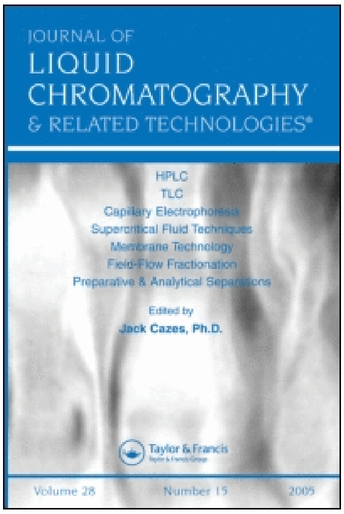

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HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY ON MICROPARTICULATE SILICAS AND ITS APPLICATION TO FORENSIC ANALYSIS

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ABSTRACT

Size exclusion chromatography (SEC) on irregular silicas of 5-10 um particle size and 60-130 Å average pore size is shown to provide a rapid method for the comparison of a variety of forensically significant samples. Use of dual detectors (UV/fluorescence of UV/Refractive Index) was found to enhance the value of the technique for forensic screening.

INTRODUCTION

Forensic analysis frequently requires the comparison of a variety of materials and although highly discriminatory methods are ultimately necessary to satisfy the stringent demands of the work it is often convenient to use rapid screening techniques at an early stage of an examination to eliminate dissimilar samples. Of the chromatographic procedures now available high performance size exclusion chromatography (SEC) offers one of the most rapid methods of comparison, but no applications to forensic analysis have yet been reported. This paper describes SEC studies undertaken in this laboratory during the last year.

EXPERIMENTAL

All samples were dissolved in tetrahydrofuran (THF) of HPLC grade. Following an investigation of different experimental parameters all samples were chromatographed under the following standard conditions:-

Column

25cm x $\frac{3}{8}$" o.d. (8.1 mm i.d) stainless steel column, slurry packed with microparticulate silica of ca 5 um particle size of an average pore size in the range 50-130 Å. The columns were terminated with specially machined end-fittings ($\frac{3}{8}$" - 1/16" reducers), either flat-bottomed or with an internal taper.

Solvent

THF + 1% water

Flow rate

2ml/min

Pressure

ca 600-700 psi

Detectors

A variable wavelength UV detector (Cecil 212, Cecil Instruments Ltd., Cambridge, England) operated at 254 nm was coupled with one other detector using PTFE microbore tubing. This was either a fluorescence detector (Perkin Elmer LC 1000, Beaconsfield, England) operated at an excitation wavelength of 364 nm and an emission wavelength of 420 nm., or a refractive index detector (Model 750/13, Applied Chromatography Systems Ltd., Luton, England).

Pump

Waters 6000 or Altex 110 A

Injection

Stop-flow syringe injection (see later)

Standards

For column testing, various polystyrene standards were used (supplied by Polymer Laboratories Ltd., Shawbury, Shrewsbury, England). An epoxy resin sample (Epikote 1001, Shell Chemical Co., Ltd) was also used for rapidly assessing column performance.

RESULTS AND DISCUSSION

The silicas

Although a number of silicas are now specifically marketed for SEC, it should be appreciated that most chromatographic grade silicas with pore sizes in the range of 50-150 Å can provide size-based separations of molecules with dimensions below that of a 20,000 to 30,000 M.Wt polystyrene (1). Forensic samples usually contain a high proportion of molecules of this size range and hence four microparticulate, irregular silicas of the appropriate pore size were studied:- Partisil 5 (Whatman Inc., Clifton, N.J), Lichrosorb SI 60 and SI 100, (E. Merck Ltd., Darmstadt, W. Germany), Syloid 74 (Davison Chemical Division, Baltimore, Md). The first three silicas are supplied specifically for HPLC, but the latter material, which is used as a low-cost filler, was found to have too wide a particle size distribution for immediate use and was graded by sedimentation to give a particle size range from 4-7 um (i.e. similar to the other supports).

Columns of the dimensions shown above were packed with the various silicas and calibrated by injecting appropriate amounts of polystyrene standards. The calibration curves are shown in Figure 1. It is apparent from these curves that the accessible pore volume ranges from 4.1ml (i.e. 10.2 - 6.1 ml) in the case of Lichrosorb SI 60 to 4.9 ml in the case of Syloid 74. The exclusion limit increases in the sequence Lichrosorb SI 60, Partisil 5, Syloid 74 and Lichrosorb SI 100 and ranges from 13,000 - 35,500 M.Wt. For most applications that were subsequently studied there was found to be little to choose between the various silicas in terms of their size exclusion performance. The chromatographic efficiencies of the four packing materials were also rather similar - see Table 1

The differences in efficiency of these silicas can be seen to parallel changes in pressure drop and it can be assumed that they are related to particle size rather than any other property

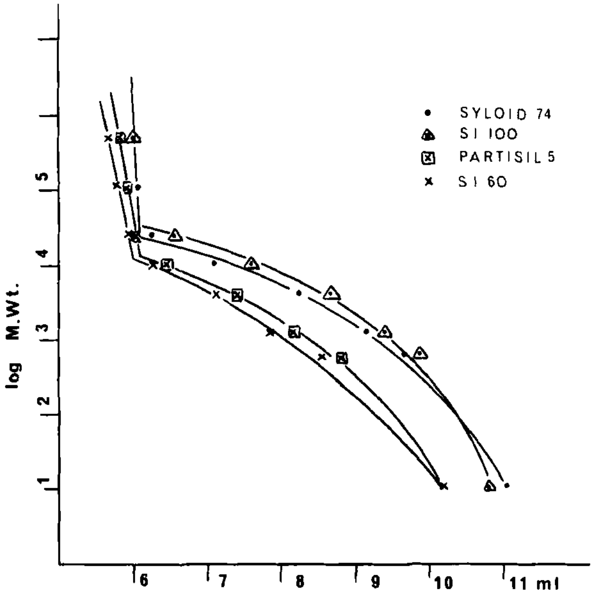

FIGURE 1 Calibration curves for polystyrene on four different silicas

TABLE 1

Comparison of the chromatographic efficiencies of columns packed with four different silicas.

Silica	Pressure drop (psi)	Retention volume of toluene (mls)	N *
Lichrosorb SI 60	575	9.63	18,000
Partisil 5	675	9.66	29,100
Syloid 74	625	10.47	22,100
Lichrosorb SI 100	625	10.53	20,450

*The efficiency was determined for the toluene peak, with 1 ul injections being made on to 25cm x 8.1 mm columns with the solvent flow rate being 2ml/min.

inherent in the packing material. Thus the packing material which gave the highest pressure drop also gave the highest efficiency and vice versa.

INJECTION TECHNIQUE

Stop-flow syringe injection is used routinely in this laboratory because it provides a low cost system of sample introduction which has the chromatographic advantages of allowing centralised on-column injection and is not pressure limited. The injectors normally used here are modified ball valves (2), but these cannot be used with THF owing to solvent attack of certain elastomers within the valve. An alternative all-stainless steel injector was therefore produced from a 1/16" tee (Swagelok) and is shown in Figure 2. The tee was capped at one end and was connected to the column with a length of wide bore 1/16" o.d. stainless steel tubing. To make an injection the cap was removed and the syringe needle pushed down to the surface of the frit terminating the column.

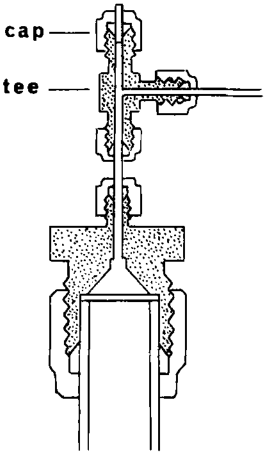

FIGURE 2 Stop-flow injector made from a Swagelok 1/16" tee.

In using this type of injector with a column fitted with either a flat-bottomed or tapered ⅛" - 1/16" reducing union three different injection procedures were studied - these are illustrated diagrammatically in Figure 3. In the case of configurations 3b) and 3c) the conical or cylindrical voids above the columns were filled with glass ballotini (<u>ca</u> 80 mesh), which allowed needle penetration, but contained and hence prevented the dispersion of the injected liquid. The results obtained with the various configurations are summarised in Table 2 (the same packing material was used in each case).

It is apparent from the results that the type of end fitting configuration makes a considerable difference to the performance of the column. Although configuration 3c) gave the best results, in practice it was found that columns terminated in this way deteriorated fairly rapidly. This was attributed to the flexible wire mesh failing to prevent the column bed from unpacking upwards with the frequent stopping and starting of the solvent flow. In contrast configuration 3b) which gave slightly poorer column efficiency was found to impart good column stability, presumably

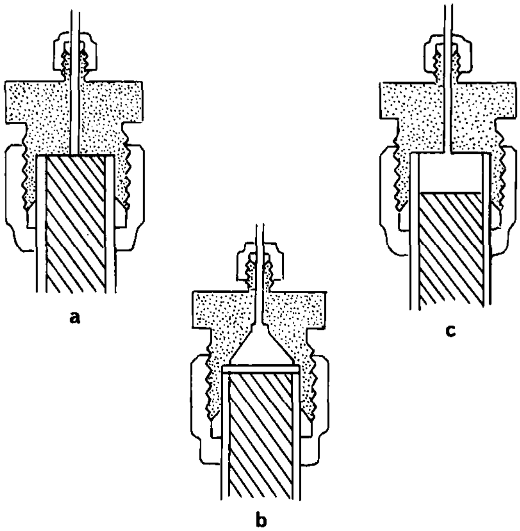

FIGURE 3 End fitting configurations used with the stop-flow injector.

a) Steel mesh disc at the top of the column. No void above.

b) Steel frit on top of the column. The conical void above the frit was filled with glass ballotini.

c) Steel mesh disc pressed down on to the packing material. The cylindrical void above the mesh was filled with glass ballotini.

TABLE 2

Variation of column efficiency with end fitting configuration

Configuration (see Fig. 3)	Column efficiency*
a	10,100
b	22,100
c	27,500

*N value determined for a 1 ul injection of a toluene standard. Column dimensions 25cm x 8.1mm i.d. Flow rate 2ml/min.

because the rigid steel frit prevented any back-expansion of the column bed. The poor performance of system 3a) has been noted by other workers when using valve injectors (3) and is probably attributable to unswept dead areas in the column bed immediately below the inlet reducing union. The applications described later were all carried out using configuration 3b).

It should be noted that the presence of glass beads was found to be essential in achieving the maximum efficiency and reproducibility with the type of stop-flow syringe injections used in this work. The beads act by containing the small volume of liquid injected and prevent any band spreading before the sample contacts the packed bed. They also help to smooth out the flow of the incoming solvent stream.

COLUMN DIMENSIONS

In using any chromatographic method for comparative analysis resolution is obviously an important factor, but in the case of SEC carried out on a given silica, resolution can only be improved by increasing the efficiency of the column, either by altering its dimensions or by using a smaller particle size packing material. In developing the described method a variety of experiments were carried out to arrive at a set of conditions which would prove adequate for the majority of samples. One of the factors studied was the column diameter and Table 3 illustrates the results obtained from columns of the same length but different diameters, with the solvent flow rate adjusted to produce approximately the same velocity down the columns.

The results show that increasing the column diameter provides a way of improving column efficiency without increasing the analysis time. This is consistent with other reported work (4) and is probably attributable to the way in which "wall effects" reduce efficiency in relatively narrow bore columns.

TABLE 3

Variation of column efficiency as a function of column diameter

Column diameter o.d. (inches)	i.d. (mm)	Column efficiency*	Flow rate (ml/min)
1/4"	4.9	14,500	1
3/8"	8.1	22,100	2
1/2"	10.5	27,000	4

*N value determined for a 1 ul injection of a toluene solution in THF. Columns were 25 cm in length and were packed with the same batch of Syloid 74. The injection configuration was b) above.

Despite the advantages of using wide bore columns there are two practical drawbacks:-

1) the quantity of packing material used becomes large and hence expensive.

2) the dilution effect on the sample makes detection more difficult.

In forensic work where samples are often very small, detector sensitivity is a constant problem and despite the attractions of the 1/2" diameter column it was found that the 3/8" column represented the best practical compromise between efficiency and minimal sample dilution. It was found possible to produce columns displaying efficiencies of 40,000 to 50,000 theoretical plates (measured on the basis of the toluene peak) by increasing the column length to 50 cm, but for most samples the higher efficiency offered no practical advantage since it was attained at the expense of increased analysis time and additional sample dilution.

SOLVENT COMPOSITION

The advantages of using THF as a solvent for SEC are that it is able to dissolve a wide variety of materials, and can be readily obtained in an HPLC grade which allows use with UV detectors at 254 nm. When anhydrous THF was used as a solvent, however, there was a tendency for silicas of the type studied to adsorb polar compounds, causing them to elute at a volume greater than would be expected from the solute size. To reduce this phenomenon small quantities of water were added to the THF to de-activate the silica. Figure 4 illustrates the change in the elution volume of aniline, phenol and toluene as a function of varying water content. It will be noticed that the least polar compound - toluene, eluted at a constant volume, whereas the two more polar compounds eluted with reduced volumes as the water content increased.

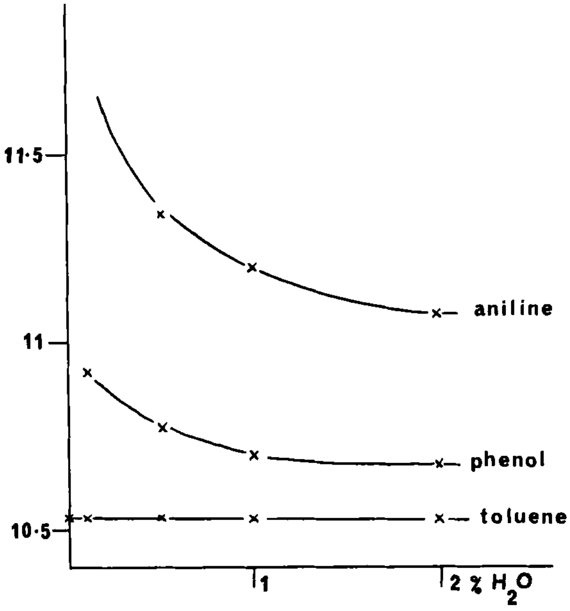

FIGURE 4 The variation of the elution volume of toluene, phenol and aniline as a function of the water content of the THF.

In practice a water content of 1% was adopted for regular use as this was found to effectively deactivate the silicas without reducing the solvent properties of the THF. In general most samples were not found to contain components that were retained beyond about 11ml; cutting oils, however, were exceptional and did give rise to some strongly retained peaks (e.g. eluting at 12-15 ml). Even when such retention occurred it was found to be reproducible and the only drawback was that it increased the time of analysis.

MONITORING WAVELENGTHS

The UV monitoring wavelength and the settings of the fluorescence detector were arrived at empirically. There is no reason why other wavelength settings should not be used and on occasion they may well provide additional information.

SAMPLE DISCRIMINATION

The chromatograms obtained in this study were found to be reproducible, independent of the sample concentration and often showed marked differences in their structural features. The shape of the chromatogram was also very often characteristic of a class of material. Despite the structural simplicity of most of the size exclusion chromatograms produced, features such as minor peaks and shoulders on major peaks had considerable significance, and with the described injection procedure such features were highly reproducible. Two criteria were used to compare samples:-

1) the shape of chromatograms.

2) the ratio of UV to fluorescence response or UV to RI response.

In many instances an analysis with the UV/fluorescence detector combination was adequate to discriminate samples, but if not a repeat injection with the UV/RI combination was carried out.

When using the RI detector it was always necessary to inject larger amounts of the sample to compensate for the insensitivity of this monitoring device.

It was found convenient to quantitate criterion 2) above in the form of a response factor (e.g. UV peak height/peak height on one of the other two detectors). Such factors have no absolute significance since they are dependent upon the detectors used, but under a standard set of conditions they provide a value which is dependent upon the chemical constitution of the sample and hence can be used to compare samples. The experimental results were found to be reproducible (i.e. within 5-10%) and independent of concentration over at least an order of magnitude. The values were found to vary, however, when measured after an interval of several weeks.

APPLICATIONS

Vehicle Indicator plastics

Optical quality plastics such as vehicle indicator light covers are usually based on polymethylmethacrylate and related polymers. Fragments of such materials are encountered in cases arising from vehicle incidents and SEC was found to be a useful method for comparing such materials. Typical chromatograms are shown in Figure 5 and it can be seen that the UV detector tended to observe low molecular weight components, whereas the RI detector was capable of also detecting higher polymers. Occasionally some UV response was observed in the higher molecular weight region and this was assumed to be due to a strongly UV absorbing species such as polystyrene. (see Fig 5a). Although some variation was observed in the low molecular weight profiles (attributable to anti-oxidants and dyestuffs) most discrimination was possible by comparing the UV/RI response ratio (i.e. low molecular weight maximum/high molecular weight maximum). In a group of indicator plastics chosen at random from case work samples the following results were obtained

Response factor*	Number of samples
1.5 - 1.99	2
1.0 - 1.49	6
0.5 - 0.99	3
0 - 0.49	4

*UV response at 0.2 sensitivity/RI response at x 4 setting

Such response factors were reproducible during a given day and independent of the concentration of sample over a wide range, hence samples could be compared without having to match concentrations too accurately. The amount of sample required for injection was about 50 ug, but this could be present in as much as 25 ul of THF.

Few of the indicator plastics examined produced a fluorescence response, but where a response was found it was at the low molecular weight end of the chromatogram and was assumed to be due to fluorescent dyestuffs. The comparative rarity of this phenomenon made it of strong evidential value when it did arise.

Oils

SEC was found to be a powerful method for discriminating both used and unused engine oils. Over 60 oil samples were examined in the survey and by using the UV and fluorescence detectors in series it was possible to uniquely characterise each sample. Typical chromatograms are shown in Figure 6. A few of the oils contained UV absorbing high molecular weight components (e.g. 6a), but this was exceptional, whereas every sample had lower molecular weight components which produced chromatograms differing both in their structural features and the response ratios. (e.g. 6b & 6c). Increasing use of an engine oil led to an increase in the intensity of the fluorescence response and it was assumed that this was due to the build up of polynuclear hydrocarbons resulting from combustion

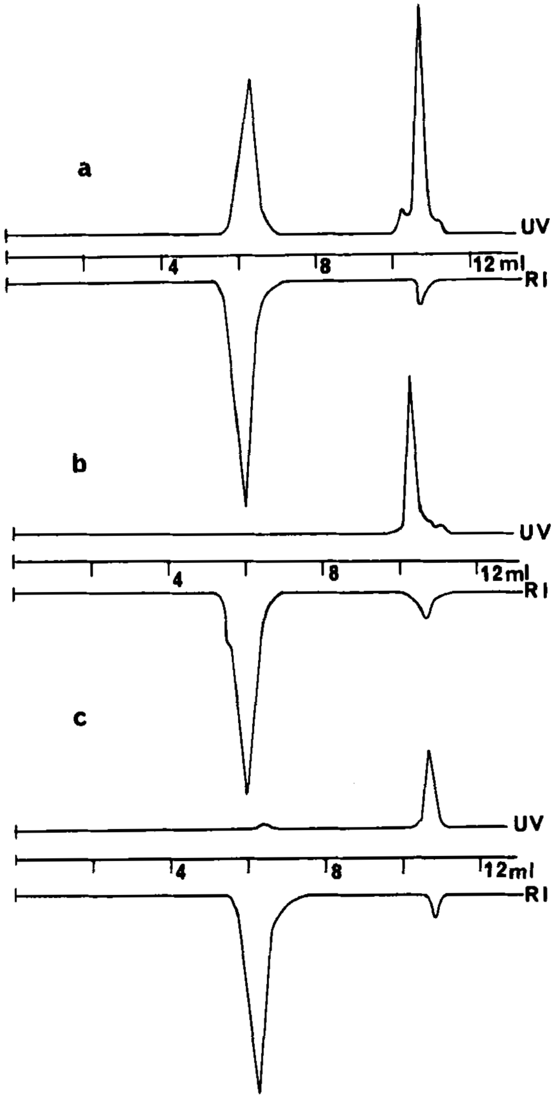

FIGURE 5 Chromatograms of vehicle indicator plastics.

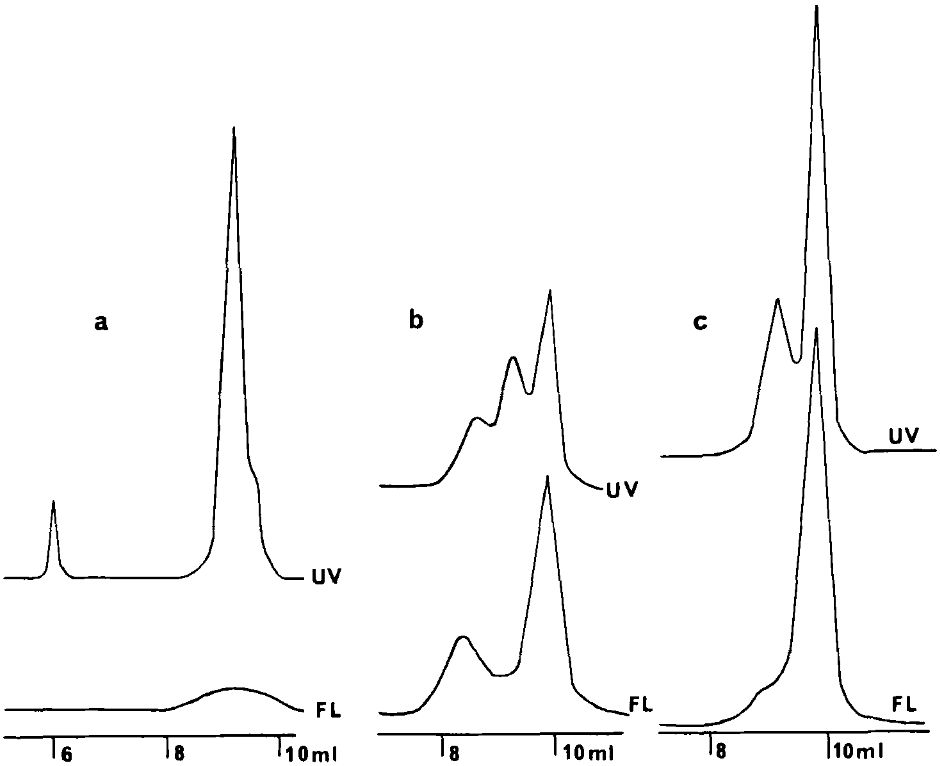

FIGURE 6 Chromatograms of used engine oils.

processes. Despite most of the engine oil sample being paraffinic in character and eluting without detection when monitoring by UV absorption or fluorescence, those oil components that are detected enable oil samples of 1-5 ug to be compared. In contrast the RI detector had far less sensitivity but was able to produce an adequate signal from samples of 5-10 ug. Although the RI detector provided little discrimination when used alone UV/RI response ratios varied considerably and are a useful discriminant.

In addition to the engine lubricating oils it was found that cutting oils i.e. oils used to cool and lubricate metals during machining processes, could also be discriminated by SEC. Such oils gave chromatograms indicative of a lower molecular weight range than found in engine oils, and displayed less structure. Despite the simplicity of the chromatograms the response ratio UV/fluorescence varied appreciably and generally required about 1-5 ug of oil. The UV/RI detector combination required 10-20 ug of oil to be injected. By using both ratios it was possible to uniquely characterise twelve different oils from one manufacturer.

Bitumens

Materials of this type are encountered quite frequently in the form of bitumen paints, vehicle underseals, insulating tape backings etc. As a group they are quite difficult to discriminate and it is fortunate that SEC provides a convenient screening technique. Typical chromatograms of bitumens are shown in Figure 7, and it can be seen that such materials contain components which eluted across the whole of the included molecular weight range. These components were strongly UV absorbing and at the lower molecular weight end of the chromatogram displayed strong fluorescence. The shape of the chromatogram was found to be quite distinctive for this class of material, but showed sufficient variation to be a powerful discriminant. In addition the UV/fluorescence response ratio was found to give added discrimination and the method could be used to compare less than 0.5 ug amounts of sample. The RI detector was far less sensitive and an injection of 10-20 ug of material was needed to produce a chromatogram. The RI trace was complicated by the high molecular weight components having RI values lower than that of THF (i.e. lower than 1.404) whereas the lower molecular weight fraction had a higher RI. Thus the chromatographic trace was distributed on either side of the axis corresponding to the RI of THF.

Plastic tapes

This type of sample occurs in a variety of cases and SEC appears to offer a rapid method of comparative analysis. Typical

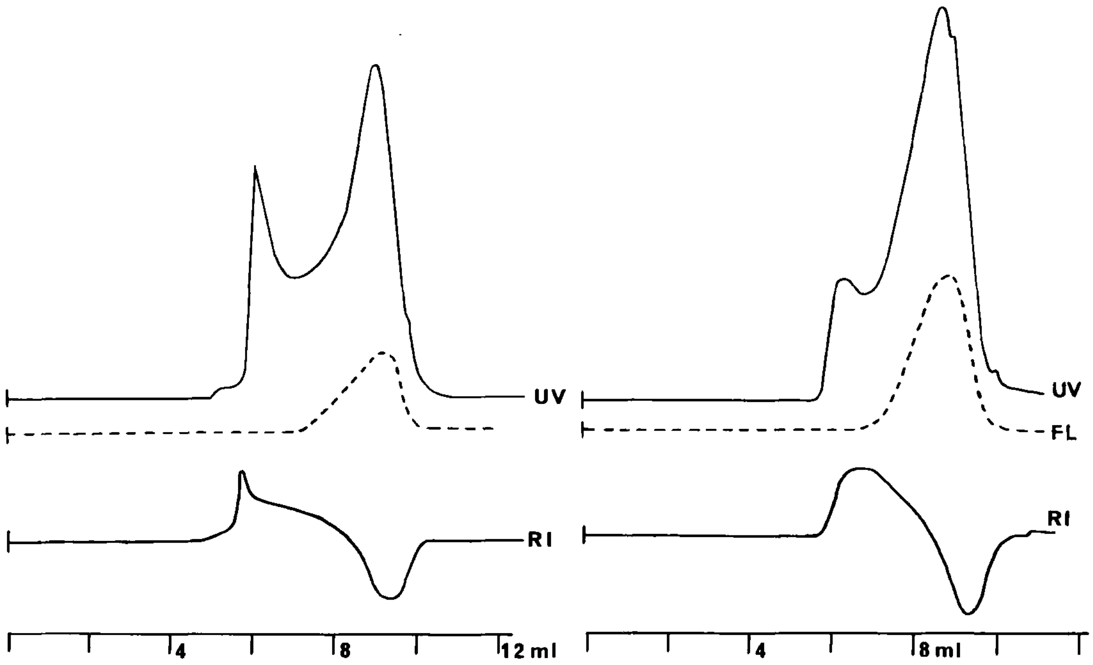

FIGURE 7 Chromatograms of bitumens.

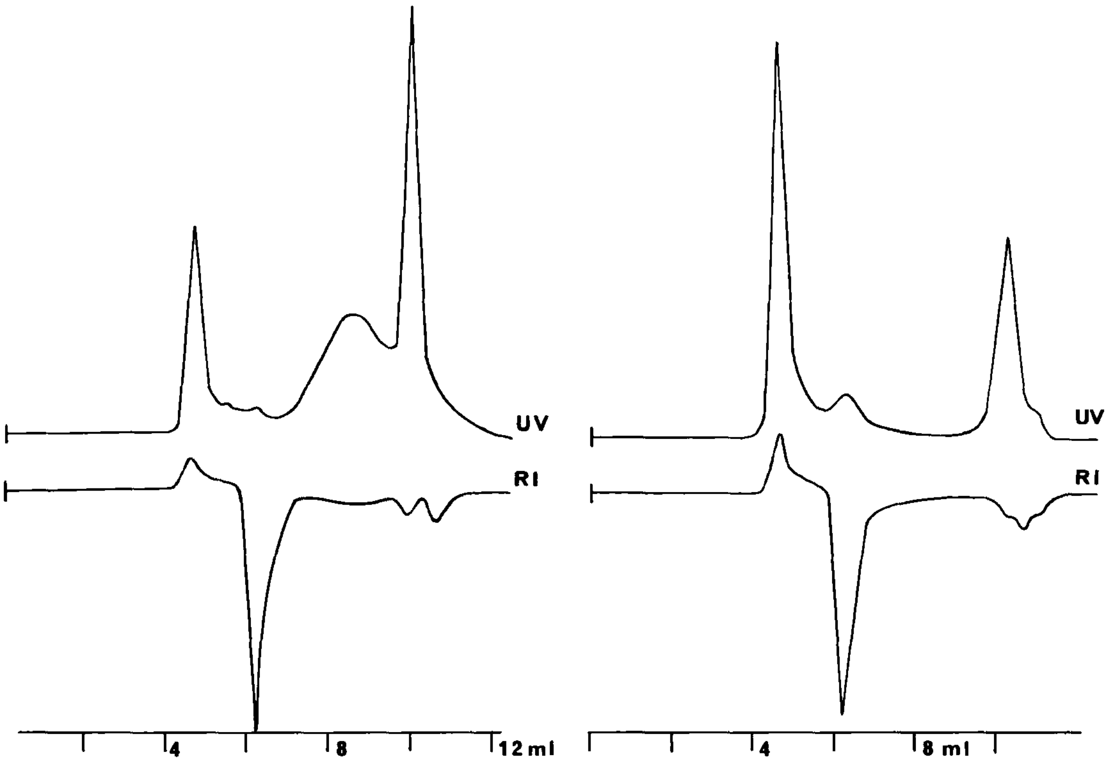

FIGURE 8 Chromatograms of plastic tapes.

chromatograms are shown in Figure 8 and about 20 ug of sample was necessary to display an acceptable RI response. Very few of the samples examined displayed fluorescence but the UV/RI combination was a powerful discriminant.

Miscellaneous samples

In addition to the samples described above a variety of other materials have been examined by SEC. Samples such as cosmetic creams, lipsticks, waxes, rubber elastomers etc have all been found to produce characteristic SEC profiles and the application of the method to such samples was limited only by the constraints of sample size and solubility in the THF solvent.

Preparative aspects of SEC

The results presented above indicate that SEC provides a rapid and reproducible method for producing chromatographic profiles for a variety of forensically important samples. When two such profiles are identical there is a probability, high in some instances, that the samples are very similar and in such circumstances a further examination by an independent technique is appropriate. Once again SEC can be of value, for the columns described can be used in a preparative mode to isolate particular components from the sample solution to aid in their characterisation by spectroscopic or other methods. Thus low molecular weight components of polymeric materials can be readily isolated from the high polymer and consequently more effectively identified. It must be concluded that SEC has a versatility that should make it of considerable significance in the forensic field.

ACKNOWLEDGMENTS

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