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Note

Electronic signal differentiation as an aid for the comparison of size-exclusion chromatograms

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The use of size-exclusion chromatography on microparticulate silica for the rapid comparison of a wide variety of samples of forensic interest has recently been reported¹. This form of chromatography, which produces a molecular size profile, often generates chromatograms which consist of a single broad peak with various shoulders and inflections and the comparison of such data can be difficult. To afford a greater level of confidence when judging the significance of such profiles it is the usual practice here to sequentially monitor the eluate with two detectors mounted in series, but a supplementary method is to enhance the visual difference between poorly structured chromatograms generated with a single detector by electronically differentiating the signal. Although derivative spectrophotometry is not new, recent developments in electronics now make it a simple technique to apply², and its use for enhancing the discriminatory capability of size exclusion chromatography is an obvious area of application.

EXPERIMENTAL

The column used in these experiments was a stainless-steel tube, 25 cm × 3/8 in. O.D. × 0.8 cm I.D., terminated with Zero Dead Volume (ZDV) reducing unions (3/8-1/16 in.) and packed with an irregular microparticulate silica of *ca.* 5 μm diameter, average pore size 13 nm, and surface area 320 m²/g. The solvent used was tetrahydrofuran containing 1% water and it was pumped at 4 ml/min. The eluate was monitored with a UV detector (Cecil 212, Cecil Instruments, Cambridge, Great Britain) operated at 254 nm. Samples were dissolved in tetrahydrofuran and injected using a stop-flow technique. The detector signal was split to provide the fundamental chromatogram and its 1st derivative form simultaneously, and both were recorded on flat-bed pen recorders.

The electronic differentiation was carried out using the circuit shown in Fig. 1 which was designed after consideration of various reference sources³⁻⁵. IC 1 is a non-inverting, unity gain buffer amplifier used to match the output impedance of the detector to the input impedance of IC 2. IC 2 is the differentiator circuit which gives an output voltage proportional to the rate of change of input voltage *i.e.*

$$E_o = - C_1 R_1 \frac{dE_{in}}{dt}$$

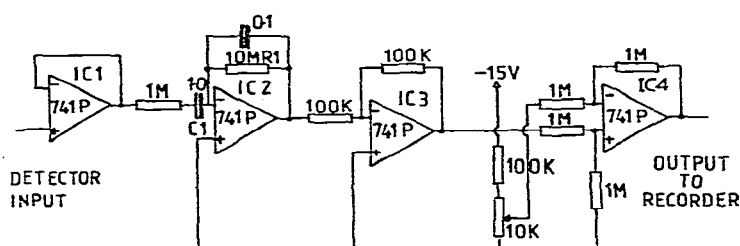


Fig. 1. The circuit used for the first-order differentiation of the detector signal produced with size-exclusion chromatograms.

As this circuit also inverts the signal a unity gain inverter IC 3 is utilised to correct the signal; the output after IC 3 then becoming $-E_0$. A d.c. level is present at the output of IC 3 due to the leakage of components in and around IC 2 and this standing current is backed off using the subtractor circuit of IC 4 to present a signal suitable for a pen recorder input.

RESULTS AND DISCUSSION

Typical chromatograms produced using the described technique are shown in Fig. 2. It can be readily seen that the first-derivative form of the chromatograms is sufficiently structured to make the visual comparison of two or more profiles a simple task; the derivatised form of the chromatogram is also highly reproducible. The speed and versatility of size exclusion chromatography already makes this a powerful method for evaluating high polymers and complex mixtures of lower molecular weight and the use of electronic differentiation can only add to its versatility.

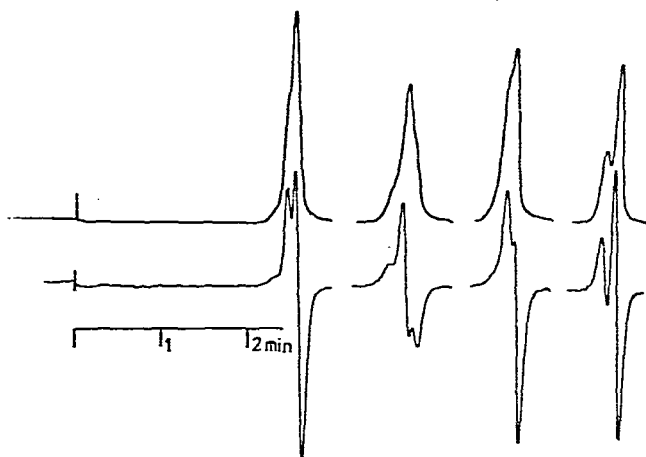


Fig. 2. Size-exclusion chromatograms of engine oils: comparison of the fundamental and first-derivative form of the UV detector response. Column: 25×0.8 cm I.D.; packing: $5\text{-}\mu\text{m}$ silica of 13 nm pore size; solvent: tetrahydrofuran-water (99:1); flow-rate: 4 ml/min; pressure: 1400 p.s.i.; detector: UV at 254 nm. The upper traces are the fundamental form of the chromatogram, the lower traces the first-derivative form.

The circuit described costs only about £ 5 to construct and despite the use of low-quality components worked very satisfactorily. One of the main problems in constructing low-frequency differentiator circuits is that of the long time constants involved and ideally low-leakage, non-electrolytic capacitors should be used in conjunction with high input impedance operational amplifiers. Although the circuit shown does not use capacitors of this type it was adequate for first-order differentiation but is unlikely to perform well if higher orders of differentiation are required. Nevertheless there is every indication that differentiation to higher orders can be advantageous² and we propose to study this area of application in due course.

ACKNOWLEDGEMENTS

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