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Note

Characterisation of nanoparticulate systems by hydrodynamic chromatography

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Abstract

Particle size and particle size distribution can have a fundamental effect on the physical properties of colloidal dispersions. For many systems the measurement of average particle size is not sufficient, the presence of different size populations will have a strong influence on properties and could be related to the production process. Hydrodynamic chromatography (HDC) provides a method for the separation of polymers in solution or particles in suspension based on their size. In a packed column, the separation takes place in the inter-particle channels and the elution order is from large to small, analogous to gel permeation chromatography. The dynamic range of packed column HDC is from molecular size up to particles of greater than 1 μm. New instrumentation which can be used to determine the particle size distribution of a range of colloidal dispersions by packed column HDC is described. Data to support accuracy and precision of average particle size determination is presented as well as a number of case studies to illustrate the applicability of the technique to samples with polydisperse or multi-modal particle size distributions.

Keywords: Particle size distribution; Hydrodynamic chromatography; Latex; Liposome; Gold particles

In the Polymer Laboratories' Particle Size Distribution Analyser (PL-PSDA), a proprietary eluent (water based and containing a mixture of salts and surfactants at a controlled pH) is continuously pumped through the system at a constant flow rate of nominally 2.0 ml/min. A carousel based autosampler enables multiple sample vials to be loaded and sampled for continuous, unattended operation. Samples are prepared in the 2 ml vials as a dilute slurry in the eluent but sample concentration is not critical. The sample under investigation, and a small molecule marker solution, are introduced into the system via a two position, electrically actuated valve such that the eluent flow is not interrupted. The PL-PSDA contains a separating 'cartridge', the standard system (cartridge Type 2) has a dynamic operating range from 20 nm to 1.5 μm, although alternative cartridges are available to cover specific size ranges up to 3 μm. The sample components are separated in the cartridge based on the different eluent velocity experienced by particles of differ-

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ent size due to the velocity gradient in the effective capillaries which exist in the inter-particle spaces in the packed bed (McHugh, 1989). Following the separation, sample components eluting from the cartridge are detected by a UV detector and in this technique detection occurs predominantly because the particles scatter the incident UV light. The chromatogram of retention time versus response is converted to a particle size distribution using a combination of calibration procedures and peak fitting models (McGowan and Langhorst, 1982). The primary calibration is determined using narrow polydispersity particle size standards employing the parameter retention factor (Rf) where

$$\mathrm{Rf} = \text{Retention time marker/Retention time sample}$$

The total analysis time is less than 10 min. Instrument control, data acquisition and data management/analysis are all controlled from one interface operating in a Windows based environment.

The hydrodynamic chromatography (HDC) separation mechanism employed in the PL-PSDA is independent of particle density and the instrument is therefore applicable to a wide range of different particle types. This is illustrated in Fig. 1 which shows a calibration plot of Rf versus square root of particle diameter for polystyrene latex, silica and melamine particles whose density values are listed below:

Polystyrene latex: $\rho = 1.05$ g/cm^3

Silica: $\rho = 2.00$ g/cm^3

Melamine: $\rho = 1.51$ g/cm^3

A polystyrene latex standard of nominal size 519 nm (ex Duke Scientific) was analysed six times. Table 1 summarises the results of the replicate analyses and illustrates the precision and accuracy of the determination of the median particle size.

Samples to be analysed using the PL-PSDA must be dispersed in the eluent which contains phosphate buffer to enable pH adjustment and stabilisation and both ionic and non-ionic surfactants. As with any suspension particle sizing technique, the sample must be completely dispersed in the eluent prior to analysis. Where samples are

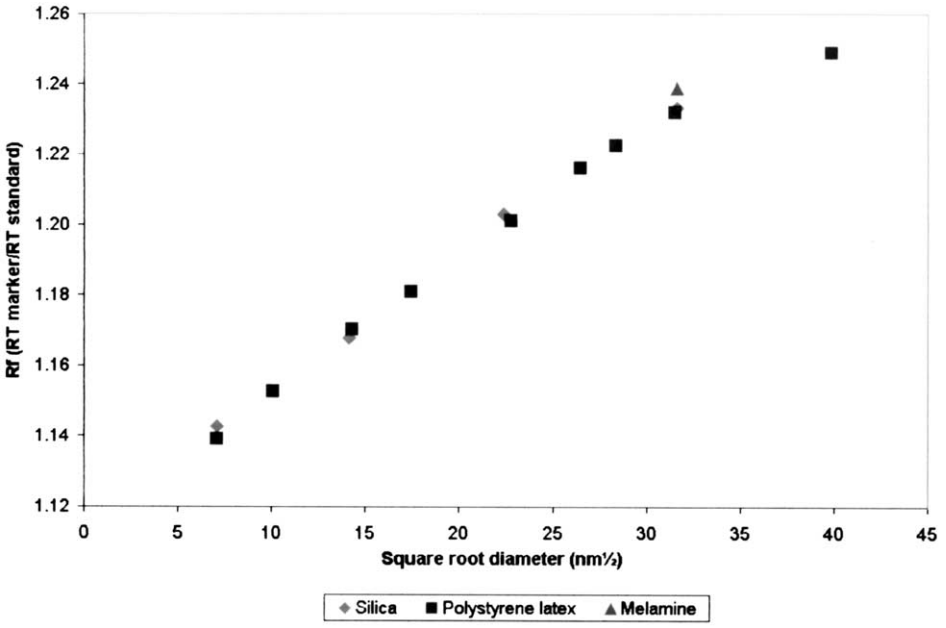

Fig. 1. Universality of HDC size calibration (Rf versus square root particle diameter) for different particle types.

Table 1
Replicate results for analysis of polystyrene latex, nominal size 519 nm

Injection number	Mean diameter (nm)
1	518
2	518
3	517
4	517
5	517
6	518
Average (nm)	517.5
Variation (%)	0.1

sensitive to pH stabilisation, the eluent pH can be adjusted by addition of acid or base.

Fractionation techniques, including packed column HDC, offer advantages over non-fractionation techniques for particle sizing in that the method produces information about the average particle size and the distribution of particle size. Non-fractionation techniques, such as photon correlation spectroscopy, are less well suited for the analysis of multi-modal samples or samples with broad particle size distributions due to the low resolution of the method. The high resolution of the technique is illustrated in Fig. 2 which shows the particle size distribution obtained for a blend of narrow polydispersity polystyrene latex standards analysed using a Type 1 cartridge (dynamic range 5–300 nm).

A variety of nano- and micro-particulate systems are employed in clinical and pharmaceutical applications. By way of illustration of the applicability of the PL-PSDA, two examples are discussed. Fig. 3 shows the particle size distribution obtained for a commercial liposome sample. In this particular case the liposome, which is used in a drug delivery application, is refined using various processes and the aim was to measure changes in the particle size distribution at various stages of processing. These particular liposome samples were analysed at pH 7 using a Type 2 cartridge.

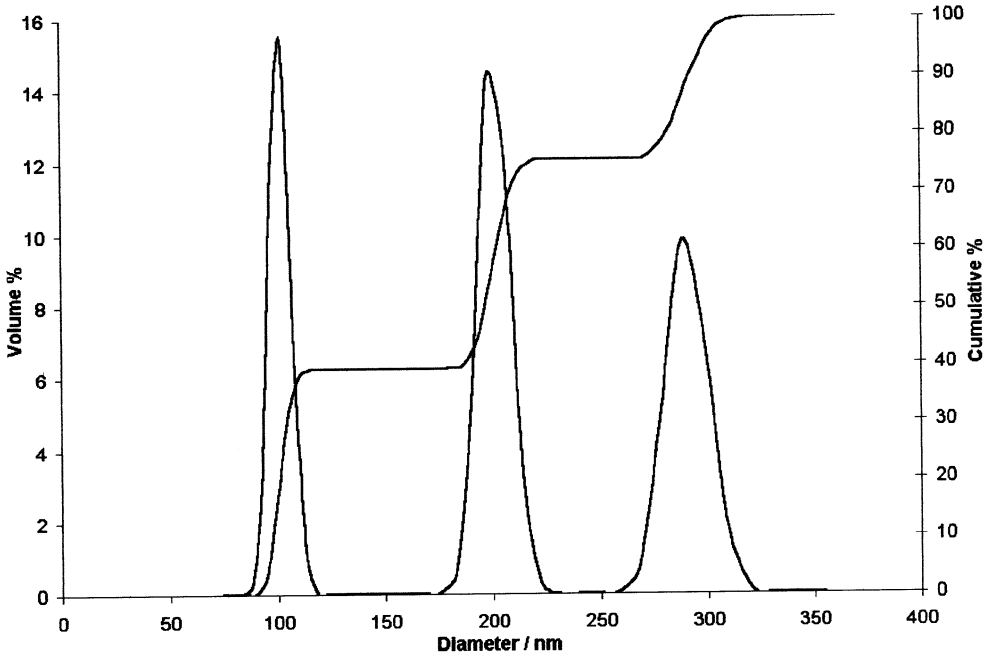

Fig. 2. Particle size distribution plot for a blend of latex standards, nominally 100, 200 and 300 nm.

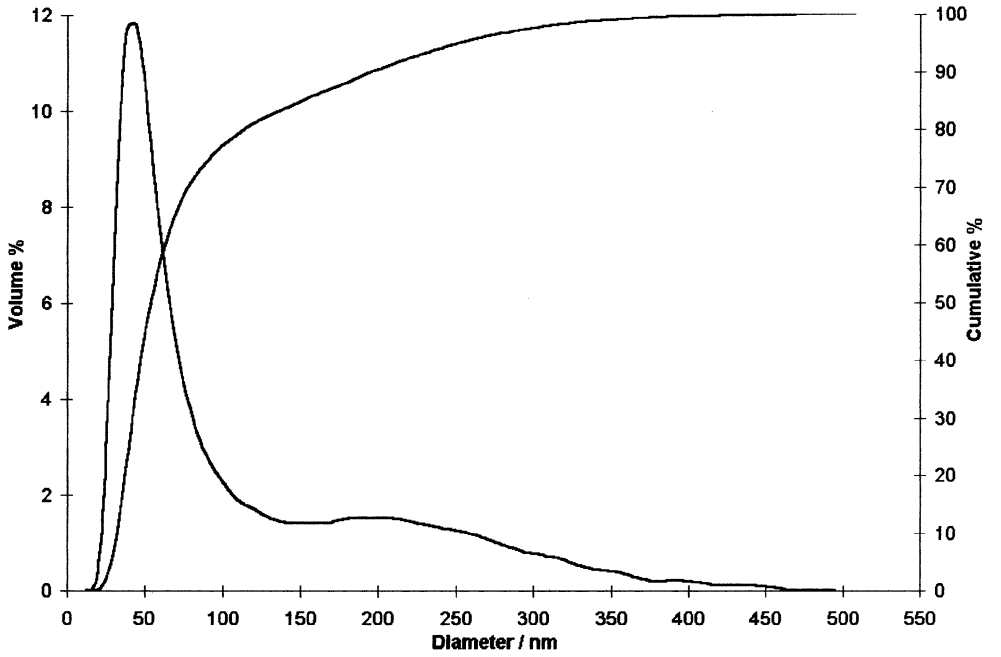

Fig. 3. Particle size distribution plot for a liposome sample.

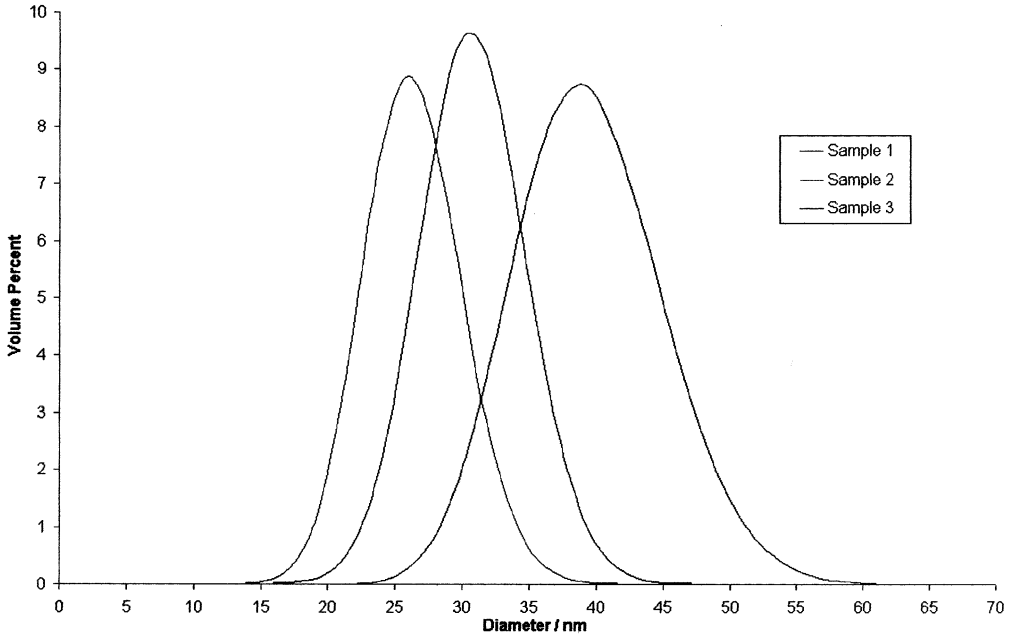

Fig. 4. Particle size distribution overlay plot for three gold colloids.

Gold colloids are widely used in clinical and diagnostic applications and the efficacy of such systems can be influenced by the particle size and its distribution. Owing to the relatively small size of such nanoparticulate system the Type 1 cartridge was employed with the eluent at pH 7. Fig. 4 shows an overlay of the particle size distributions obtained for three different samples

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