent polarity. The main application of this detector will therefore, in our view, be

found in the screening of straight-phase column systems, where the universal response is useful, and the dynamic characterization of injection devices and highly efficient columns where the rapid response of the detector is very valuable.

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