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Analysis of colloids VII. ☆Wide-bore hydrodynamic chromatography, a simple method for the determination of particle size in the nanometer size regime

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Abstract

Wide-bore hydrodynamic chromatography in a polyether ether ketone (PEEK) capillary (I.D. 0.7 mm, length 20 m) was used to determine the weight average diameter d_w of colloidal particles. The method was applied to cadmium sulphide and gold sols in the diameter range between 3 nm and 27 nm. The method is based on the radial distribution of the analyte in the capillary due to the hydrodynamic flow profile in the capillary and due to the diffusion coefficient of the particles, which is dependent on their diameter. The diameter was calculated from the ratio of the heights of convection peak and diffusion peak. The size-quantization effect of small semiconductor particles made it possible to visualise the separation inside of the capillary. One important advantage of the applied method is the very much reduced adsorption, which often causes serious problems in the HPLC especially of inorganic colloids. The results of wide-bore hydrodynamic chromatography, size exclusion chromatography and transmission electron microscopy were compared.

Introduction

The study of colloidal semiconductor and metal particles in the nanometer size regime is a steadily growing field in chemistry. Because ultra small particles exhibit unusual physical and chemical properties, e.g. blue-shifted absorption and fluorescence spectra with decreasing diameter and because of their possible application in solar energy technology and microelectronic devices they have become the focus of much recent physicochemical research [1,2]. However the investigation of size dependent properties requires good and reliable size analysis. The classical technique is transmission electron microscopy (TEM), where the particle diameters are measured directly. Nevertheless, problems can arise from radiation damage due to the high energy applied to the material [3]. If no expensive image processing system is available, the measurement of the sizes on the micrographs is also rather tedious and often quite subjective. Size exclusion chromatography (SEC) has also recently been shown to be a very convenient method for the size determination for inorganic colloids [4,5]. In the case of HPLC–SEC, the system measures

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☆ For part VI see Ref. [8], for part V Ref. [7] and for part IV Ref. [26].

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size distributions within a few minutes, once the initial calibration has been carried out [6,7]. Speed is important when kinetics of the growth of very unstable colloids is investigated [6]. Further advantages of the chromatographic method are the good statistics of the result, on-line coupling with diode array detectors for studies of size depending UV/Vis spectra [6] and the possibility of scaling-up the system for preparative separations [8]. However, with some kinds of colloids, e.g. PbS, adsorption or particle growth during the passage through the column causes difficulties even in the presence of the stabilisers in the eluent. The hydrodynamic chromatography in packed columns showed the same limitations [9]. In the classical hydrodynamic chromatography (HDC) in *narrow* capillaries or with other name capillary hydrodynamic fractionation (CHDF) organic particles could be separated according to the diameter with good resolution [10–13]. Silebi [11] described the analysis of latex particles with diameters down to 88 nm (diameter range of the inorganic particles under investigation: 3 nm–27 nm). Adsorption was less pronounced than in packed columns, but still a problem. Therefore a chromatographic technique could be helpful, where there is a smaller surface area than in a packed column or in a narrow capillary.

In 1978 Mullins and Orr [14] and in 1979 Noel et al. [15] reported the fractionation of micrometer-sized particles in a capillary with an internal diameter of 250 μm. Submicrometer particles could not be distinguished.

In 1984 Kelleher and Trumbore [16] described an easy method for the determination of the molar weight of biopolymers just by pumping a sample plug through a rather thick capillary. The internal diameter was some tenths of a millimetre and therefore much thicker than in classical hydrodynamic chromatography (capillary hydrodynamic fractionation) where the diameter is typically some microns. The experiment was carried out initially with a normal UV/Vis detector, but later with special RI detectors which measured the radial concentration gradient [17–19]. Unlike normal chromatography, it is the peak shape, rather than the retention time, which was used for the calculation of the molecular weight. The method is based on the dependence of the diffusion coefficient on the molecular weight. Conversely, diffusion coefficients of species with known molecular weight can be calculated from the peak width [20]. Vanderslice et. al. investigated the concentration profiles in such flow systems and calculated them under various conditions [21].

In the past the method in question has been contributed to the group of flow injection analysis (FIA). However, most often in FIA concentrations are measured by peak size either after mixing with special reagents or without mixing by the use of specific detectors. Though the described method has doubtless similarities with FIA, it is based on the hydrodynamic flow profile in the capillary, which is generated by the flow resistance of the walls. The species to be analysed interact dynamically with the flow medium and indirectly with the walls. This is typical for chromatography, though not a spectrum of various (particular) retention times is measured. Therefore we prefer the name *wide-bore* hydrodynamic chromatography (HDC) in order to stress similarities and differences to the *classical* HDC.

Experimental

Chromatography

Wide-bore HDC

The experimental set-up consisted of a Merck–Hitachi L6000 HPLC pump, a Merck Autosampler A2000 (sample volume 100 μl) and a Waters 990 diode array detector. Autosampler and detector were connected via a 20 m long, 0.7 mm I.D. polyether ether ketone (PEEK) capillary. The flow rate was 0.8 ml/min. The eluent for the cadmium sulphide sols was 10^{-3} *M* cadmium perchlorate (Ventron)/$6 \cdot 10^{-3}$ *M* sodium polyphosphate (based on the phosphate units, Riedel de Haen), and for gold sols 10^{-3} *M* sodium citrate was used.

SEC

Two 125 × 4 mm columns (Knauer Säulentechnik, Berlin, Germany) in series were used: For cadmium sulphide Nucleosil 500C4 (7 μm) and Nucleosil 1000C4 (7 μm) and for gold Nucleosil 500 (15–25 μm) and Nucleosil 1000C4 (15–25 μm). Eluents, pump and detector were the same as for HDC.

Preparation of the colloids

Cadmium sulphide sols

Hydrogen sulphide gas or aqueous sodium hydrogen sulphide solution was injected through a septum into an aqueous solution of 10^{-3} *M* $Cd(ClO_4)_2$ and $6 \cdot 10^{-3}$ *M* sodium polyphosphate, through which nitrogen had been bubbled for ten minutes. The solution was shaken prior to use. The particle size was controlled by the initial pH value of the solution before sulphide addition. A lower starting pH leads to smaller particles [22]. Aged samples containing larger colloids were also used in some experiments.

Gold sols

Gold sols were prepared by using a mixture of trisodium citrate and tannic acid (Mallinckrodt product no. 8835) as reducing agent [23]. $KAuCl_4$ (85 ml, 0.1%) was heated to 60°C and stirred rapidly. A second reducing solution was prepared by mixing trisodium citrate (4 ml, 1%) tannic acid (0–5 ml, 1%) and an equivalent amount of K_2CO_3 (0–5 ml, 10^{-2} *M* and making up to 25 ml. This solution was also heated to 60°C and then added rapidly to the chloroaurate solution. The colour of these sols developed almost instantly. The solution was then boiled for several minutes and allowed to cool. Tannic acid increases the rate of nucleation, thereby generating smaller particles. The higher the tannic acid:citrate ratio, the smaller the particle size. The lowest size limit achievable was found to be about 2–3 nm. The sols so prepared were stable for months, although sometimes a slow sedimentation was observed over time, which could be removed by centrifugation. The particle size distributions were measured from electron micrographs.

Electron microscopy

A small drop of sample was adsorbed onto a 400-mesh copper grid coated with a 50 Å thick carbon support film. After 10 seconds of contact time the fluid was blotted off. The grids were dried under argon and examined in a Philips CM 12 transmission electron microscope with an acceleration voltage of 120 kV. The microscope was equipped with a supertwin lens and an EDAX detector. For imaging, axial illumination was used as well as the "nanoprobe mode" with a beam spot size of 1.5 nm, to enable diffraction patterns of the individual clusters to be obtained. All images were made under conditions of minimum phase contrast and low electron dose with a magnification of 120 000 and 430 000 ×.

Results and discussion

Method

When a liquid passes through a capillary under laminar flow conditions, a parabolic flow profile is formed, i.e. fast flow in the centre and decreasing velocity towards the walls. Dissolved species are transported forward with the liquid flow, but they can also move in other directions by diffusion. Here the motion perpendicular to the flow direction is of particular importance. It brings the solute from the centre to the walls and vice versa, i.e. from faster streams to slower flowing parts of the cross-section. However, this motion is dependent on the diffusion coefficient of the sample. When a sample of very big colloidal particles or very large macromolecules is injected into such a flow system with appropriate flow rate, their diffusion is low compared to the speed of the forward stream. Therefore the radial movement is negligible. In Fig. 1a the axial and radial concentration distribution of sample species in the capillary is shown schematically, based on the calculations of Van-

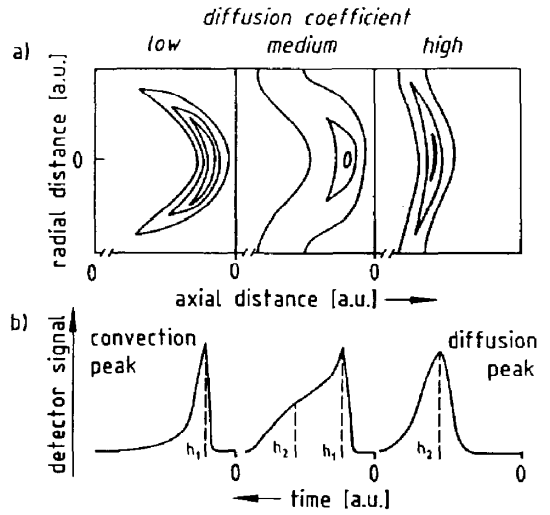

Fig. 1. Behaviour of material with small, medium and high diffusion coefficient during laminar flow through a "wide-bore" capillary. (a) Axial and radial concentration distribution expressed in equiconcentration lines, based on the theoretical calculations of Vanderslice et al. [21]. (b) Corresponding elution profiles.

derslice et al. [21]. The left part of Fig. 1 represents the situation just described for low diffusion coefficient. The analyte follows the laminar flow profile, apparently without any additional motion. On the other hand species with a very high diffusion coefficient exchange efficiently between slow and fast areas (Fig. 1, right part), and therefore the concentration is more uniform over the diameter and the average speed is slower than in the first case. Species with medium diffusion coefficient (Fig. 1, centre) show a behaviour between the extreme cases. These distributions cannot be easily visualised. But with a simple experimental set-up consisting of a pump, an injection valve with sample loop, the capillary and a detector with a through-flow cell, an elution profile can be obtained which is similar in principle to a chromatogram. The response gives the integral radial concentration at a certain axial distance from the injection point. Due to the flow, the whole distribution is pushed through the detector cell with time. The result is an early eluting, steeply increasing, but strongly tailing and therefore asymmetric convection peak for slowly diffusing species and a relatively symmetric late-eluting diffusion peak for material with a high diffusion coefficient. Materials with a medium diffusion coefficient show an elution profile with the elements of both kinds of peaks, the ratio depending on the size of the diffusion coefficient. Fig. 1b shows the elution profiles corresponding to the upper situations. The diffusion coefficient of dissolved organic polymers depends on the size of their coils which is proportional to the molecular weight. In the case of colloidal particles their diameter is the important parameter. The experimental set-up is very simple (Fig. 2). A pump delivers the eluent, and the sample is introduced by a sample valve and pumped through the capillary to the detector.

Application

Cadmium sulphide sols

For the first experiments cadmium sulphide sols were used. Much experience exists in SEC of these semiconductor colloids, so that the results could easily be compared. Stabilisers such as polyphosphates have to be added to these aqueous colloids in order to reduce particle growth. These molecules form complexes with the surface of the particles and protect it against direct contact with others and therefore against coagulation. On the other hand it also reduces adsorption on the surface of the column and the stationary phase.

A series of CdS sols of different particle size, 10^{-3} *M* each, were prepared, whereby the size was controlled via the pH value before the sulphide addition. These sols were injected simultaneously in the HDC capillary and onto the SEC column. The eluent compositions of both methods were the same: 10^{-3} *M* cadmium

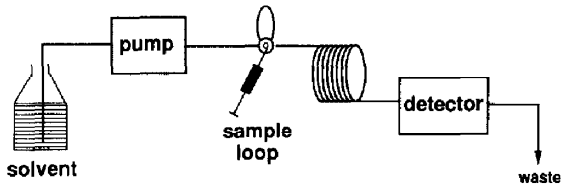

Fig. 2. Scheme of experimental set-up in wide-bore HDC.

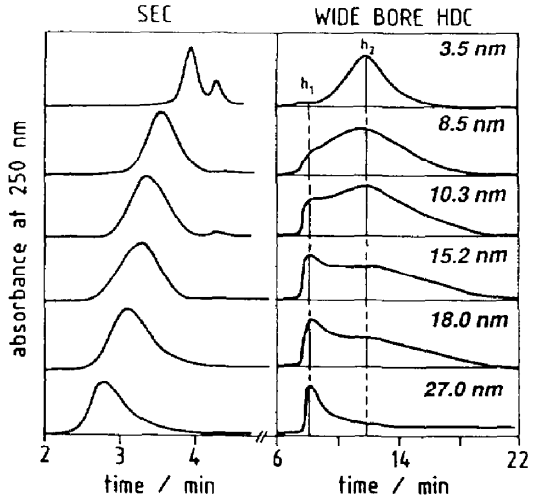

Fig. 3. Cadmium sulphide sols of different particle sizes analysed by SEC (left) and wide-bore HDC (right). The samples are sorted, the smallest particles on top, largest at the bottom. The weight average diameters d_w are given on the right hand side.

perchlorate/$6 \cdot 10^{-3}$ M sodium polyphosphate. Fig. 3 shows the results. On the left, the size exclusion chromatograms and on the right the corresponding hydrodynamic chromatograms. The samples are sorted with respect to increasing particle diameter. As the SEC peak shifted to shorter retention volumes, the HDC peak became less symmetric. In addition to the late diffusion peak, the early convection peak grew. Finally only the latter remained with a pronounced tailing.

For proof of the separation inside a wide capillary the size quantization effect (Q effect) of the nm-sized semiconductor particles could be used. For these particles, the onset of absorption shifts to shorter wavelengths with decreasing particle size [24,25]. When a CdS sol with a broader size distribution was analysed, different chromatograms were obtained depending on the observation wavelength. At shorter wavelengths (250 nm and below) all sizes are detected and have the same molar extinction coefficient, whereas at longer wavelengths smaller particles absorb less than larger ones. Consequently we found in Fig. 4a at 250 nm a large diffusion peak next to a small shoulder due to a convection peak. With increasing wavelength, the shoulder grew and at 500 nm the convection peak was pronounced, because smaller particles do not absorb any more in this wavelength range. The separation is also evident from the spectra taken with the diode array spectrometer during the run (Fig. 4b). The spectrum at 8.7 min had an onset

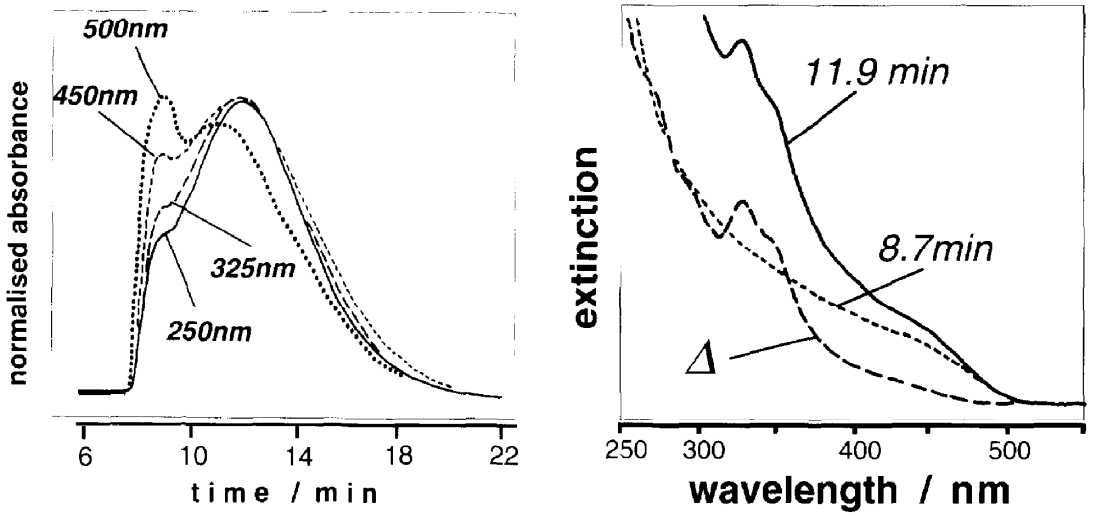

Fig. 4. Wide-bore HDC with diode array detection and the size quantization effect of a cadmium sulphide sol with a relatively broad size distribution. Left: Chromatograms obtained at different wavelengths. Right: UV–Vis spectra measured at 8.7 min (dotted line), at 11.9 min (solid line) and the difference Δ between both spectra (dashed line).

of absorption near 500 nm and no further fine structure, typical for rather large CdS particles. The spectrum obtained at 11.9 min showed a similar onset, but also a small maximum at 330 nm and a shoulder at 350 nm. This spectrum is a superposition of smaller and bigger particles, since the bigger particles of the sample are still eluting. As is evident from the chromatogram at 500 nm they are only of medium size with convection and diffusion peaks of about the same height. Therefore a spectrum with a pronounced maximum typical for particles below 3 nm is obtained, when the first spectrum is subtracted from the second (spectrum Δ in Fig. 4b). The maxima in this region are due to so-called magic agglomeration numbers, i.e. energetically very stable agglomerates [1,2].

Gold sols

Gold sols were prepared by the reduction of tetrachloroaurate with citrate in the presence of tannic acid [23]. Increasing concentration of the latter yields smaller particles. A series of these sols was also investigated by transmission electron microscopy (TEM). In the micrographs, the diameters of a sufficiently high number of particles, i.e. more than 150, was measured and the size distribution constructed (Fig. 5, left). Then SEC was carried out (Fig. 5, centre) on Nucleosil 500 and Nucleosil 1000 (15–25 μm) [26]. When smaller silica material was utilised, the gold sol was irreversibly adsorbed on the column. There are some samples with bimodal distribution in the SEC and only a single size population in TEM. It shows that sometimes in the TEM less frequent populations can be overlooked. For the wide-bore HDC of gold sols, a 1 m*M* sodium citrate solution was used as the eluent, the same as that used with SEC. Again, the same trend in the chromatogram shape from pure diffusion to pure convection peak was observed (sample a→e in Fig. 5, right). These parallel experiments allow a good comparison of all three methods.

Calculation

In the past different approaches have been used in the determination of the molecular weight from the obtained elution profiles. When a gradient detector was used, the molecular mass M_r was determined by means of the asymmetry ratio of the derivative signal [27]. Trumbore et al. [28] suggested for the normal elution profiles, the ratio R of the height of the convection peak h_1 to that of the diffusion peak h_2 (Eq. 1) for the determination of the molar weight M_n of a polymer by empirical correlation. When one of the both peaks is not sufficiently pronounced, the height of the chromatogram at the position in question is taken for the calculation.

$$R = h_1/h_2 \tag{1}$$

We also used the height ratio R, but as a function of the weight average *diameter* d_w, since colloid chemists prefer the use of diameter, considering that the solid particles are rigid and non-swelling as opposed to the organic polymer coils. For the calibration, a number of colloids were prepared and their weight average diameter was determined by computer evaluation of the SEC chromatograms or in the case of gold sols directly by TEM. In Fig. 6 the ratio R as a function of the diameter d_w is given for both colloids CdS and Au and there is a clear dependence. From these calibration plots the average particle size of unknown samples of the same material can easily be determined. The slopes of both curves are quite different. Two reasons can be given to explain this. Firstly, the eluent composition was different and secondly, the true size of the solid particles is different from the effective particle size due to the electrical double layer, which is formed at the solid–liquid interface by electrolytes of the solution. The thickness of this layer is dependent on the solid material but also on the particular electrolyte. Under the conditions of Fig. 6 the electrical double layer of the gold particles seems to be thicker and therefore diffusion plays a less important role than in the case of cadmium sulphide.

Finally it should be mentioned that the concentration of the colloid itself also has an effect on the diffusion rate and therefore on the result of size determination. The higher the concen-

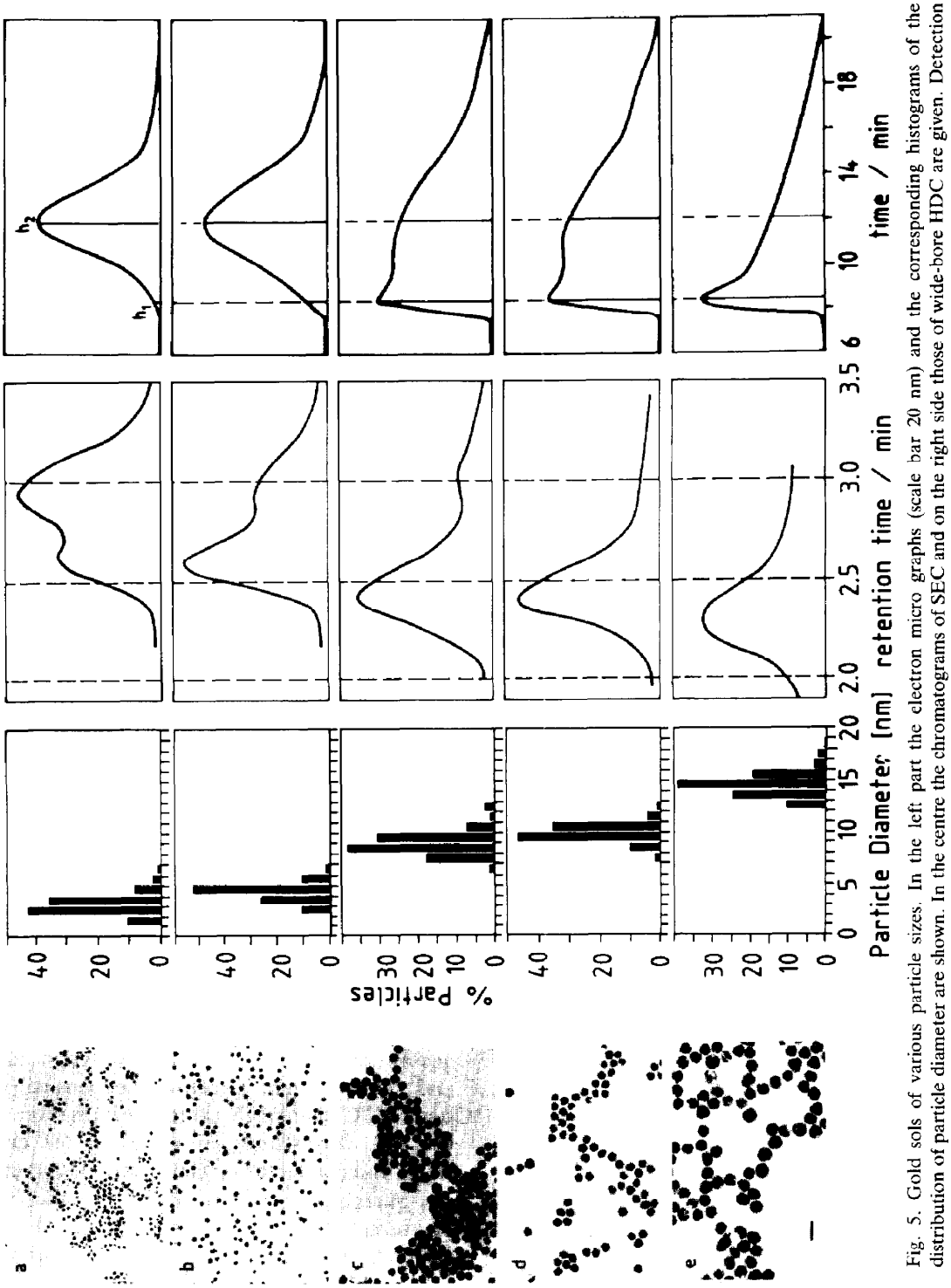

Fig. 5. Gold sols of various particle sizes. In the left part the electron micro graphs (scale bar 20 nm) and the corresponding histograms of the distribution of particle diameter are shown. In the centre the chromatograms of SEC and on the right side those of wide-bore HDC are given. Detection wavelength in both cases was 520 nm.

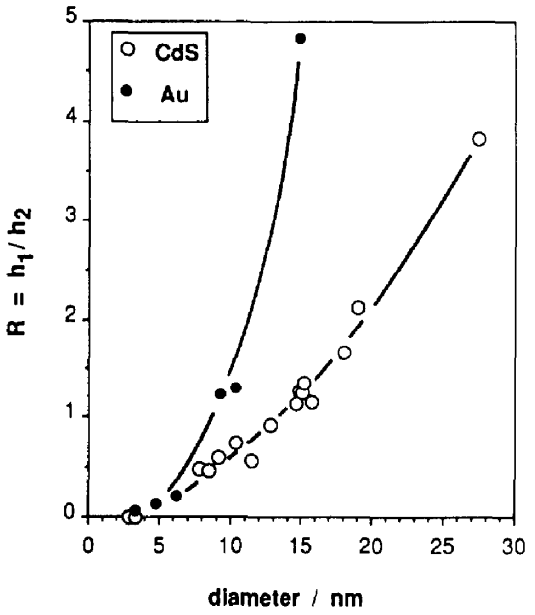

Fig. 6. Calibration plot for the wide-bore HDC of gold (●) and cadmium sulphide (○) sols. It shows the ratio $R = h_1/h_2$ of the height of convection peak over diffusion peak as a function of weight average particle diameter d_w, determined by SEC or electron microscopy, respectively. Experimental conditions are given in the text.

tration of the colloidal particles, the faster is their diffusion between slowly and fast flowing parts of the cross-section of the capillary and the more pronounced is the diffusion peak. This is demonstrated with a sol containing $5 \cdot 10^{-3}$ *M* CdS, which was diluted with water stepwise down to $1 \cdot 10^{-4}$ *M* (Fig. 7). From the particular height ratio *R* of the chromatogram and the calibration curve (Fig. 6) diameters between 89 nm and 104 nm were calculated. Although the influence of the concentration is not dramatic one should try to work always with identical colloid concentrations as those used for the calibration. For higher concentrations the effect is smaller than for more dilute ones. On the other hand highly concentrated colloidal solutions might become unstable, or particle growth may occur. A 10^{-3} *M* solution is recommended as a good compromise. Then a fivefold higher or a tenfold lower concentration would cause an error of only 7 or 8 percent, respectively.

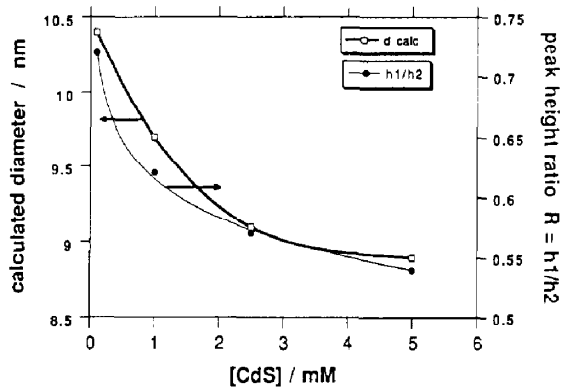

Fig. 7. Effect of concentration on the result of wide-bore HDC. A 5 m*M* cadmium sulphide sol was diluted stepwise down to 0.1 m*M*. Height ratio $R = h_1/h_2$ of convection peak to diffusion peak (●) and the particle diameter d_{calc} calculated from *R* as a function of CdS concentration (□).

Comparison of the methods and conclusion

TEM shows directly the size and shape of particles, but radiation damage can occur [3] and alter the original particle size. Furthermore, less frequent populations could be overlooked [26] and sometimes during sample preparation, smaller and larger particles separate to some extent by diffusion, so that the statistics in an observed part of the whole sample is not perfect any more. Without a digital analyser the analysis is time consuming and tedious. SEC gives a statistically more accurate view of the true size distribution as long as all colloidal material elutes. The analysis is very fast. However, the chromatography columns have a limited lifespan. A new column must then be calibrated again by TEM, because the colloids in question are not stable over long periods. Further drawbacks are that adsorption can occur with very active colloids and the equipment is relatively expensive. Wide-bore HDC cannot give direct information about the size *distribution*. However, in the case of small semiconductor or metal particles where the size-quantization effects allow an independent measure of the particle size, it can be done indirectly via the chromatograms at various detection wavelengths (see Fig. 3). But the wide-bore HDC has many advantages. Whereas in the

classic HDC equipment and handling are quite sophisticated and detection is difficult due to the small dimensions, the *wide-bore* HDC uses cheap, empty, standardised capillaries. Therefore one calibration can be directly transferred to any other capillary of the same type. Although this work was done with HPLC pumps, these sophisticated instruments are not necessary for this technique. A precise flow rate is not required because only the ratio of the peak *heights* is measured, and not the retention time. The method is fast and less calculation is necessary than in SEC. But most important is the lack of any packing material, the large surface of which often causes problems of reversible and irreversible adsorption. In a relatively wide capillary of 0.8 mm I.D. the surface does not play a significant role. Therefore the method is especially recommended for colloids with high surface activity.

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