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Separation of nanometer gold particles by size exclusion chromatography

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Abstract

Size-exclusion chromatography is employed for the separation of gold nanoparticles in the range from 5.3 to 38.3 nm with a polymer-based column of 100 nm pore size. The sorption problem of gold nanoparticles by high surface of stationary phase can be solved with the addition of anionic surfactant to the eluent. Excellent linearity from the plot of logarithm of the particle size as a function of elution time is obtained. Also, the reproducibility of separation for the entire range of calibration curve is high. The size resolution (R_s=1.0) of 10 nm, without considering particles size distribution, is obtained from optimal conditions.

Keywords: Mobile phase composition; Gold particles; Sodium dodecylsulfate

1. Introduction

Nanometer-sized metal materials have attracted extensive attention in the fields of physics, chemistry, and biology [1], because particle size of colloidal metal particles is an important character that dramatically affects the physicochemical properties, such as catalysis [2]. Intensive investigations to effectively control the size of different types of metals particles are undergoing [2–4]. Transmission electron microscopy (TEM) is generally employed to characterize the size and shape of metal clusters to understand the correlation between particle size and physicochemical properties [2–4]. However, TEM is a time-consuming technique and does not involve any separating process. Since size depending optical spectra of nanoparticles can be easily obtained by chromatographic systems with diode array detection (DAD) [5–6]. Hence, chromatographic methods can be utilized in helping the characterization of size-dependent properties of metal nanoparticles.

Size-exclusion chromatography (SEC) has been employed and combined with TEM to characterize nanometer-sized gold particles ranging in size of 3 to 20 nm successfully [6]. It was also employed for the analysis of other types of colloidal particles, such as silica [7] and semiconductors [8–11]. SEC also has the potential to generate monodisperse colloidal from a polydisperse one with fractional collection [12]. These reports indicate that the use of chromatographic methods for the separation of nanoparticles is feasible for various types of nanoparticles.

SEC separations are generally accomplished by the selection of a suitable stationary phase, that has a proper pore size, along with the use of proper eluent.

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However, irreversible adsorption of stationary phase toward nanoparticle is a problem for employing chromatographic technique for nanoparticles separation, because the high surface area of the stationary phase and high surface activity of nanoparticles increase the problem of irreversible sorption. Capillary electrophoresis (CE) technique was proposed to have the potential of reducing this problem by decreasing the surface effect of separation system [13]. However, sample collection to obtain monodispersed particles by CE is a big challenge. An alternative approach is proposed here, to reduce the surface sorption of nanoparticles in SEC by the use of surfactant in the mobile phase.

Surfactant is known to associate with stationary phase, subsequently, resulting in the effect of retention behavior of the analyte [14]. The use of surfactant above critical micellar concentration (CMC) in SEC has been developed earlier for the separation of inorganic ions [15] and small molecules [16]. Elution behavior of SEC with micelle generally follows the three-phase model of micellar liquid chromatography (MLC) developed by Armstrong et al. [17]. Extensive reviews about the use of micellar solution in MLC have been reported [14,18,19].

Surfactants are also widely applied to control the size and shape of nanoparticles [1,20–23]. The association of surfactants with particles stabilizes the particles by electrostatic or steric effect to prevent further agglomeration of particles. In the present work, the use of surfactant in the SEC eluent is employed for separating different sizes of gold particles. The separation behavior along with the separation performance of SEC are discussed to demonstrate the advantages of ionic surfactant in the mobile phase of SEC for nanoparticles separation.

2. Experimental

2.1. Apparatus

An HP 1050 chromatograph with an HP-1050 or 1100 DAD detection system and an HP Chemstation datastation (Hewlett-Packard, Palo Alto, CA, USA) were used in SEC separation of gold nanoparticles. A Nucleogel GFC 1000-8 column (Macherey–Nagel, Düren, Germany) of 300×7.7 mm, that has 100 nm pore size and 8 μm particle size, with a pre-column filter (0.45 μm, Rheodyne, Cotati, CA, USA) were employed for the analytical separation. The flow-rate was 0.5 ml/min, and the injection volume was 10 μl. High-resolution TEM data were acquired on a Hitachi (Tokyo, Japan) HF-2000 field emission TEM system operated at 200 kV accelerating voltage. Samples containing Au particles were prepared by dip-coating of colloidal solution on formvar/carbon film Cu grids (200 mesh; 3 mm) obtained from Argar Scientific (Essex, UK). The pH value of the electrolytes were measured with an Orion model 420A pH meter (Boston, MA, USA).

2.2. Particles samples and chemical reagents

Gold colloids with mean diameters of 5.3±0.38, 9.8±1.1, and 19±1.4 nm were obtained from Sigma (St. Louis, MO, USA), and with mean diameter of 29.3 and 38.3 nm were obtained from British BioCell (Cardiff, UK). Gold particles from Sigma were produced by the reduction of 0.005% gold chloride ($KAuCl_4$) solution with a mixture of trisodium citrate and tannic acid mixture [24]. Gold particles from BioCell were produced similarly with the method mentioned above from 0.01% gold chloride solution. Particle concentrations are in the range of 9.0×10^{9}–3.1×10^{13} gold particles per ml, depending on the size of diameter. Sodium dodecyl sulfate (SDS, purity >95%) was provided by Sigma (St. Louis, MO, USA). Trisodium citrate dihydrate (>99.5%), sodium chloride (99.5%), hydrochloric acid (37%), ethylene glycol (>99.5%) and methanol (>99.8% HPLC grade) were obtained from Merck (Darmstadt, Germany). Sodium hydroxide (>97%) was obtained from Hanawa (Osalca, Japan). Stock solutions of 100 m*M* SDS and 100 m*M* NaCl were prepared by dissolving the required amount of SDS and NaCl in water. Stock solutions and water were used to obtain different compositions of mobile phase by mixing each component with the required ratio from an HP-1050 quaternary pumping system. All eluents were filtered through a 0.45μm filter from Alltech (Deerfield, IL, USA).

3. Results and discussion

3.1. Surfactant effect on the separation

Sorption of the particles by column packing materials is one of the disadvantages of using SEC for the separation of nanometer particles. This problem limits the types of column to be used. Nucleosil 500 and 1000 columns (Macherey–Nagel) were successfully employed in a series to separate gold particles with 1×10^{-3} *M* trisodium citrate [6]. In that report, citrate was used not only as an eluent, but also as a stabilizer. Severe adsorption occurred in our experiment by employing Nucleogel column with citrate as the eluent. It is worth noting that Nucleosil is a silica based packing material and Nucleogel is a polymer (polystyrene–divinylbenzene) based column. The same conditions employed by Nucleosil are not suitable for Nucleogel column even though both have a similar pore size. This is mainly due to the sorption problem of nanoparticles. Different concentrations of SDS solution were employed as the mobile phase to reduce the sorption of packing material. The effect of different concentrations of SDS solution as mobile phase on the sorption and separation of gold nanoparticles are demonstrated in Fig. 1. The data indicate that small signal and no separation were observed for the eluent without the addition of SDS. The signal size and separation resolution were improved with the increase of SDS concentration. The data clearly demonstrate that the sorption problem and separation performance of nanoparticles are improved with the addition of SDS in the eluent. The role of SDS in this separation system and how it can be utilized for size separation of nanoparticle has been an interesting aspect. SDS concentrations in the mobile phase at a range from 0.1 to 40 m*M* were employed to investigate the concentration of SDS effect on the elution time of gold particles, as shown in Fig. 2. These results indicate that the elution time decreased at the beginning, followed by an increase at higher SDS concentrations. It is possible that several types of effect take place on the retention time with the addition of SDS in the eluent. At low SDS concentration, the interaction of SDS with gold nanoparticle and SDS with packing material may occur at the same time. The interaction of SDS with

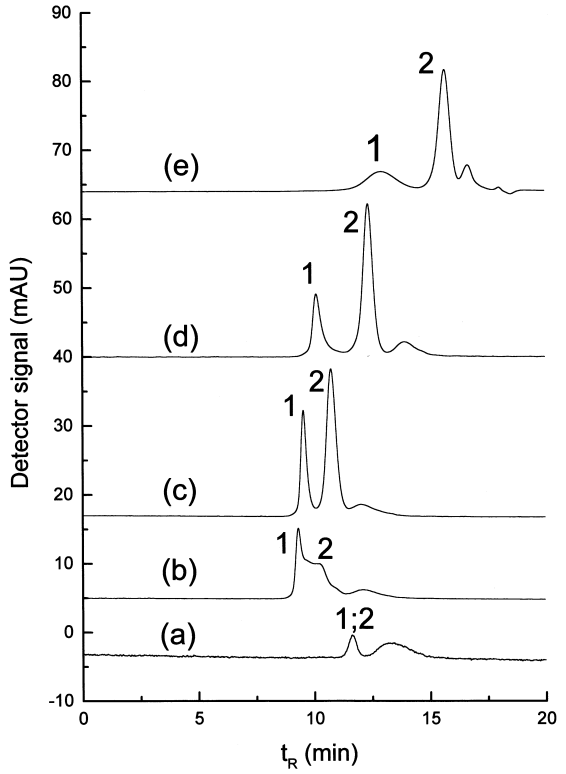

Fig. 1. SDS concentration effect on the separation of 5.3 and 38.3 nm gold nanoparticles by SEC. (a) 0, (b) 0.1, (c) 1, (d) 5, and (e) 80 m*M* SDS; 1: 38.3 nm gold particle, 2: 5.3 nm gold particles. Sample volume: 10 μl gold particles solution; flow-rate: 0.5 ml/min.

packing material that reduces the sorption of gold nanoparticles decreases the retention time and increases the analyte signal, as shown in Fig. 1.

To further confirm the signal improvement is due to the sorption of nanoparticles by packing material, nanoparticle solution was diluted with different concentrations of SDS solution at 1:1 ratio, and then passed though packing material, supported on the top of a filter paper, and the filtrates were measured by a HP-1100 DAD system. The absorption spectra of filtrates are shown in Fig. 3. It is obvious that the existence of SDS in gold solution increases the absorbance over that without SDS. Also, the color of gold nanoparticles was observed on the packing material after passing the gold solution without SDS through the packing material. These gold particles adsorbed on the packing material can be stripped

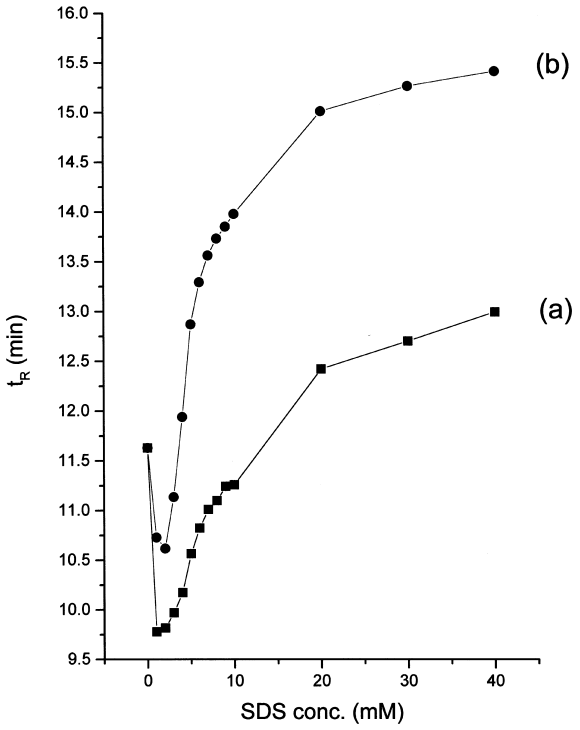

Fig. 2. SDS concentration effect on the retention time of gold nanoparticles in SEC. (a) 38.3 nm gold nanoparticles, (b) 5.3 nm gold nanoparticles. Sample volume: 10 μl gold particles solution; flow-rate: 0.5 ml/min.

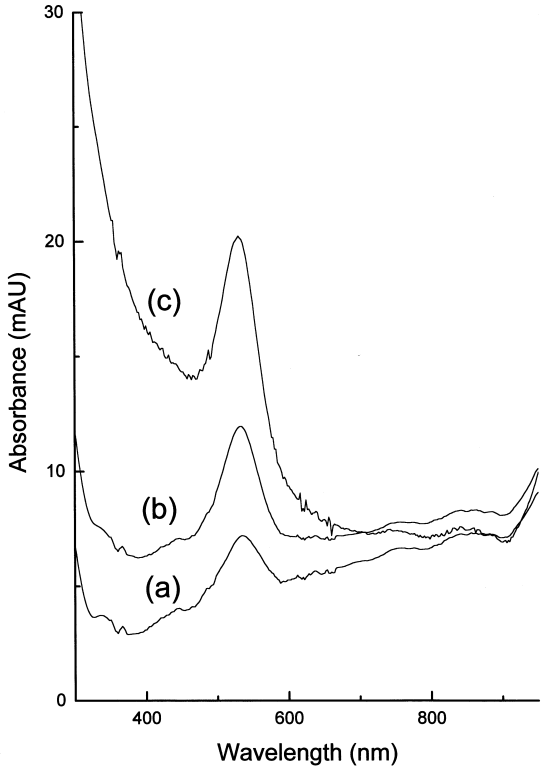

Fig. 3. SDS concentration effect on the sorption of 5.3 nm gold nanoparticles by packing material. (a) 0, (b) 0.1, and (c) 5 m*M* SDS. Particle solution was diluted with different concentration of SDS solutions at 1:1 ratio.

down by SDS solution. This clearly confirms that the addition of SDS in the sample can reduce the sorption of nanoparticles by packing material. The improvement in the signal is attributed to the interaction between surfactant and packing material resulting in a negative charge on the surface of packing material and preventing the sorption of gold particles. Since electrostatic interaction between negatively charged SDS, that adsorbed onto the packing material, and gold particle, also negatively charged, arising from the sorption of citrate and chloride ions, reduce the possible adsorption of gold particles. The sorption of surfactants by packing materials was demonstrated before [15].

Also, the theory of size exclusion chromatography is based on the fact that larger gold particles pass through the column quicker and have less elution volume. A decrease of elution time with the increase of SDS concentration, as shown in Fig. 2, suggests that the addition of SDS may enlarge the apparent particle size, consequently, the elution time is decreased. This implies the interaction of SDS with gold particle at low SDS concentration that enlarges the apparent particle size. The interaction between nanopaticles and surfactant was investigated by UV–Vis absorption spectra of gold particles at different SDS concentration, as shown in Fig. 4. Gold nanoparticles (5.3 nm) diluted with water and SDS solutions to keep the same particle concentration were compared to examine the effect of SDS on the surface plasmon absorption band. An increase in intensity and blue-shifting of the peak position can be observed with the addition of SDS. This implies the interaction between SDS and gold particles may take place [25]. In addition, SDS in the eluent is possibly causing the change in the size of gold nanoparticles, thus, changing the peak position of the plasmon band in UV–Vis and elution time in SEC.

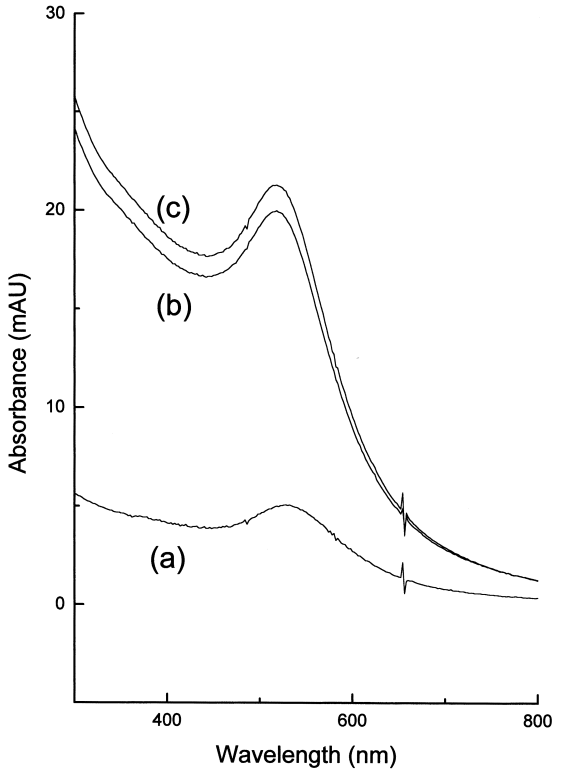

Fig. 4. SDS concentration effect on the UV–Vis absorption spectra of 5.3 nm gold particles. (a) 0, (b) 0.1, and (c) 5 m*M* SDS. Particle solutions were diluted with different concentration of SDS solutions at 1:1 ratio.

The blue-shift of the band maximum implies a decrease of size distribution of gold particles. Transmission electron microscopy was employed to examine the size of 5.3 nm gold particles with and without the mixing with SDS. No change in size distribution of particles was observed. Thus, no change in the elution time should be observed for the interaction between SDS and gold particles. Therefore, the interaction between SDS and column packing material plays a more important role of SDS effect on the elution time at low SDS concentration in Fig. 2.

At high SDS concentration, the ionic strength effect on the double layer of particles plays a more important role. Therefore, further increase of SDS concentration may behave like the increase of ionic strength of the eluent, as indicated in Fig. 2. Ionic strength effect on the elution behavior of colloid has been well-documented [26]. Basically, the electric double layer of charged particles is inversely proportional to the ionic strength of electrolyte. Smaller double layer of gold particles at high SDS concentration has a smaller apparent size and longer elution time. Electrolyte was added to the eluent for increasing the ionic strength and changing the thickness of the electric double layer to verify the argument of double layer decreasing at high SDS concentration. The effects of NaCl and SDS concentration on the elution time of 5.3 nm gold particles were compared, as shown in Fig. 5. In this experiment, 5 m*M* SDS solution was added to each NaCl solution to prevent the adsorption of gold particles by packing material. The effects of NaCl and SDS concentration on the elution times are similar, i.e. an increase of elution time with the increase of electrolyte concentration. It clearly ex-

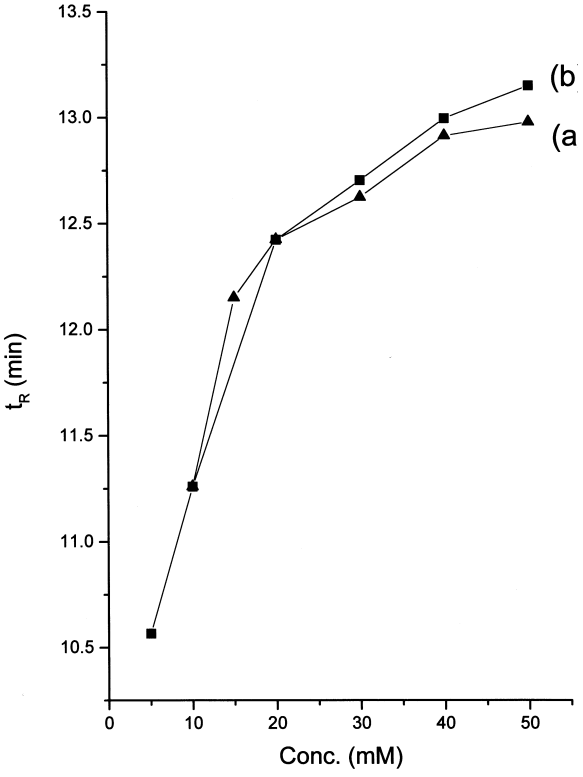

Fig. 5. Comparison of the effect of NaCl and SDS concentrations on the retention of 5.3 nm gold nanoparticles in SEC. (a) NaCl solution with 5 m*M* SDS, (b) SDS. Sample volume: 10 μl gold particles solution; flow-rate: 0.5 ml/min.

plains that an increase of ionic strength with the increase of electrolyte concentration decreases the double layer of charged gold particles and results in longer elution time. Also, the charge effect on the elution time of 5.3 nm gold particles is examined (not shown here). The elution time increases in a faster rate with the increase of $MgCl_2$ concentration than that of NaCl concentration. Because the ionic strength of eluent depends on the charge of the electrolyte, the thickness of double layer, therefore, decreases in a faster speed with the increase of $MgCl_2$ concentration than that of NaCl concentration.

Surfactant is a strong electrolyte at low concentration. However, the micelle formation occurs at high concentration, i.e. above CMC value. Hence, the effect of the SDS concentration (above CMC) on the elution (in Fig. 2) may not entirely be due to the ionic strength effect on the double layer even though there is a similarity between SDS and NaCl. The other possible mechanism for the increase of elution time at high SDS concentration can be referred to in the separation model of micellar SEC. Linear relationship between $V_i/(V_r-V_0)$ and SDS concentration would be obtained with the association of micelle and analyte, where V_i, V_r, and V_0 are internal pore volume of the packing, retention volume, and void volume or volume of mobile phase in interstices between packing materials, respectively. V_0 was obtained from retention volume of 100 nm gold nanoparticles. Dependence of $V_i/(V_r-V_0)$ on the SDS concentration was not linear for gold nanoparticles, as illustrated in Fig. 6. A similar relationship of $V_i/(V_r-V_0)$ on the SDS concentration has already been reported for anionic solutes in micellar SEC [15]. Similar plots have also been reported in MLC [14,16]. Those reports attributed the exclusion effect between analyte and micelle with the same size of charge. Both gold nanoparticle and SDS micelle have negative charges that may partially explain the results of Fig. 6.

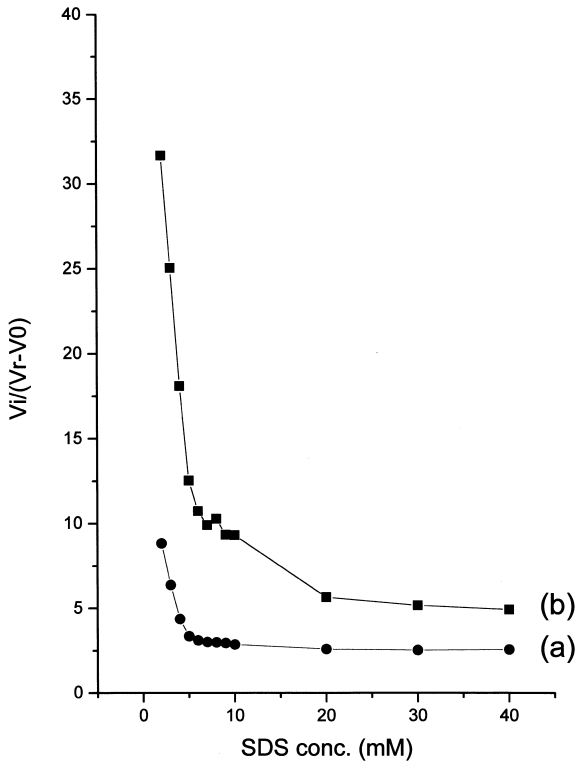

Fig. 6. Plots of $V_i/(V_r-V_0)$ vs. SDS concentration for 5.3 and 38.3 nm gold nanoparticles in SEC. (a) 5.3 nm gold nanoparticles, (b) 38.3 nm gold nanoparticles. Sample volume: 10 μl gold particles solution; flow-rate: 0.5 ml/min.

3.2. Separation performance

A plot of the logarithm of the particle size range from 5.3 to 38.3 nm as a function of elution time is shown in Fig. 7. Excellent linearity ($r^2>0.99$) in this plot suggests that the particles are being separated purely by steric exclusion, similar to the results reported by other researchers without employing surfactant in the mobile phase [5]. In addition, examined herein is the reproducibility of the elution time from 21 consecutive runs of a mixture of 5.3 and 38.3 nm gold particles. The calculated precision on the migration time of 5.3 and 38.3 nm particles are 0.07 and 0.14%, respectively. It should be pointed out that the reproducibility of the elution depends on the performance of the column. The column performance was degrading after more than 6 months of adding different types of surfactant in the mobile phase. The best separation performance, in terms of resolution (R_s), was obtained at 5 m*M* SDS. The calculated R_s value is about 3.2 for 5.3 and 38.3 nm particle mixture with a single column only. Estimated size resolution ($R_s=1.0$) without considering the size distribution of analyte is about 10 nm, compared with 20–60 nm without SDS employed in

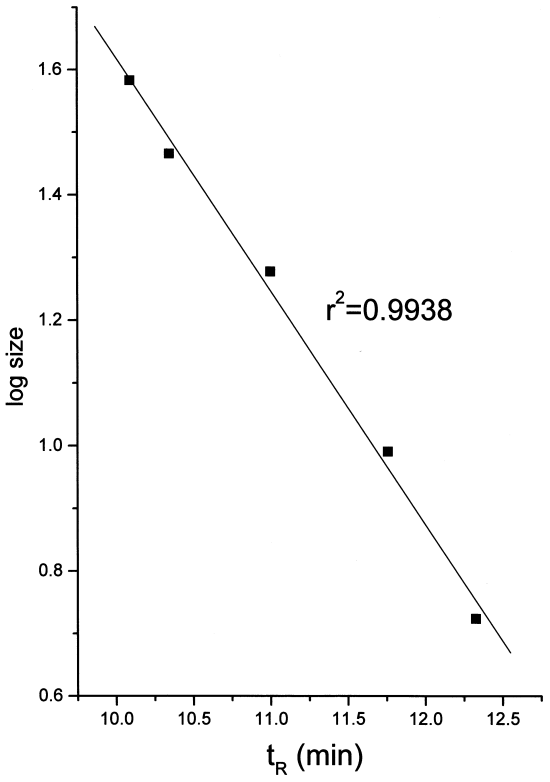

Fig. 7. Semilogarithmic plot of the diameter as a function of the retention time for gold nanoparticles in SEC. Sample volume: 10 μl gold particles solution; mobile phase: 5 m*M* SDS; flow-rate: 0.5 ml/min.

other reports with two GFC columns in a series [6]. These results demonstrate the advantage of surfactant in SEC for the separation of nanoparticle gold particles.

4. Conclusions

The addition of surfactant to the mobile phase was successfully applied to SEC for the separation of gold nanoparticles. Interaction between surfactants and packing materials reduced or eliminated the sorption of nanoparticles by packing materials. Separation performance was also improved with the addition of SDS in the mobile phase. The effects of SDS on nanoparticles separation in GFC column were discussed. Excellent linear relationship for the logarithm of the particle size range from 5.3 to 38.3 nm as a function of elution time was obtained. This implied that the separation mechanism in this work was based on steric exclusion.

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