Size separation of colloidal nanoparticles using a miniscale isoelectric focusing technique

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Abstract

A new method for the isolation of nanometer scale metal particles according to size is demonstrated in this paper. The colloids were derivatized with carboxylic acid groups using self-assembled bifunctional surfactant molecules. The pH at which onset of ionization occurs for the surface carboxylic acid groups was determined for silver and gold colloidal nanoparticles of different sizes using a miniscale isoelectric focusing unit. The pH at which ionization begins is a strong function of the surface curvature of the colloidal particles. It is demonstrated that this property can be used to separate mixtures of colloidal particles of different sizes. In addition to the ability to separate different sized nanoparticles, this technique may also be used to improve the polydispersity of colloids which is an important prerequisite for many applications. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Colloidal particles are increasingly receiving attention as important starting points for the generation of micro- and nanostructures [1–12]. Much of the interest may be traced to the unusual physicochemical and optoelectronic properties exhibited by such nanoscale systems [13] and their application potential in non-linear optics [14], in light-emiting diodes [15] and in quantum dot lasers [16] to name just a few.

Many applications of nanostructured thin films grown from colloidal particles depend critically on the polydispersity of the colloids. Therefore, much research effort has been directed towards control of the shape and size distribution of the colloidal particles. Separation of CdS nanoparticles with a mean diameter between 40 and 60 Å and a relatively narrow size distribution was achieved by controlled precipitation at a definite pH by Spanhel et al. [17]. Lianos et al. [18] have described the utility of inverted micelles in producing CdS (5 Å) particles of very low aggregation number. Fischer et al. have isolated polydisperse CdS colloidal particles into fractions of narrower size distribution by low- and high-pressure size-exclusion chromatography [19]. The use of laponite, porous vycor glass and molecular sieves, faujasite X and sodalite to prepare small

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particles of CdS with limited dimensions has been demonstrated by Stramel et al. [20]. Preparative gel electrophoresis was applied to separate Q-size CdS colloids into fractions containing highly monodisperse particles by Eychmuller et al. [21].

Isoelectric focusing (IEF) is a technique commonly employed to determine the isoelectric point of proteins and enzymes [22,23]. Proteins introduced into the column move under the influence of an electric field in a pH gradient and come to rest at the isoelectric point (pI), which is the pH at which the proteins are electrically neutral. Taking a cue from this, we show that colloidal metal and semiconductor particles of different sizes derivatized with carboxylic acid groups can be rapidly separated from their mixtures in a miniscale IEF unit developed in-house [23]. Presented below are details of the investigation.

2. Experimental

2.1. Preparation of gold colloids

Gold colloidal particles of three different sizes were synthesized as detailed below.

2.1.1. Gold particles of 130 Å diameter

The gold colloidal particles of 130 ± 30 Å size were synthesized by citrate reduction of $HAuCl_4$ as described elsewhere [9]. This yielded a clear, deep violet hydrosol at a pH of ≈3. After cooling, the hydrosol pH was increased to 8.5 using ammonia and the gold colloidal particles were capped with 4-carboxythiophenol (4-CTP) by mixing 9 ml of the gold sol with 1 ml of 4-CTP in absolute ethanol. The concentration of the 4-CTP in ethanol was adjusted to yield a capping concentration of 10^{-5} M in the hydrosol. Thiol groups are known to bind strongly to gold clusters [9,24] thereby yielding carboxylic acid derivatization of the gold particles with 4-CTP. The capping of the colloidal particles was followed by optical absorption spectroscopy. Optical absorption measurements were carried out on a Hewlett-Packard 9542 diode array spectrophotometer at a resolution of 2 nm. A shift in the surface plasmon resonance from 524 nm for the bare gold colloid to 530 nm on capping was observed indicative of co-ordination of

the bifunctional molecule to the gold cluster surface [9,24].

2.1.2. Gold colloidal particles of 40 Å diameter

Clusters smaller than those obtained by citrate reduction of chloroauric acid described above could be routinely prepared by borohydride reduction of the gold salt. More specifically, a solution of chloroauric acid prepared from $1.5\cdot10^{-3}$ g $HAuCl_4$ dissolved in 100 ml water was reduced using 0.01 g $NaBH_4$. A clear, red solution of pH 8.5 was obtained. These clusters were then capped with 4-CTP as described above for the citrate reduced gold colloid. A red shift in surface plasmon resonance from 510 nm for the bare gold sol to 514 nm on capping with 4-CTP was detected. TEM measurements carried out on the 4-CTP capped sol using a Philips TEM 301 T instrument (operating voltage= 80 kV; magnification=57 000) yielded a cluster diameter of 40 Å and a standard deviation of 7 Å.

2.1.3. Small gold particles (22 Å)

Small gold colloidal particles of diameter 22 ± 4 Å were synthesized by the reduction of 100 ml of an aqueous solution of chloroauric acid ($HAuCl_4$, $1.25\cdot10^{-4}$ M concentration) with 0.01 g of sodium borohydride ($NaBH_4$). The reduction was carried out at room temperature in the presence of 10^{-4} M 4-CTP, which was dispersed in the aqueous salt solution using ethanol. This procedure reproducibly gave gold colloids of the size mentioned above with the clusters being capped by a monolayer of 4-CTP molecules [24]. UV–Vis spectroscopy measurements on the gold sol gave a strong surface plasmon resonance at 512 nm. At the pH of synthesis (≈9), the carboxylic acid groups are fully ionized, leading to exceptional stability of the sol.

2.2. Preparation of silver colloids

Silver colloidal particles of 70 ± 12 Å diameter were grown and capped with 4-CTP as described elsewhere [8]. The sol was clear yellow in color (pH≈9) with a surface plasmon resonance at 388 nm. After capping with 10^{-4} M 4-CTP, the resonance shifted to 398 nm [8,25].

2.3. Electrofocusing unit

After synthesis of the different carboxylic acid derivatized colloidal particles, the hydrosols were subjected to electrofocusing using a modified electrofocusing apparatus built in-house [23]. Details of the IEF unit used in this study are illustrated in Fig. 1. The unit essentially consists of a single main column which is filled with a mixture of suitable electrolytes, density gradient solutions and an ion-conducting polymer at the anodic end. On application of an electrostatic potential (Fig. 1), a pH and density gradient is set up in the solution within the column with the lowest pH value occurring near the anode. If an ampholyte is added to the column, it will move under the influence of the electrostatic field until it reaches a pH at which it is electrically neutral (the pI point of the species). In this study, the carboxylic acid derivatized colloidal particles which are fully ionized at basic pH conditions (pH>9), would move towards the anode and stabilize at the pH at which the surface charge is zero, or in other words, at the pH at which onset of ionization occurs. The 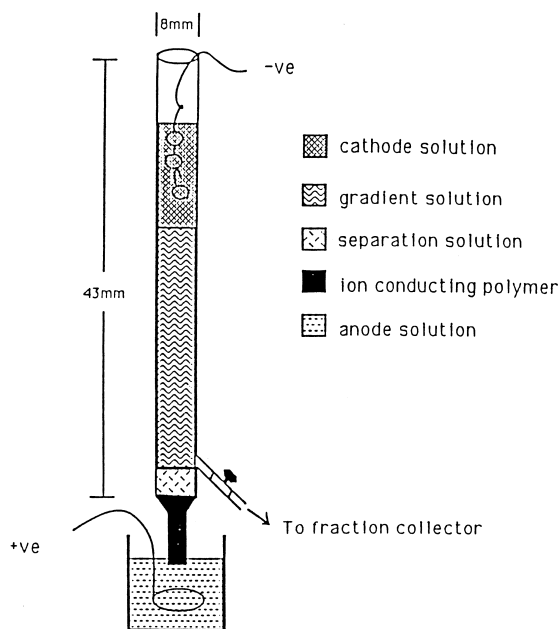density gradient present in the column would immobilize the colloidal particles at the point of zero charge and can thus be effectively used to separate the particles. This, in essence, was the principle used to determine the variation in ionization of the different carboxylic acid derivatized colloids with change in particle size. The details of the various solutions used in the IEF units are described below.

(A) Electrolyte solutions: (i) 0.1 M ortho-phosphoric acid as anode solution and (ii) 0.1 M NaOH as the cathode solution.

(B) Separation solution: 1.5 ml of glycerol with 1 ml of water.

(C) Gradient solutions: (i) high density solution: 3 ml glycerol with 2 ml of the different hydrosols (to be loaded in the IEF unit) together with 0.2 ml carrier electrolyte, and (ii) low density solution: 3 ml water with 2 ml of the hydrosol and 0.2 ml of the carrier electrolyte.

The column solutions were adjusted to enable separations in the pH range 6–8 and 8–10.5 individually. The apparatus was placed in a cold room (5±2°C) and the separation solution was layered above the conducting polymer up to the level of the sidearm (Fig. 1). The gradient solutions were layered on the separation solution using a gradient mixture. The cathode solution was then layered gently above the gradient using a 10-ml syringe after which the platinum electrodes were dipped in the respective solutions. IEF was carried out for 5 h at 400 V and yielded a current of 1.5 A. After clear separation had formed, electrofocusing was discontinued and the fractions of ≈0.2 ml each were collected by opening the tap on the side arm. The pH of the eluted fractions was measured and the optical absorption spectra of the various colloidal fractions recorded. In the absence of surface charging, the colloids in the eluted fractions were observed to flocculate within 30 min. Care was taken, therefore, to minimize the pH and optical absorption measurement time to the extent possible and thereby preclude any aggregation effects on the pH shifts studied. The column used in this study enabled separation of fractions with pH variation as small as 0.02 pH units [23].

Fig. 1. Diagram of the isoelectric focusing unit used for size separation of the colloidal particles with details of the various components.

3. Results and discussion

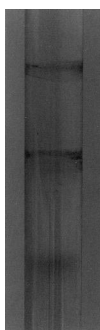The silver and three different gold colloids were

Fig. 2. Picture of the IEF unit showing, from top to bottom, the well separated bands comprising the 130 Å gold particles, 70 Å silver particles and 40 Å gold particles. The band due to 22 Å gold particles was faint and could not be imaged adequately.

mixed in an equivolume ratio and the resulting solution allowed to stabilize for ≈3 h. During this period, no aggregation of the colloidal particles could be observed. After this stabilization period, the colloidal mixture (2 ml) was carefully introduced into the IEF unit. Within 4–5 h, clearly resolved colored bands could be observed in the column

indicating electrofocusing of the sols at well-defined pH values (Fig. 2). The colloidal fractions were eluted out and the optical absorption spectra as well as the pH of the fractions measured. Very little aggregation of the colloidal particles was found to occur even though the electrostatic stabilization of the particles is no longer expected to be operative at the point of zero charge. This is borne out by optical absorption spectroscopy measurements of the sols measured before and after separation and is shown in Fig. 3 for the small gold particles (22 Å). Similar behavior was also observed for the other colloids. It can be seen that except for the dilution factor, there is very little broadening of the plasmon resonance or growth of a long wavelength component which would indicate aggregation of the particles. Therefore, any effects of the aggregation of the particles on the pH at which ionization occurs can be safely ruled out.

The separated bands in the IEF unit were small, typically 0.04 pH units in all cases. It is interesting to investigate the variation in pH at which ionization occurs for the different size colloidal particles since
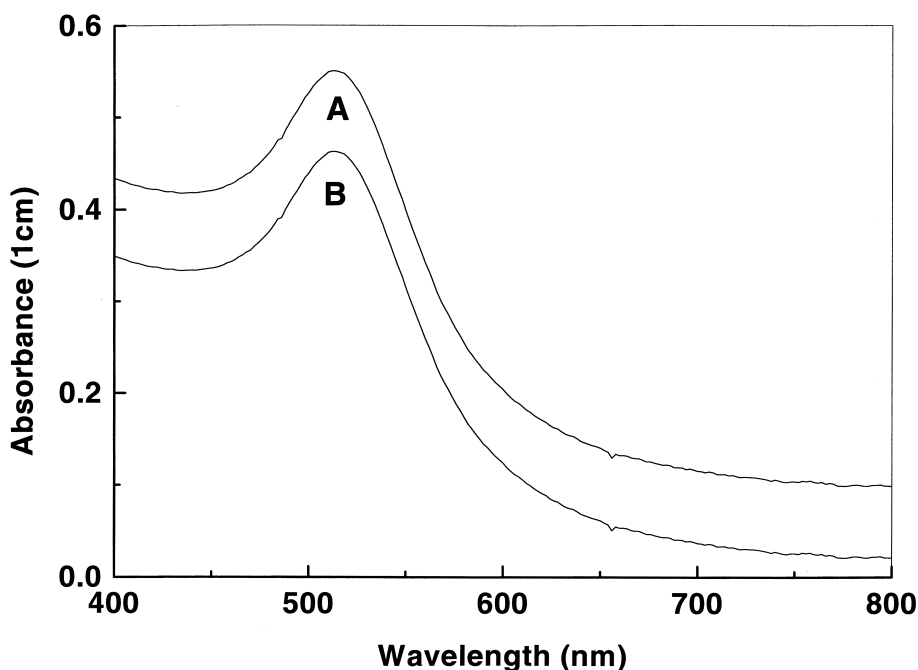

Fig. 3. Optical absorption spectra recorded from the 22 Å as-prepared gold hydrosol (A) as well as the hydrosol after elution from the IEF unit (B).

Table 1
pH values at which onset of ionization occurs for the different sized colloidal particles of this study

Colloidal system	Radius[a] (Å)	Area/carboxylic acid group	pH
Gold particles capped with 4-CTP	16	56.25	7.30
Gold particles capped with 4-CTP	25	39.06	7.40
Silver particles capped with 4-CTP	40	32.65	7.53
Gold particles capped with 4-CTP	70	28.99	7.80

[a] These radii are calculated as the cluster core radius+the length of the 4-CTP molecule taken as 5 Å.

the technique presented in this paper is based on this phenomenon. Table 1 lists the pH values at which onset of ionization occurs for the different colloids together with the radii of the particles. The size of the 4-CTP molecule was taken as 5 Å in calculating the effective radii of colloids capped with this molecule. A strong colloidal particle size dependence on the pH at which ionization of the carboxylic acid groups in the three-dimensional self-assembled monolayers (SAMs) occurs is seen. While the overall pH variation between the smallest and largest colloidal particles is not very large (0.5 pH units), it is much larger than the resolution of the IEF unit used

(0.02 pH units). The individual pH variation between colloidal particles closest in size is also many times this resolution. We would also like to remark here that separate IEF measurements of the individual sols yielded onset of ionization pH values within this resolution and the values obtained are therefore highly reproducible. The pH of ionization increases, as the particle becomes larger or stated differently, the pH increases as the curvature (inverse of the radius) decreases. The variation of the pH of ionization with colloidal particle surface curvature is illustrated in Fig. 4. The inset of Fig. 4 shows a plot of the onset of ionization pH with nanoparticle
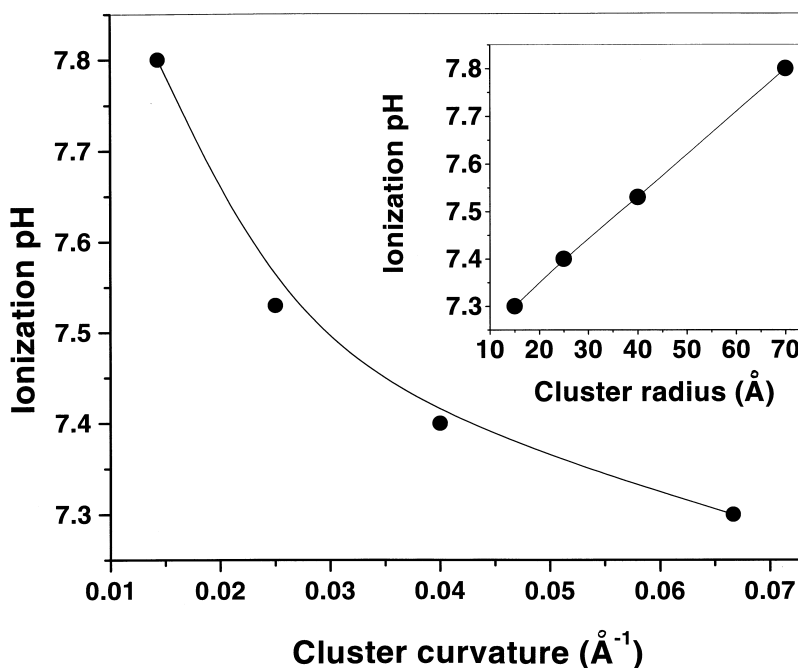

Fig. 4. Variation in pH at which onset of ionization occurs as a function of the curvature of the particles. The inset shows the onset of ionization pH values plotted as a function of colloidal particle radius.

radius. A linear dependence of the pH of ionization on the colloidal particle radius appears to hold for the systems studied herein, recollecting that the concentration of 4-CTP was such that complete surface coverage occurs. Therefore, any variation in the pH at which onset of ionization of the carboxylic acid occurs for the three-dimensional SAMs of this investigation may be attributed purely to particle size effects.

Simple calculations can be performed to show that as the particle curvature increases (particle size decreases), the areal density of the carboxylic acid groups on the particle surface would decrease. We observe that as the colloidal particle surface curvature increases, there is an increase in surface acidity (lower onset of ionization pH). The two-dimensional analogue of this process is the dilution of surface carboxylic acid groups in mixed SAMs [26,27]. Whitesides and co-workers observed that the surface acidity decreased as the density of carboxylic acid groups in the mixed SAMs decreased [26,27]. The results obtained in this study and rationalized in terms of a simple curvature-dependent model is clearly contrary to the observations in mixed SAMs. The assumption that the particles are spherical may not always be justified and faceting of the particle surface needs to be accounted for [28,29]. It is clear that further detailed measurements are required before the role of colloid size on the onset of ionization of surface-bound carboxylic acid groups is understood.

In conclusion, it has been shown that a simple, miniscale IEF unit commonly employed in the determination of the isoelectric point of proteins can be used in the separation of colloidal particles of different sizes. The method is fast, inexpensive and highly sensitive. It shows potential for future modification and development in improving the monodispersity of colloidal particles.

Acknowledgements

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