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CAPILLARY HYDRODYNAMIC CHROMATOGRAPHY —AN INVESTIGATION INTO OPERATIONAL CHARACTERISTICS

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SUMMARY

Three capillary tubes of equal length (50 m) but different internal diameter (182, 242, 450 μm) have been examined as columns for capillary hydrodynamic chromatography The tubes were calibrated for particle size analysis by elution of standards in water, methanol and tetrahydrofuran and applied to the fractionation of paint, pollen and used engine oil

Analytical separation of particles in the diameter range 0 07–40 μm was achieved. Column resolution improved dramatically at higher flow-rates of eluent, contrary to usual chromatographic behaviour.

INTRODUCTION

Hydrodynamic chromatography (HDC), as recently introduced by Small and co-workers[1 2], is an experimentally simple method for the fractionation of colloids, emulsions and particle suspensions In its classical form, particulate samples in the diameter range 0 01–1 0 μm are eluted with water or methanol through a packed column of uniform impermeable beads. The particles are fractionated and emerge in order of decreasing diameter A variant of the technique, described by Noel *et al*[3] and Mullins and Orr[4], uses a long open capillary tube to achieve a similar separation of particles. The range over which fractionation is achieved in this case is 0 5–30 μm These two techniques, packed column HDC and capillary HDC, form the beginnings of a bridge between conventional techniques of particle size analysis, such as sieving, microscopy, centrifugation and Coulter counting, and molecular size analysis of soluble high polymers, emulsions and viruses by permeation chromatography

In both forms of HDC, the order of particle elution is not yet fully explained but several mechanisms have been postulated. For packed column HDC, Small and co-workers[1 2] suggested that the separation must occur in the void volume of the column by a combination of three effects First, particles to be separated vary in their ability to penetrate the interstices between beads of column packing; the largest particles, being unable to move into all interstices, would remain longer in the eluent stream and emerge early This would be supplemented by a second effect involving electrostatic forces of repulsion between the surfaces of the eluting colloid and the packing material; such forces were shown to be present by variation of retention time with ionic strength. Lastly, at high ionic strength, the order of elution was reversed, suggesting the presence of Van der Waal's forces of attraction.

The separation mechanism in capillary HDC is thought to arise purely from hydrodynamic forces within the system. Particles in a liquid filled capillary under laminar flow are known to move away from the walls and the axis of the tube into an annular region whose radius is dependent on particle and capillary diameter. This "tubular pinch effect", described by Segré and Silberberg[5,6], may arise from the balance of a "spin-slip" force moving particles towards the axis of the tube and a "shear-slip" force towards the wall (Mullins and Orr[4]). However, current theoretical treatments cannot predict the elution pattern from this system.

In the present paper, we have sought to extend the useful fractionation range for capillary HDC by studying parameters such as capillary diameter, eluent viscosity and eluent flow-rate. The technique is even simpler than that for packed columns and lends itself easily to the analysis of a wider variety of particulate materials from natural and artificial sources.

EXPERIMENTAL

Particle standards

Polystyrene (latex) particles (0.067, 0.1, 0.23, 0.45 μm) and Polybead® carboxylated particles (0.15, 0.765 μm) were obtained from Phase Separations (Queensferry, Great Britain). Other latexes, pollens and particles (1.15, 2.03, 3.07, 4.88, 4.91, 10.5, 15.0, 20.5, 26.44, 39.8, 40.5 μm) were obtained from Coulter Electronics (Harpenden, Great Britain) and spherical metals (aluminium 7–15, nickel 6–18 and tungsten 6–9 μm) from Particle Services (Grants Pass, OR, U.S.A.). Triton X-100 and Universal Indicator were obtained from BDH (Poole, Great Britain).

Chromatographic system

Eluent was delivered from a constant pressure syringe pump (Applied Research Labs., Great Britain; Model 750/01) of maximum delivery pressure 1350 p.s.i., to a loop injection valve (Specac, Great Britain; Model 30-100, sample volume 5–50 μl). The analytical columns consisted of stainless steel or of PTFE capillary tubes 50 m in length (Phase Separations and Jones Chromatography, Great Britain). The stainless steel capillaries, nominally 203 or 254 μm (0.008 or 0.010 in.) I.D. and 1.6 mm (1/16 in.) O.D., were formed into a coil 30 cm in diameter. The PTFE capillary was nominally 152 μm (0.006 in.) and 1.6 mm (1/16 in.) O.D., coiled to a diameter of 15 cm. The effluent from this was detected in a UV or visible spectrophotometer (Cecil Instruments, Great Britain; Models CE212 or CE272) fitted with a flow cell (8 μl), and the output was fed to a standard chart recorder.

Column volume and internal diameter

The column volume was measured accurately by filling the capillary with aqueous benzyl alcohol (1 %, w/v), washing the solution into a flask of known volume with distilled water and measuring the optical density at 257 nm. The volume of the capillary and its average internal diameter were calculated by comparison with a series of standard solutions.

Sample preparation and chromatography

The columns were eluted in water (containing Triton X-100, 0.1 %, w/v), methanol or tetrahydrofuran. These eluents were passed through a 0.6-μm filter before use. Particle standards (50–500 μl or 1–10 mg dry wt.) were suspended in eluent (10–40 cm^3) and introduced on to the flowing column through the injection valve. The void volume was marked using Universal Indicator.

Column parameters

The resolution factor, R_s, between two peaks was calculated from the usual expression

$$R_s = 2\frac{(V_2 - V_1)}{(v_1 + v_2)}$$

where V_1, V_2, and v_1, v_2 are respectively the elution volumes of each peak and the related peak volumes. Values of R_s greater than unity correspond to complete resolution of two adjacent peaks.

The height equivalent to a theoretical plate (HETP) in mm of the column was calculated from the expression

$$\text{HETP} = \frac{L}{16}\left(\frac{V_w}{V_e}\right)^2$$

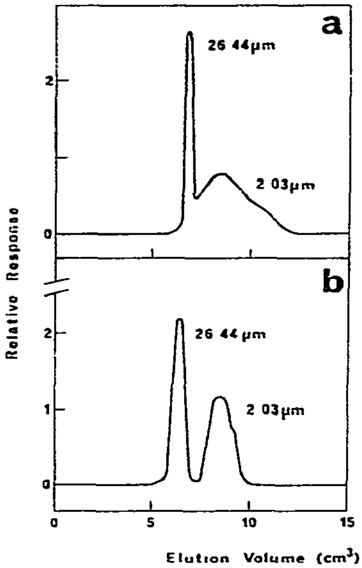
where V_e is elution volume of a particle standard, V_w is peak volume and L is the column length in mm.

RESULTS AND DISCUSSION

Capillary hydrodynamic chromatography (HDC) as described by Noel *et al.*[3] and Mullins and Orr[4] showed promise as a new and rapid method for particle size analysis. However, the technique had been demonstrated only in water and methanol eluents and had a somewhat limited operational range of particle diameters (0.5–30 μm). These restrictions limited the usefulness of the technique to solve problems encountered in these laboratories. It was therefore decided to attempt to extend this range, particularly in the region less than 1 μm, and to operate capillary columns in a less polar organic solvent for analysis of paint dispersions and used engine oils.

Experimental and theoretical[3] considerations suggested that the fractionation range for a capillary column was controlled by the ratio of particle size to tube internal diameter. This relationship was studied using three capillary columns of equal length (50 m) but different internal diameter (nominally 152, 203, 254 μm but by measurement 182, 242, 450 μm) by elution of particle standards in water, methanol or tetrahydrofuran (THF).

The effect of eluent flow-rate on column resolution was studied using the 450-μm column with water–Triton X-100. A mixture of polyvinyl toluene latex (2.03 μm) and lycopodium particles (26.44 μm), only partially resolved at 1.4 cm^3 min^{-1} in water, was completely resolved at 7.5 cm^3 min^{-1} (Fig. 1). Plots of peak volume and resolution factor, R_s, against flow-rate (Fig. 2) show that the best peak separation is



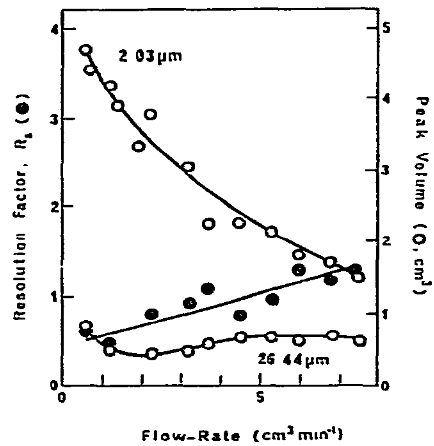


Fig. 1 Separation of polyvinyl toluene latex (2 03 µm) from lycopodium particles (26.44 µm) in water–Triton X-100 at 1 4 cm³ min⁻¹ (a) and 7 5 cm³ min⁻¹ (b)

Fig 2. Variation in resolution factor, R_s (●), and peak volume (○) for the 2.03- and 26.44-µm particle standards in water–Triton X-100

obtained at the highest possible flow-rates consistent with laminar flow and practical analysis times. The plate heights (HETP) of this column for the 2.03- and 26.44-µm standards were 640 and 14 mm respectively at 2.25 cm^3 min^{-1} and 112 and 28 mm at 7.5 cm^3 min^{-1}.

The three capillary columns (182, 242, 450 µm) were calibrated for particle size analysis using water–Triton X-100 at the highest flow-rates (0.2, 0.9 and 10.5 cm^3 min^{-1}) obtainable from the constant pressure pump. Plots of logarithm particle diameter *versus* retention ratio V_e/V_s, where V_e and V_s are respectively the elution volumes of the particle standard and a small molecule (Universal Indicator), can be seen in Fig. 3. These curves show that each tube fractionates in a different particle size range namely 0.07–1.0 µm, 0.5–5.0 µm and 1.0–25 µm. The tubes also show both an upper and lower size limit for particle separation. The upper limit in each case was between 5 and 10% of the tube internal diameter, the lower limit being less predictable.

The 450-µm capillary was calibrated for use with methanol and THF by elution of the same particle standards. A typical separation is given in Fig. 4. Plots of logarithm particle diameter *versus* V_e/V_s (Figs. 5 and 6) are broadly similar in shape to those obtained in water although somewhat displaced in fractionation range. Suitable insoluble particle standards were not available for the diameters less than 5 µm in THF. The similar trends of elution shown in all three eluents suggest that electrical interactions between the particles or the particles and the capillary wall are of little importance compared with the hydrodynamic forces of separation.

Limited quantitative analysis may be performed using capillary HDC. At a

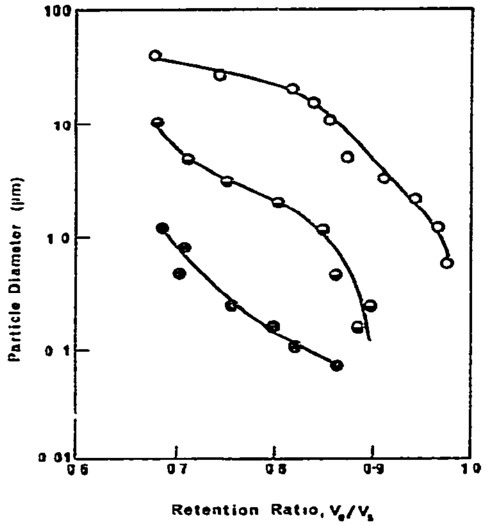


Fig 3. Calibration for capillary columns of 182 (●), 242 (⊖) and 450 μm (○) I.D eluted with water–Triton X-100 at flow-rates 0 2, 0 9 and 10 5 cm^3 min^{-1} respectively.

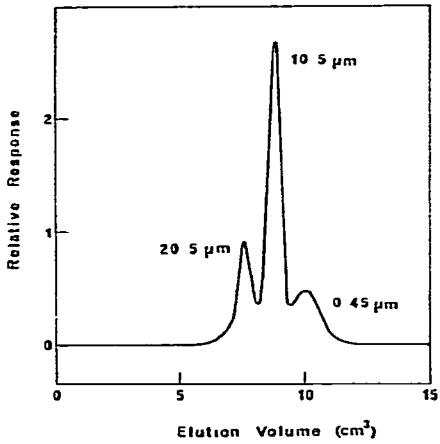
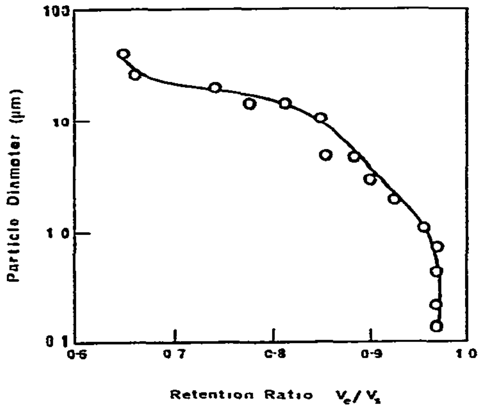


Fig. 4. Separation of 20.5-, 10 5- and 0 45-μm particle size standards in methanol (50 m × 450 μm capillary) at 13.4 cm^3 min^{-1}.

given wavelength, the absorption spectrophotometer response was almost linear to particle concentration up to 1.0 absorbance units and, for a given separation, chromatograms were repeatable. However, instrument response was found to be a complex function of particle diameter and wavelength of incident light, containing unexpected maxima and minima. In addition, the elution volume of particles was found to vary according to density, so that particles of aluminium (ϱ = 2.7 g cm^{-3}; 7–15 μm) were eluted before nickel (ϱ = 8.9 g cm^{-3}; 6–18 μm) and tungsten (ϱ = 19.4 g cm^{-3}; 6–9 μm). These effects prevent an easy interpretation of chromatograms from samples which are polydisperse in either size or density. Nevertheless, the qualitative results have potential value as shown in the following applications. Using the present



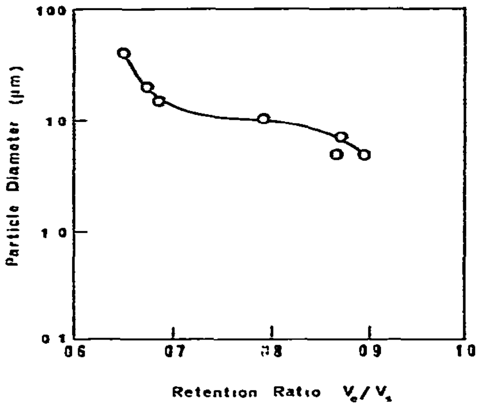


Fig. 5. Calibration curve for stainless steel capillary (50 m × 450 μm I.D.) in methanol

Fig. 6. Calibration curve for stainless steel capillary (50 m × 450 μm I.D.) in THF.

system, the lower detection limit for a 0.45-μm standard (optimum response at 300 nm) was found to be about 10 μg cm^{-3} (*ca.* 2 × 10^8 particles per cm^3).

Analysis of water-based emulsion paint was performed using the 450-μm capillary in THF (Fig. 7). The bimodal nature of this chromatogram was repeatable on successive samples and could be used to "finger print" successive batches of such paint.

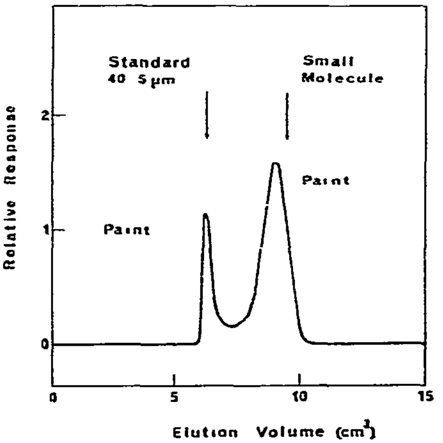


Fig. 7. Elution profile for an aqueous emulsion paint in THF (capillary 50 m × 450 μm) at 13.4 cm^3 min^{-1}.

A used multigrade engine oil from a Petter W1 test engine (gasoline fuel, 36 h) was examined on the 242-μm I.D. column in THF (1.9 cm^3 min^{-1}). It was discovered that at the usual test wavelength (300 nm) light absorption by dissolved molecular species obscured any peaks arising from the HDC of particles. However, measurements made at a longer wavelength (550 nm) (Fig. 8) showed the elution of two partially resolved peaks, one due to molecules in solution the other due to suspended

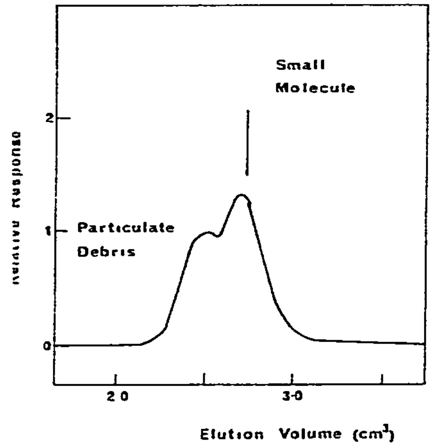


Fig 8 Elution profile for used engine oil in THF (capillary 50 m × 242 μm) at 1 9 cm^3 min^{-1} detected at 550 nm

particulate debris (carbon, metals, polymeric products). The maximum of the latter would represent a mean particle diameter of about 1 μm.

HDC of the 15-μm particle standard (paper mulberry pollen) showed this to be trimodal in nature (Fig. 9) with peak maxima at V_e/V_s values 0.64, 0.78, 0.99. Optical microscopy of this sample revealed significant quantities of unspecified debris in the sample. This could also account for the fact that this particle standard did not fall precisely on the calibration curve (Fig. 5).

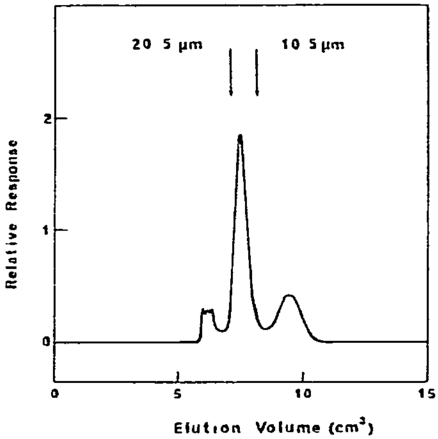


Fig 9. Elution profile of paper mulberry pollen (15 μm) in methanol (capillary 50 m × 450 μm) at 13 4 cm^3 min^{-1}.

CONCLUSIONS

The present work has extended the useful analytical range of capillary HDC down to particle diameters of 0.07 μm and shown that common organic solvents may be used to achieve fractionation of commercial and industrial suspensions of particles such as are found in paint and used engine oil. Elution conditions of high flow-rate produced both smallest HETP values and best separations. This effect emphasizes the uniqueness of the chromatographic separation mechanism and suggests that even higher flow-rates would yield correspondingly better results. However, such conditions would require much higher pump pressures than are at present available.

Quantitative measurements of mean particle diameter and standard deviation would require a formal mathematical relationship between parameters such as particle diameter, shape and density and wavelength response of the detector. This would be difficult to achieve using a standard absorption spectrophotometer or nephelometer. More suitable detectors for HDC might be found in laser light scattering or particle counting instruments which could discriminate individual particles. Despite these limitations, the present form of capillary HDC shows promise for quality control of industrial particulate materials in the sub-micrometer region.

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