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IMPROVED HIGH-FREQUENCY PERMITTIVITY DETECTOR FOR THE CHROMATOGRAPHY OF ALCOHOLS AND OTHER POLAR SPECIES

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SUMMARY

A permittivity detector for high-performance liquid chromatography (HPLC) and flowstream analysis employing three oscillators is described. The cell circuit is of the Franklin type, and the third oscillator allows operation over the complete range of permittivities and prevents locking. A new variable-spacing conical plate cell is described and the detector applied to amino acids and HPLC of alcohols.

INTRODUCTION

The principle of detecting an eluting solute by means of the change in permittivity of the carrier solvent is well known. The theory of such detectors has been developed by Haderka[1,2]. Most work published has involved low-permittivity, low-conductivity eluents with great emphasis on thermal considerations[3–5]. The permittivity detector offers universal sensitivity with a different sequence of sensitivities from the refractive index monitor together with no theoretical lower limit to the cell volume. A comparison of the relative changes of several bulk physical properties in Table I shows permittivity to be one of the most favourable.

Three techniques of measurement have been described, in all of which the flow cell forms a capacitor whose value varies with eluent permittivity. The heterodyne method[7] measures a change in the resonant frequency of an LC circuit with respect to a stable reference oscillator. Alternating current bridge methods[3] typically measure the out of balance signal of a bridge incorporating the flow cell in one arm. A time constant based detector that measures the resistance–capacitance product has been described[8], but it cannot distinguish between changes in these two properties. Heterodyne systems offer potentially the highest resolution which should be proportional to their frequency of operation.

The device described is a three oscillator heterodyne system, operating around 25 MHz designed to allow the use of solvents of any permittivity.

In the conventional two oscillator approach[7] the difference frequency between analyser and reference oscillator is passed to a frequency-to-voltage converter to produce a voltage output. Often the reference oscillator contains a second flow cell

TABLE I

A COMPARISON OF BULK PHYSICAL PROPERTIES (P) IN TERMS OF RANGE AND FRAC-
TIONAL CHANGE WITH TEMPERATURE, $(dP/P)/dT$[6]

	Range P_{min} to P_{max}	P_{max}/P_{min}	Range 10^{-3} $(dP/P)/dt$
Static permittivity	1.9–80+	40	20
Thermal conductivity (mW cm^{-1} °K^{-1})	1.0–6.1	6	3.0
Refractive index (5893 nm)	1.3–1.65	1.26	6.0
Viscosity (cP)	0.3–10	30	1.6
Density (g cm^{-3})	0.7–1.2	1.7	9.0
Ultrasonic velocity (km sec^{-1})	1.1–1.9	1.7	1.1
Magnetic susceptibility	0.99999–1.00001	—	—

through which a reference flowstream runs. Mounting both cells in the same block offers the advantage of thermal compensation[5], but the added complexity of maintaining two streams makes it unattractive. In the work described here a crystal controlled oscillator is used to provide the reference frequency; to permit frequency–voltage conversion the difference frequency is made less than 100 kHz.

Two oscillators of similar frequency will tend to lock such that the small potential change in frequency due to a small change in cell capacitance will not be observed at the output. This tendency to lock can be reduced by buffering and careful shielding, but locking occurred over several kHz with previous devices[9] and it is better to adopt a design which eliminates the problem totally.

If a single frequency quartz reference oscillator is employed the difference frequency will vary greatly with the eluent used, due to the wide change in cell capacitance. Such a system can only be optimised for a small range of solvent permittivities unless the plate spacing can be varied to achieve the desired capacitance — which, although possible, is not the best solution.

To overcome the "locking" problem it was initially arranged to run the reference and cell oscillators about 667 kHz apart. This first difference frequency was then mixed with a second crystal oscillator operating at 667 kHz and a second difference frequency obtained which was converted to a voltage output as before. (There is no scientific significance to the choice of difference frequency; anywhere in this region would be all right.) The operating restrictions of such a device were as severe as before and are shown in Fig. 1. The cell oscillator frequency must lie within ±100 kHz (the range of the frequency–voltage converter) of $f_2 \pm$ 667 kHz. By varying the plate spacing this can generally be achieved, but perhaps only at undesirably large cell volumes with consequent degradation of resolution.

To achieve greater flexibility, this fixed intermediate frequency oscillator was replaced by a frequency synthesizer allowing operation between $f_2 \pm$ 5 MHz. This wider operating range is shown in Fig. 1 and allows the use of whatever plate spacing is best from the views of mechanical stability and minimum cell volume for any eluent. This work describes the development of an improved capacitance cell and
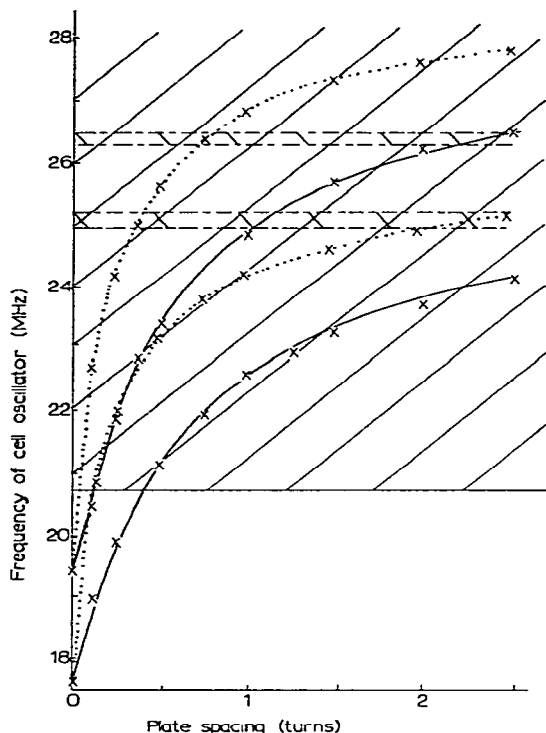
Fig. 1. Operating range of detector with crystal (cross-hatched) and frequency synthesizer (shaded) as intermediate frequency oscillator. ....., Propan-2-ol; ———, methanol–water (60:40).

measurement circuit for high-performance liquid chromatography (HPLC) and its application is shown for the chromatography of alcohols.

THEORETICAL

The theory of permittivity of mixtures (ε_m) is only fully understood for non-associating mixtures of non-polar liquids whose permittivities (ε_1, ε_2) are additive in terms of volume fractions (v_1, v_2):

$$\varepsilon_m = \varepsilon_1 v_1 + \varepsilon_2 v_2 \tag{1}$$

For dilute solutions of polar molecules such as the effluent from HPLC columns, ($v_x < 10^{-3}$), this may provide a reasonable approximation. If, however, the solute is a solid or changes on dissolution, i.e., ionises, dimerises or forms zwitterions, no such theoretical approximation is justified. It is found in practice that at sufficiently low concentrations the increase in relative permittivity is linear[10]

$$\varepsilon_m = \varepsilon_1 + \delta m_2 \tag{2}$$

where m_2 is the solute molarity and δ is a constant, the dielectric increment of the
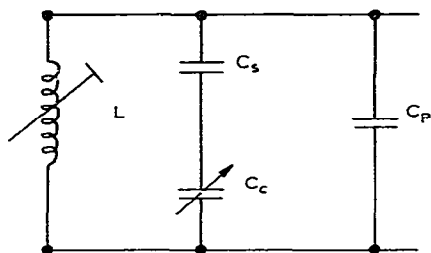
Fig. 2. Frequency determining components of an inductance–capacitance (LC) oscillator.

solute. Values for δ can be predicted, *e.g.*, Oster's equation[10] and the same reference gives a selection of values for amino-acids and other compounds in aqueous solution.

For the present study the predicted response of the detector, Δf, will be derived in terms of δ.

The frequency determining portion of the Franklin oscillator is shown in Fig. 2. The parallel capacitance, C_p, is the stray capacitance of leads, etc., amounting to about 0.5 pF. The frequency (f) will be given by

$$f = (1/2\pi)\,(LC_\tau)^{-\frac{1}{2}} \tag{3}$$

where L is the inductance and the total capacitance, C_τ, is given by:

$$C_\tau = C_p + \left(\frac{C_c C_s}{C_c + C_s}\right) \tag{4}$$

C_c is the cell capacitance with solvent in the cell and C_s the series capacitance. Rearranging eqn. 4 and substituting into eqn. 3 gives:

$$f = \frac{1}{2\pi} \times \left(\frac{C_c + C_s}{L\,[C_c(C_p + C_s) + C_s C_p]}\right)^{\frac{1}{2}} \tag{5}$$

When the cell is full of solvent of permittivity ε_1, $C_c = A\varepsilon_1$, $f = f_1$, where $A =$ constant. When the cell is filled with the mixture, $f = f_m$ and:

$$C_m = A\varepsilon_m = A\varepsilon_1 + A\delta m_2 \tag{6}$$

Substituting eqn. 6 into eqn. 5 for the condition $f = f_m$ results in:

$$f_m = f_1 \left(1 + \frac{A\delta m_2}{A\varepsilon_1 + C_s}\right)^{\frac{1}{2}} \left(1 + \frac{A\delta m_2(C_p + C_s)}{A\varepsilon_1(C_s + C_p) + C_s C_p}\right)^{-\frac{1}{2}} \tag{7}$$

If $m_2 \ll 1$ the latter two terms can be expanded by the binomial theorem:

$$f_m = f_1 \left(1 - \frac{A\delta m_2 C_s^2}{2\,[A\varepsilon_1(C_s + C_p) + C_s C_p]\,(A\varepsilon_1 + C_s)}\right) \tag{8}$$

Subtracting f_1 to obtain the difference frequency (Δf) and replacing $A = C_c/\varepsilon_1$ yields:

$$\Delta f = \frac{f_1 \delta m_2 C_c C_s^2}{2\varepsilon_1 [C_c(C_s + C_p) + C_s C_p] (C_s + C_p)} \tag{9}$$

Using eqn. 3 this gives:

$$\frac{\Delta f}{m_2} = \frac{f_1 \delta C_c C_s^2}{2\varepsilon_1 C_r (C_c + C_s)^2} \text{ Hz l mol}^{-1} \tag{10}$$

Defining a circuit constant $Z = C_c C_s^2 / C_r(C_c + C_s)^2$ and converting into concentration (X) units where M_2 is the molecular weight of the solute such that $X = m_2 M_2/1000$ yields:

$$\frac{\Delta f}{\Delta X} = \frac{500 f_1 \delta Z}{\varepsilon_1 M_2} \text{ Hz ml g}^{-1} \tag{11}$$

To obtain a high sensitivity one requires high operating frequency, low permittivity solvents and maximum Z. This last condition means making cell capacitance, C_c, small compared with C_s and eliminating as much stray capacitance, C_p, as possible. A complication is that the cell capacitance is composed of two parts and is better represented by $C_c = C_1 + B = A\varepsilon_1 + B$ where C_1 is that due to the filling liquid and B is due to stray paths through the air. Substituting C_1 for C_c leads to a reduced value (Z') for the circuit constant Z. For the cell described $B = 4$ pF (measured on a 0.1 % bridge at 1 MHz) and the typical value of $C_c = 17$ pF used means that the uncorrected value of Z is 24 % high. Thus cell design must attempt to minimise stray capacitance.

EXPERIMENTAL

A block diagram of detector circuitry is shown in Fig. 3; the power supply used was a ± 15-V encapsulated unit (R.S. Components Ltd.) with 0.1 % voltage regulation. The cell oscillator, of the Franklin design[11], operated nominally at 25 MHz, but was able to swing between 17 and 31 MHz. The reference was a crystal oscillator working on the third harmonic of an 8.579-MHz crystal to give an output of 25.736 MHz. The frequency synthesizer was programmed from a set of binary-coded decimal-
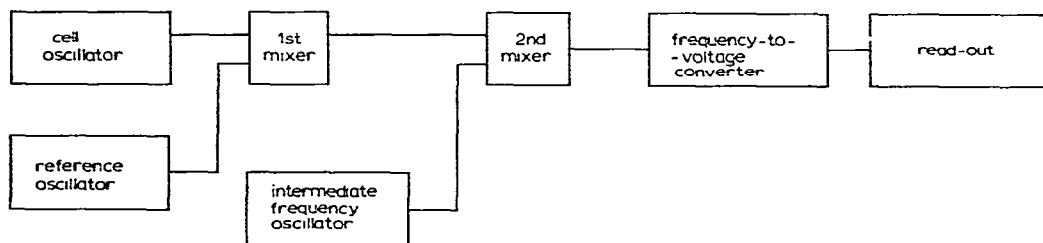

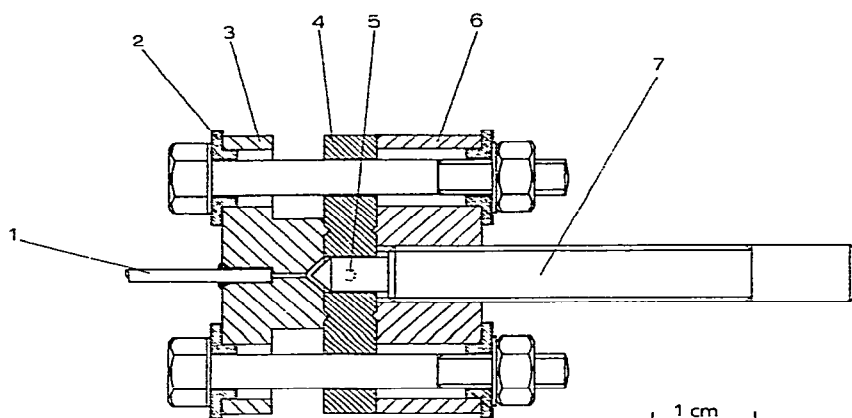Fig. 3. Block diagram of three-oscillator detector.

Fig. 4. Conical plate permittivity cell (section across diameter). 1 = Capillary inlet from column; 2 = insulating bush; 3 = ground plate; 4 = PTFE spacer; 5 = cell outlet and PTFE tubing; 6 = cell body; 7 = threaded rod forming moveable "high" plate.

encoded logic switches mounted on the front panel of the device. It produced a square-wave output between 3 kHz and 5 MHz in 1-kHz steps. The frequency-to-voltage converter (R.S. Components Ltd.) had a linearity of 0.1 % up to 100 kHz.

The pumps used were an HPL-HM (MPL Ltd.) metering pump, with a house-built pump damper for chromatographic studies and a Masterflex (Cole-Palmer Inc.) peristaltic pump with continuously variable pumping speed for development work. Solvents and chemicals used were of the highest purity obtainable and were degassed by boiling under reduced pressure to avoid bubble formation within the cell. All amino acids used were "chromatographically pure grade" (BDH, Poole, Great Britain).

The cell shown in Fig. 4 was of PTFE and stainless-steel construction. The column effluent entered through a short length of 0.006-in. or 0.010-in. I.D. stainless steel tubing (Phase Separations, Queensferry, Great Britain) into a conical cavity impinging onto a matching conical plate. The spacing could be varied by screwing-in this plate by 0.025-in. per turn. The volume between the conical plates was 5.5 μl per turn and the stray capacitance in air was 4 pF. The effluent passes out between the plates and a PTFE spacer into a PTFE exit tube.

In the chromatographic work a Specac 30-100 sampling valve (Specac-Sidcup) fitted with a 10-μl sample loop was used to introduce samples onto a 10-μm Li-Chrosorb RP-8 column (20 cm × 4.6 mm I.D.).

RESULTS

Experiments were performed to verify that response was proportional to δ/M (eqn. 11).

Amino acids were selected for their high δ/M values typically $0.1 < \delta/M < 0.6$ due to the formation of zwitterions. Solutions were made up in methanol–water (50:50) in the range 1–10 g l^{-1} and passed sequentially through the cell using a peristaltic pump, the solutions being first equilibrated in a thermostatted bath. The
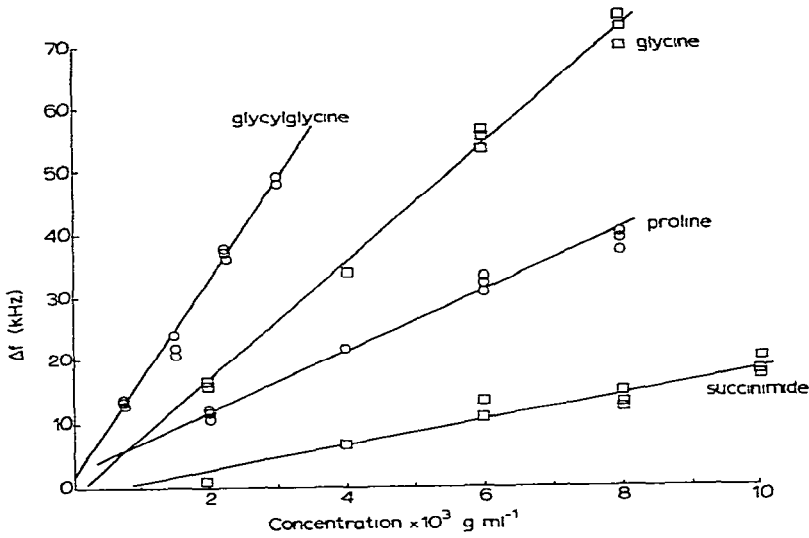
Fig. 5. Calibration curves for amino acids in methanol–water (50:50) (parallel plate cell, solvent as reference blank).
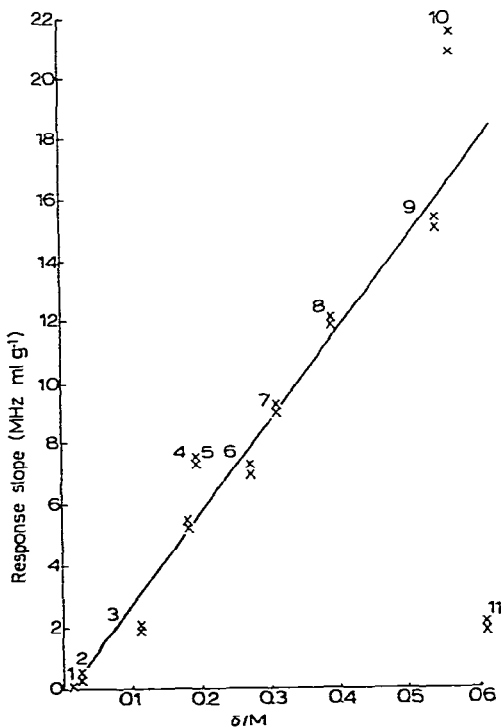

Fig. 6. Response found plotted against predicted sensitivity in methanol–water (50:50). 1 = Sorbitol; 2 = glucose; 3 = succinimide; 4 = proline; 5 = norvaline; 6 = alanine; 7 = glycine; 8 = β-alanine; 9 = glycylglycine; 10 = 6-amino-*n*-hexanoic acid; 11 = benzoic acid.

change in frequency, Δf, was measured with respect to the solvent blank. To avoid errors due to changes in the methanol–water ratio and hence carrier permittivity, the solvent was not heated to dissolve the acid but rather stirred under sealed conditions. It was not possible, generally, to make a stock solution in this way containing more than 10 g l^{-1}. The solvent was degassed beforehand whilst stirring under water pump vacuum. Over this limited concentration range linear calibrations of response were obtained as shown in Fig. 5 and the slopes of these are plotted against δ/M in Fig. 6 and presented in Table II together with values for glucose, sorbitol and benzoic acid.

TABLE II

LIST OF RESPONSES TO AMINO ACIDS AND OTHER COMPOUNDS IN METHANOL–WATER (50:50) (25.1 MHz)

All amino acids are BDH "chromatographically pure grade".

Compound	Slope (MHz ml g^{-1})	T (°C)	Range (g l^{-1})	δ/M
Succinimide	2.0	21.0	0–10	0.11
	1.9		0–10	
L-Tryptophan	4.0		0–5	
	4.9	22.8	0–3	?
β-Phenylalanine	4.3	22.6	0–3	
	4.4	23.1	0–3	?
L-Proline	5.4	25.1	0–10	0.18
	5.3		0–10	
L-Alanine (α)	7.0	21.4	0–10	0.27
	7.3		0–10	
Glycine	9.2	24.0	0–10	0.31
	9.0	25.6	0–10	
β-Alanine	12.0	20.1	0–10	0.39
	11.8	20.1	0–5	
Glycylglycine	15.3	25.0	0–3	0.54
	18.1		0–3	
6-Amino-	20.8	19.0	0–8	0.56
n-hexanoic acid	21.5	23.8	0–5	
Norvaline	7.5	19.2	0–5	0.19
	7.8	21.6	0–5	
Benzoic acid	1.9		0–10	0.61
	2.1	25.2	0–10	

It can be seen that with the notable exception of benzoic acid a reasonable straight line is obtained. Individual slopes were averaged over four runs using a least-squares fitting programme, run on a Nascom 2 microcomputer, to evaluate the relative standard deviation as between 9 and 16%.

The gradient of Fig. 6 is 30 MHz ml g^{-1} as against a predicted value ($C_c = 17$ pF, $C_p = 7.5$ pF, $C_s = 15$ pF) of 43 MHz ml g^{-1}. It is important to note that published values[10] of δ/M were obtained in aqueous solution while these results are for methanol–water (50:50). It has been shown[12] that the dielectric increment of the

amino acid α-butyrine is largely independent of solvent permittivity (between 35.4 and 134.9) or solution water content so that this approximation is of some value. The same source indicates that the increment is also nearly independent of temperature and concentration. This is presumably due to the fact that charge transfer is internal and complete so that solution effects are unimportant. Benzoic acid gives less response than expected, presumably since the ionisation (which gives rise to solvent ordering and hence a reduction of permittivity) is suppressed on the addition of methanol. The sugars give about a quarter of the predicted response, perhaps due to changes in equilibria between the various ring forms and conformational isomers in solution.

It is usual[13] to buffer solutions of amino acids to increase retention on reversed-phase columns but the conductivity of the buffer acts as a large admittance in parallel with the cell and causes spurious changes in the oscillator frequency. It was not possible to gain any significant separation of amino acids (as indicated by a fixed 254-nm UV detector operated in series with the permittivity detector) at concentrations below 0.025 M acetic acid which was found to be the maximum tolerable
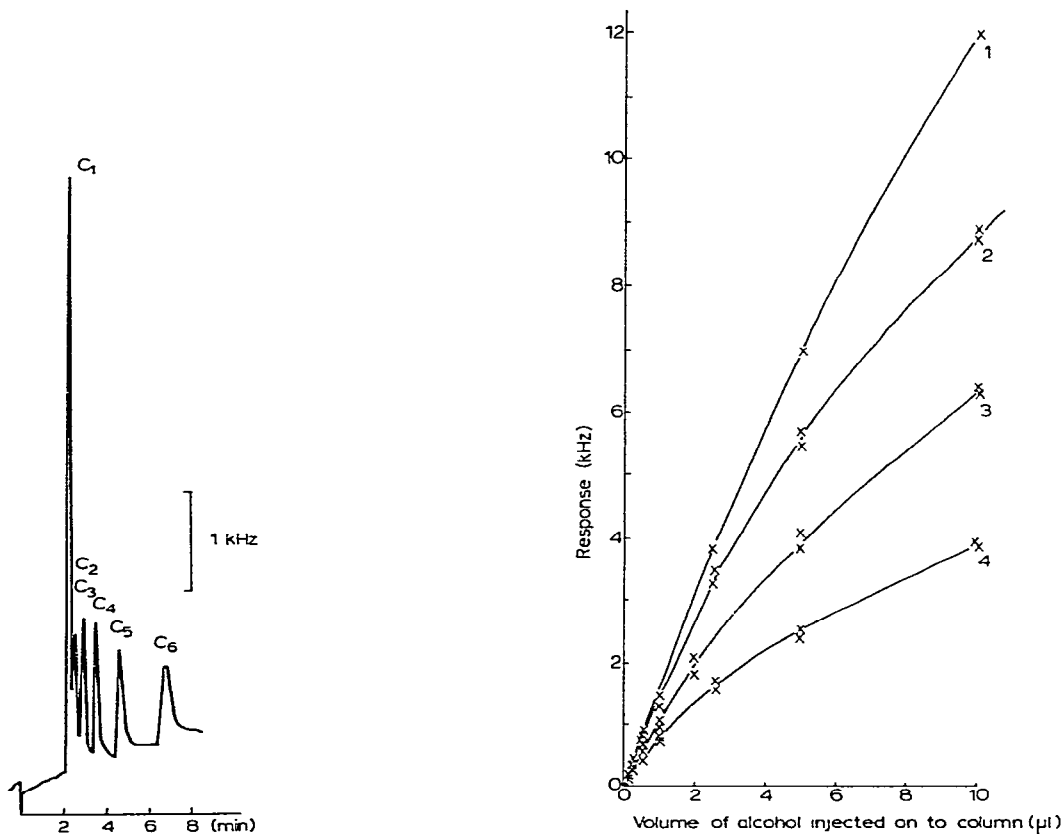
Fig. 7. Chromatogram of primary alcohols on LiChrosorb RP-8, methanol–water (60:40), 1.1 ml min^{-1}.

Fig. 8. Variation of peak height obtained in methanol–water (50:50) against volume injected (C_s = 30 pF). Curves: 1 = propan-1-ol; 2 = butan-1-ol; 3 = pentan-1-ol; 4 = hexan-1-ol.

level. This means that although there is sufficient sensitivity, the detector is unsuitable for the detection of amino acids in practical HPLC.

Primary alcohols are miscible with methanol–water mixtures and have a wide range of permittivities. Solutions were made up in the range 0.2–100% (v/v) and introduced via a 10-μl sampling valve into an eluent of methanol–water (60:40). The resulting chromatogram is shown in Fig. 7. Graphs of peak height against volume injected are shown in Figs. 8 and 9. The expected order of sensitivities is reversed due to the order of elution which spreads the less polar, higher alcohols more.

Since sensitivity should be linear at low concentrations the area under the peak should be proportional to the weight injected $\times$ δ/M. Solutions of alcohols (10%, w/v) were made up in methanol and 10-μl samples injected onto the column at a flow-rate of 1.5 ml min^{-1} of methanol–water (70:30 or 50:50). The area of the peak was found by cutting and weighing, and plotted against δ/M, a measure of predicted response (Fig. 10). (Relative standard deviation on cutting and weighing was 2.8%.)
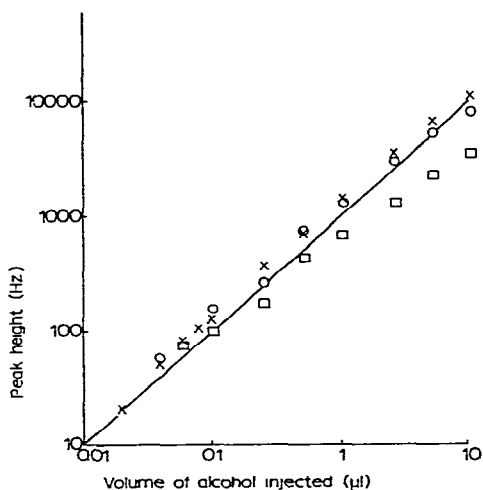

Fig. 9. Log–log graph of peak height against amount injected in methanol–water (50:50) (C_s = 30 pF). $\times$ = Propan-1-ol; $\bigcirc$ = butan-1-ol; $\square$ = hexan-1-ol. Line drawn has a slope of $+1$ for comparison.
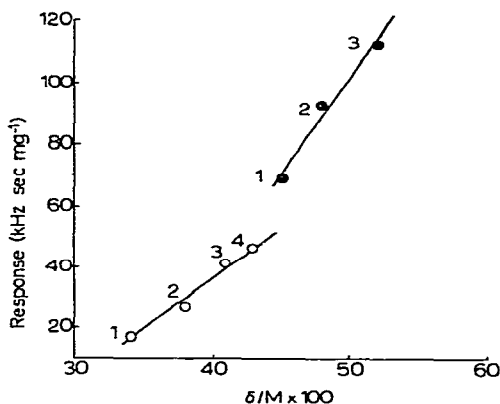
Fig. 10. Peak area response against δ/M for several primary alcohols in methanol–water (70:30) (full circles) and methanol–water (50:50) (open circles). 1 = Propan-1-ol; 2 = butan-1-ol; 3 = pentan-1-ol; 4 = hexan-1-ol.

For the individual solvents the relationship is as might be expected; the reason for the different gradients between the two solvent systems is not obvious but is possibly connected with the differing degree of solvation of the alcohols in the more aqueous medium. (Hexanol was not adequately chromatographed in the lower permittivity medium to warrant plotting the point.)

CONCLUSIONS

The instrument described, employing a very stable Franklin type oscillator for the cell circuit and second intermediate frequency to prevent locking, is a significant

improvement over the earlier devices. The new cell employed has better stability characteristics than the parallel plate cell used earlier, is less prone to bubble formation and has minimal dead volume. The detection system has shown itself most useful for the sample alcohols and amino acids, the conductivity of the buffering media, however, prevents its exploitation at present.

ACKNOWLEDGEMENTS

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