

Asymmetrical flow field-flow fractionation coupled with a liquid waveguide capillary cell for monitoring natural colloids in groundwater

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ABSTRACT

This paper describes an asymmetrical flow field-flow fractionation (AF4) technique combined with a flow-through cell having a long optical path length, called a liquid waveguide capillary cell (LWCC). The performance of the AF4-LWCC system for separation and spectrophotometric detection of nanoparticles was examined using size-standard silver nanoparticles. The limit of detection for silver nanoparticles of 30 and 60 nm diameter was highly improved to 2.5 and 7.8 $\mu\text{g L}^{-1}$ (from 220 and 850 $\mu\text{g L}^{-1}$, respectively) by applying a 100-cm LWCC, compared to a commercial UV/Vis detector. This improvement of detection sensitivity enabled investigating natural nanoparticles in granitic groundwater extracted from an underground research tunnel in the Republic of Korea. Only a small injection volume (0.5 mL) of groundwater was required to obtain the size distributions of trace amounts of natural colloids without pre-concentration. This study demonstrates that the proposed AF4-LWCC system could be applied as a convenient and efficient tool for detection and size-determination of aquatic nanoparticles at trace-level concentrations.

1. Introduction

Organic and inorganic colloids such as humic substances, silica, clay minerals or metal hydroxides are ubiquitous in natural water (Degueldre et al., 1996a, 1996b). Various toxic elements such as heavy metals and radioactive nuclides have often been observed in association with colloidal materials, which can accelerate their transport through fractured media in natural environments (Kersting, 2013; Ryan and Elimelech, 1996). Therefore, the physicochemical properties of natural colloids are important for understanding the migration behavior of toxic elements through geological media.

Among various parameters affecting the migration behavior of nanoparticles, their size distribution is a key parameter for discovering their unique physicochemical properties. Various techniques including electron microscopy, atomic force microscopy, and size-exclusion chromatography have been developed to obtain information about the size of colloids in natural water (Baalousha and Lead, 2007; Kozai et al., 2013; Lead et al., 2006). One of difficulties in sizing natural colloids is that natural nanoparticles are present in trace level concentrations ($< 100 \mu\text{g L}^{-1}$); thus pre-concentration is usually required. Recently, ultrafiltration and cross-filtration methods have been used for enriching nanoparticles (Aosai et al., 2015; Hassellöv et al., 2007). These methods

are time-consuming and result in sample loss owing to adsorption onto filter membranes or in aggregation of nanoparticles. Therefore, it is necessary to develop a sensitive and efficient method for the trace-level analysis of nanoparticles.

Field-flow fractionation (FFF) is a separation technique useful for analysis of nanoparticles and macromolecules according to their diffusion coefficients (or their sizes) (Giddings, 1993). FFF has been widely used for the characterization of various natural colloids in surface water, soil suspensions, and groundwater (GW) (Baik et al., 2007; Beckett et al., 1987; Eum et al., 2007; Gimbert et al., 2005; Hassellöv et al., 1999; Saito et al., 2015). In FFF, an on-line detector following separation is incorporated to obtain the particle size information. Light scattering (LS) (Baalousha et al., 2006), inductively coupled plasma mass spectrometry (ICP-MS) (Baik et al., 2007; Hassellöv et al., 1999; Saito et al., 2015), and laser-induced breakdown detection (LIBD) (Baik et al., 2007) have been used for investigating natural nanoparticles. However, the LS becomes quite insensitive for particles smaller than 100 nm, because the intensity of scattered light is low (Bundschuh et al., 2001). Using ICP-MS connected to a FFF, elemental distributions in a colloidal fraction can be obtained in trace levels, while the concentration of nanoparticles cannot be directly determined. Though LIBD is a sensitive detection method, especially for small colloids (Bundschuh

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et al., 2001), LIBD measurements are limited for quantitative analysis due to material-dependent breakdown responses (Lapresta-Fernández et al., 2014). In addition, all of the detection methods mentioned above have the shortcomings of high cost and complicated operation.

Spectrophotometry is the most commonly used technique for detection of nanoparticles, proteins, and macromolecules fractionated by FFF owing to its low cost and convenience of operation. However, the detection sensitivity of spectrophotometry is lower than those of the aforementioned techniques. An on-line concentration method before separation by FFF was developed by adopting an opposed flow sample concentration mode (OFSC) to investigate natural nanoparticles using spectrophotometry (Lee et al., 1998). However, a large volume (from 10 to 100 mL) of sample solution had to be loaded. This might enhance the interaction between the particles and the membrane, and distort the size determinations. When a highly sensitive absorption measurement system following fractionation is developed, it will be useful in several research fields and industries for analysis of nanoparticles in dilute concentration.

In this study, an asymmetrical flow field-flow fractionation (AF4) was combined with a liquid waveguide capillary cell (LWCC) for analyzing trace amounts of aquatic nanoparticles. LWCC with a long optical path length (OPL) was adopted to enhance the detection sensitivity in absorption measurements, as reported elsewhere (Waterbury et al., 1997; Wilson et al., 2005). Performance test of the AF4-LWCC system was carried out using silver nanoparticles, which absorb light in the visible wavelength range. Variation of the limit of detection (LOD) and separation characteristics were examined depending on the OPL of the applied flow-through cells. The AF4-LWCC system was employed to analyze trace amounts of natural nanoparticles in GW extracted from an underground research area in the Republic of Korea.

2. Experimental section

2.1. Chemicals and reagents

All solutions were prepared with deionized water ($> 18.2 \text{ M}\Omega\text{-cm}$) purified using a Milli-Q element system (Millipore). A NIST traceable size standard of polystyrene in particle diameter of $20 \pm 1.5 \text{ nm}$ was obtained from Duke Scientific (Palo Alto, CA, USA) to determine the thickness of the separation channel. Polyvinylpyrrolidone-coated silver nanoparticles (AgNPs) having nominal diameters of 32.7 ± 4.3 and $60.8 \pm 6.6 \text{ nm}$ were purchased from nanoComposix, Inc. (San Diego, CA, USA) for the performance test of the proposed AF4-LWCC system. Sodium dodecyl sulfate (SDS) purchased from Sigma Aldrich and FL-70 purchased from Fisher Scientific, Co. were applied as surfactants in the carrier solutions. Sodium azide (NaN_3) obtained from Sigma Aldrich was used to control the ionic strength of the carrier solutions, which was essential to minimize adsorption of particles onto the AF4 membrane.

2.2. Groundwater sampling

The location and geological formation of the granitic GW used in this work were described elsewhere (Baik et al., 2015). Briefly, the GW was sampled from the 199.7–228.7 m depth of a borehole (DB-1) where a multi-packer system was installed in granite bedrock located at KURT (KAERI Underground Research Tunnel) in Daejeon, Republic of Korea. The GW sample was collected in a cylindrical stainless steel bottle filled with Ar gas. The collected GW was stored and handled in a glove box filled with Ar gas. The GW was filtered through a $0.45\text{-}\mu\text{m}$ membrane filter (Millipore) to remove larger particulate matter. The concentration of dissolved organic carbon and SiO_2 was 1.13 and 17.9 mg L^{-1} , respectively, as listed at Table 1 in the literature (Baik et al., 2015). For the dynamic light scattering (DLS) measurement, the GW sample was pre-concentrated by ultrafiltration using an Amicon Ultra-15 centrifugal filter with a molecular weight cutoff (MWCO) of 10 kDa (Millipore).

Table 1

AF4 sample recovery and ζ -potential measured for 30-nm AgNPs dispersed in carrier solutions with various compositions.

Carrier solution	Recovery (%)	ζ -potential (mV)
Water with 0.02% NaN_3 + 0.02% FL-70	92.9	-30.8 ± 4.4
Water with 0.02% NaN_3 + 0.01% SDS	97.5	-30.2 ± 2.3
Water with 0.02% NaN_3	78.3	-21.6 ± 3.2

2.3. Instrumentation

2.3.1. AF4-LWCC

The AF4 system, using an Eclipse AF4 module from Wyatt Technology Europe GmbH (Dernbach, Germany), was assembled with a $350\text{-}\mu\text{m}$ -thick Mylar spacer and a regenerated cellulose membrane (MWCO of 10 kDa , Millipore). The geometry of the channel was trapezoidal with a tip-to-tip length of 17.5 cm and breadth at the inlet and at the outlet of 2.2 and 0.3 cm , respectively. The carrier solution was delivered into the channel using a HPLC pump (Shimadzu LC-20AD, Japan). The rate of cross-flow was measured using an HPLC liquid flowmeter (Model-502500, GJC Instrument Ltd., Cheshire, UK). Size standards of nanoparticles (polystyrene and AgNPs) were injected into the channel using a $20\text{-}\mu\text{L}$ loop injector (Rheodyne, Cotati, CA, USA). The syringe pump (Model 100, KD Scientific, Holliston, MA, USA) was used at a flow rate of 0.2 mL min^{-1} for 60 s to inject the standard samples through the loop injector into the channel. The GW sample was directly injected into the AF4 channel using a syringe pump at a flow rate of 0.2 mL min^{-1} while focus flow rate was maintained at 1.5 mL min^{-1} for the on-line enrichment of natural nanoparticles, as described in a previous study (Lee et al., 2015). After focus step, cross and channel flow rates were maintained at 0.5 and 1.01 mL min^{-1} , respectively. The channel thickness (w) of AF4 was determined from the retention time of 20-nm polystyrene beads, to be $238 \pm 2 \mu\text{m}$. A commercial UV/Vis detector (SPD-20A, Shimadzu, Japan) with a flow-through cell having OPL of 0.05 cm was connected to the outlet of the separation channel using PEEK tubing. Absorbance of eluted samples as a function of time was recorded using a home-made signal acquisition program based on LabVIEW (National Instruments). For a sensitive absorption measurement, a LWCC with an OPL of 10 cm (LWCC-M-100) or 100 cm (LWCC-3100) obtained from WPI Inc. (Sarasota, FL, USA) was applied by connecting to the separation channel. A UV/Vis spectrophotometer (Cary5, Varian) was coupled with the LWCC using a fiber optic interface (Hellma) and fiber optic cables ($600 \mu\text{m}$ in core diameter, Hellma). Absorbance at the wavelengths of 254 , 405 , or 307 nm was measured for monitoring polystyrene, silver, or natural nanoparticles, respectively, on the basis of the absorption features of each material. As the absorption measurement setup is a single beam configuration, signal measured during sample fractionation was corrected with absorbance of carrier solutions measured before the injection of sample.

2.3.2. Dynamic light scattering and ζ -potential measurements

DLS and ζ -potential measurements were carried out at the scattering angles of 90° and 17° , respectively, with a Zetasizer (NanoZS90, Malvern instrument, Worcestershire, UK) equipped with a He-Ne laser (633 nm) as a light source. All measurements were repeated five and ten times for DLS and ζ -potential measurement, respectively.

2.4. Hydrodynamic diameter and sample recovery

The hydrodynamic diameter (d_H) of a particle in AF4 is related to its retention time (t_r) by

$$d_H = \frac{2V^0 k T t_r}{\pi \eta w^2 F_c t^0}, \quad (1)$$

where V° is the void volume of the AF4 channel; k is the Boltzmann constant; T is the absolute temperature (K); η is the viscosity of the carrier solution; w is the channel thickness, F_c is the volumetric cross-flow rate (mL min^{-1}), and t° is the void time (the retention time of non-retained species). Using Eq. (1), an AF4 fractogram of a sample can be converted directly to its particle size distribution.

Sample recovery (R , %) in AF4 was determined by

$$R = \frac{A}{A_o} \times 100 (\%), \quad (2)$$

where A is the AF4 peak area obtained with a cross-flow. The determination of A_o was performed by elution of a sample through the channel without the cross-flow. For determination of the sample recovery in AF4 for GW samples, A and A_o were determined by eluting the same sample through the channel with and without the cross-flow, respectively, after the on-line enrichment of the GW samples.

3. Results and discussion

3.1. Selection of carrier solutions

The selection of carrier solutions is an important factor for the separation of nanoparticles by AF4 to avoid unexpected inter-particle or particle-membrane interaction in the separation channel. The adsorption and repulsion of particles in the AF4 channel can influence the efficiency of separation. Aqueous solutions of 0.02% NaN_3 with or without surfactants (such as 0.01% SDS and 0.02% FL-70) were examined for the separation of AgNPs. For the sample elution, the AF4 channel and cross flow rates were 0.50 and 1.01 mL min^{-1} , respectively. Fig. 1 shows AF4 fractograms of 30-nm AgNPs obtained in three different carrier solutions (aqueous solutions of 0.02% NaN_3 , 0.02% NaN_3 + 0.02% FL-70, and 0.02% NaN_3 + 0.01% SDS) under the same experimental conditions. The calculated sample recoveries and the measured ζ -potentials when using different carrier solutions are listed in Table 1. With 0.02% NaN_3 without surfactant, the fractogram shows tailings (dashed line in Fig. 1) and the sample recovery was 78.3%. More symmetrical and narrower fractograms (solid lines in Fig. 1) were obtained when 0.02% FL-70 or 0.01% SDS was added. The ionic strength was 3.4 or 3.6 mM for the carrier solution containing 0.02% FL-70 or 0.01% SDS. The ζ -potential of 30-nm AgNPs dispersed in the carrier solution containing 0.02% FL-70 or 0.01% SDS was -30.8 ± 4.4 or -30.2 ± 2.3 mV. Despite similar ionic strengths and

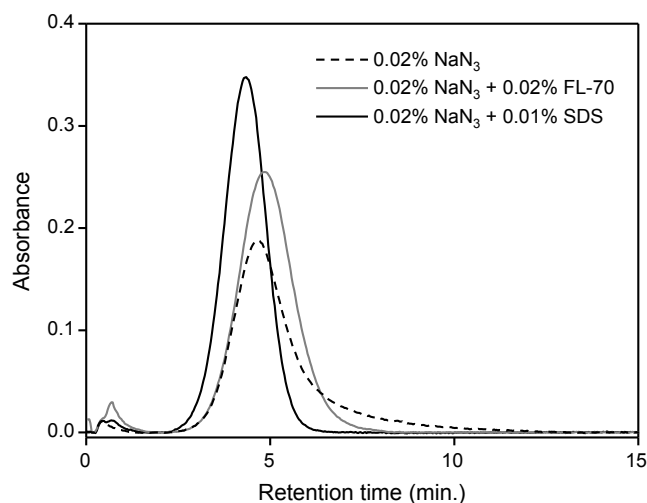


Fig. 1. AF4 fractograms of 30-nm AgNP obtained with aqueous carrier solutions containing 0.02% NaN_3 , 0.02% NaN_3 + 0.01% SDS, and 0.02% NaN_3 + 0.02% FL-70. The channel and the cross-flow rates were 0.50 and 1.01 mL min^{-1} , respectively.

ζ -potentials, AgNPs were eluted later and broader with the carrier solution containing FL-70 than with that containing SDS. The mean diameter of 30-nm AgNPs determined by Eq. (1) is 34.6 nm for using the carrier solution containing SDS, which agrees well with the nominal value of 32.7 ± 4.3 nm. Moreover, the highest sample recovery of AgNPs was observed with 0.01% SDS. Thus, an aqueous solution with 0.02% NaN_3 and 0.01% SDS was selected as the carrier solution for AF4 analysis of AgNPs in this study.

3.2. Performance of AF4-LWCC system

Fig. 2 shows AF4 fractograms obtained using a flow-through cell with OPL of 0.05 cm (a and b), 10 cm (c and d), and 100 cm (e and f), respectively, for AgNPs of 30 and 60 nm. The sample concentration was varied from 0.74 to 3.70 mg L^{-1} for 30-nm AgNPs, and from 3.76 to 9.40 mg L^{-1} for 60-nm AgNPs. As expected, the absorbance increased with increase of both the concentration of the nanoparticles and the OPL of the cells. As shown in Fig. 2 (a and b), absorbance measured using the commercial UV/Vis detector and the signal to noise ratios (S/N ratio) were low. On the other hand, when LWCCs with longer OPLs of 10 and 100 cm were used, the S/N ratios for the same samples were increased and the detection sensitivities were improved, as shown in Fig. 2 (c–f). The retention times for 30- and 60-nm AgNPs were remained near 4.4 and 7.5 min regardless of the OPLs of the applied flow-through cells. Also the full-width-at-half-maximum (FWHM) of 30-nm AgNPs did not change with OPLs, as listed in Table 2. Only the FWHM of 60-nm AgNPs was slightly increased with increasing OPL. The internal sample volumes of the applied flow-through cells with OPLs of 0.05, 10, and 100 cm were 81, 25, and 250 μL , which were small enough to investigate 30- and 60-nm AgNPs without peak broadening in the fractograms.

Absorbance at the peak maximum increased 172 ± 1 times for AgNPs when the OPL was increased from 0.05 to 10 cm. This indicates that the OPL of the flow-through cell installed in the commercial UV/Vis detector is approximately 0.058 cm. With further increase of OPL from 10 to 100 cm, the absorbance was increased ten-fold. The limits of detection (LOD) of both 30- and 60-nm AgNPs, determined as a function of OPL are summarized in Table 2. The LOD was determined from 3σ of the measured absorbance for a blank solution. The LOD for 30-nm AgNPs was determined to be 220, 16, and 2.6 $\mu\text{g L}^{-1}$, with OPL of 0.05, 10, and 100 cm, respectively. For 60-nm AgNPs, the LODs were 850, 49, and 7.8 $\mu\text{g L}^{-1}$. The LODs of both 30- and 60-nm AgNPs were increased approximately 100 times when a commercial UV/Vis detector was replaced by a highly sensitive absorption measurement system with a 100-cm LWCC. Unlike the absorbance, LOD does not proportionally increase with OPL. The main reason for this is the difference in background noise level resulting from the performance of detectors, the coupling efficiency between a flow-through cell and a detector with optical fibers, and the transmission efficiency of light through LWCCs of varying OPL (Cho et al., 2010; Wilson et al., 2005). The 3σ of absorbance at 405 nm for a blank solution was 2.6×10^{-5} , 2.5×10^{-4} , or 3.9×10^{-4} for cells with OPL of 0.05, 10, or 100 cm, respectively, in this work.

Recently, a high performance AF4-UV/Vis-ICP-MS system (Geiss et al., 2013) and SdFFF-UV/Vis system (Cascio et al., 2014) had been developed for trace analysis of AgNPs. In these works, LODs were determined to be 6.7 and 29 $\mu\text{g L}^{-1}$ for AgNPs of 60 nm diameter by using the commercial UV/Vis detectors having a flow cell with the OPL of 1 cm. It is clear that the better detection sensitivity for the commercial UV/Vis detector was obtained due to the increase of the OPL from 0.05 to 1 cm. It is not simple to compare detection sensitivities of different measurement systems. However, when the previously reported separation conditions are applied with a LWCC with a longer OPL, the enhancement of LODs below $\mu\text{g L}^{-1}$ is expected.

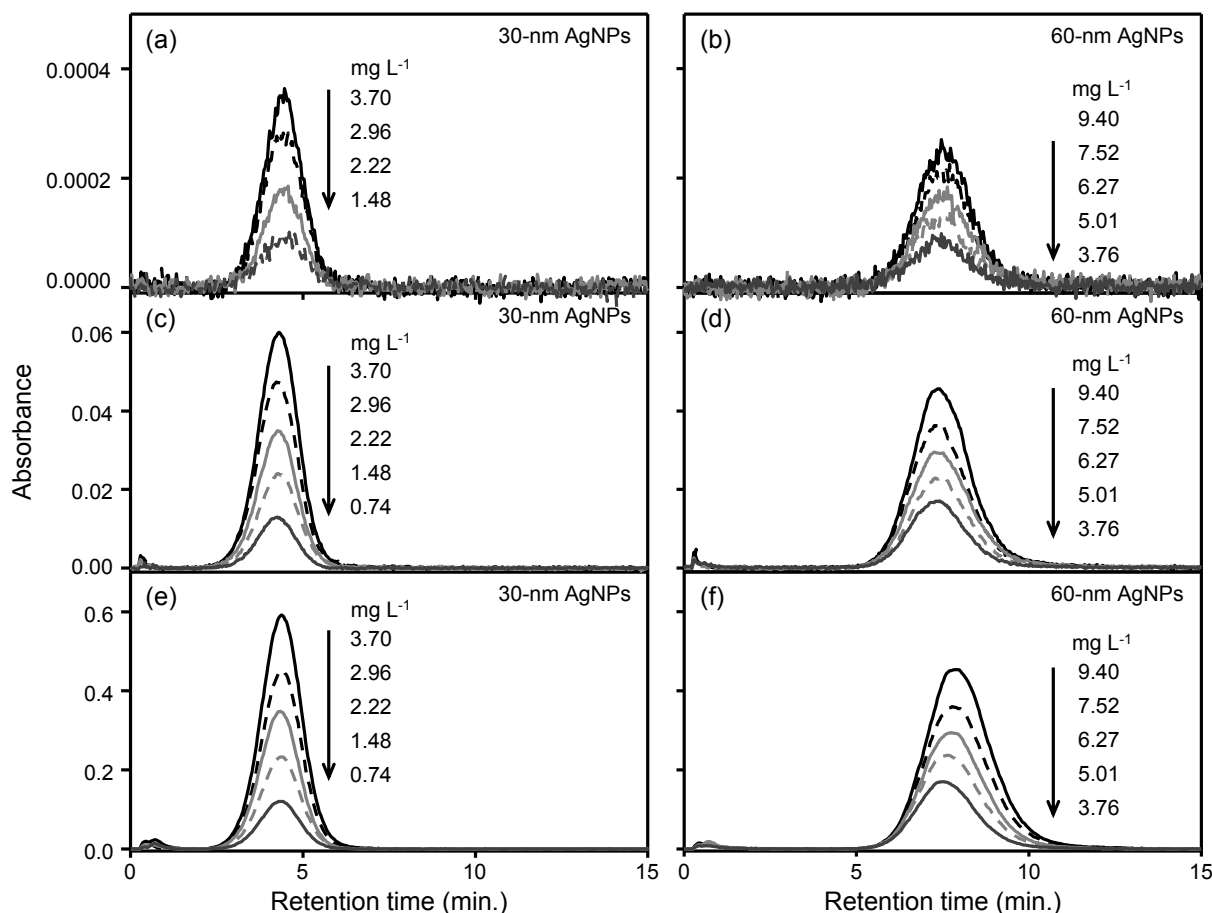


Fig. 2. AF4 fractograms were obtained using flow-through cells with OPL of 0.05 cm (a and b), 10 cm (c and d), and 100 cm (e and f), respectively, for 30- and 60-nm AgNPs at various concentrations.

Table 2

Full-width-at-half-maximum (FWHM) and limit of detection (LOD) of 30- and 60-nm AgNPs.

Optical path length (cm)	LOD ($\mu\text{g L}^{-1}$)		FWHM (mL)	
	Particle size (nm)		Particle size (nm)	
	30	60	30	60
0.05	220	850	1.34	1.89
10	16	49	1.32	1.91
100	2.5	7.8	1.34	2.06

3.3. Application of AF4-LWCC to GW colloids

Various carrier solutions have been tested for AF4 separation of natural nanoparticles (Lee et al., 1998; Neubauer et al., 2011). Water containing SDS generally yields better sample recovery than that containing FL-70, probably because SDS consists of a single chemical moiety. It has been also reported that the use of carrier solutions having lower ionic strength tends to yield better sample recovery in AF4 for natural colloids (Dubascoux et al., 2008). In this study, water containing 0.02% NaN_3 and 0.01% SDS was selected as the carrier solution for the analysis of nanoparticles in granitic GW.

Fig. 3 (a) and (b) show AF4 fractograms of nanoparticles in KURT-GW measured with a commercial UV/Vis detector (OPL of 0.05 cm) and the AF4-LWCC system (OPL of 100 cm), respectively. A 2-mL sample was injected into the AF4 channel by applying the on-line concentration method (Lee et al., 1998, 2015). Absorbance at the peak maximum ($t_r = 2.1$ min) and detection sensitivity for the same GW sample were

significantly increased by adopting the AF4-LWCC system. The fractogram in Fig. 3(b) shows that the nanoparticles in granitic GW exhibited two main distinguishable populations, eluted at 1–5 min and 10–15 min, with a broad continuous distribution eluting until 30 min for relatively low concentrations. The first main peak was not completely separated from the void peak. The size distribution determined from the fractogram using Eq. (1) ranged from a few nm to 40 nm. The second population in a range of 70–100 nm was not detectable with the commercial UV/Vis detector due to its low detection sensitivity. The sample recovery determined using Eq. (2) was $88.5 \pm 6.5\%$. It is obvious that smaller natural nanoparticles than the pore size of a cellulose membrane (10 kDa) were lost during the focusing and eluting steps. A membrane with a smaller pore size (≤ 1 kDa) is preferred for investigation of natural samples containing extremely small nanoparticles (Baalousha and Lead, 2007; Saito et al., 2013).

The size distribution of nanoparticles in a GW sample was investigated using the conventional analysis method using DLS, after pre-concentration by ultrafiltration (Aosai et al., 2015; Hassellöv et al., 2007). As shown in Fig. 4, a broad and bimodal size distribution with a main population in a few hundreds of nanometers was observed. Smaller particles than 20 nm were not observed in contrast with that observed using the AF4-LWCC system. This discrepancy between the size distributions obtained by DLS and AF4-LWCC can be explained with physicochemical changes of nanoparticles during ultrafiltration (Aosai et al., 2015), different sensitivity for detecting nanoparticles of DLS and absorption measurement, and the limitations of DLS for analysis of natural samples (Domingos et al., 2009; Filella et al., 1997). The GW sample was concentrated from 50 mL to approximately 0.5 mL using a regenerated cellulose membrane with MWCO of 10 kDa.

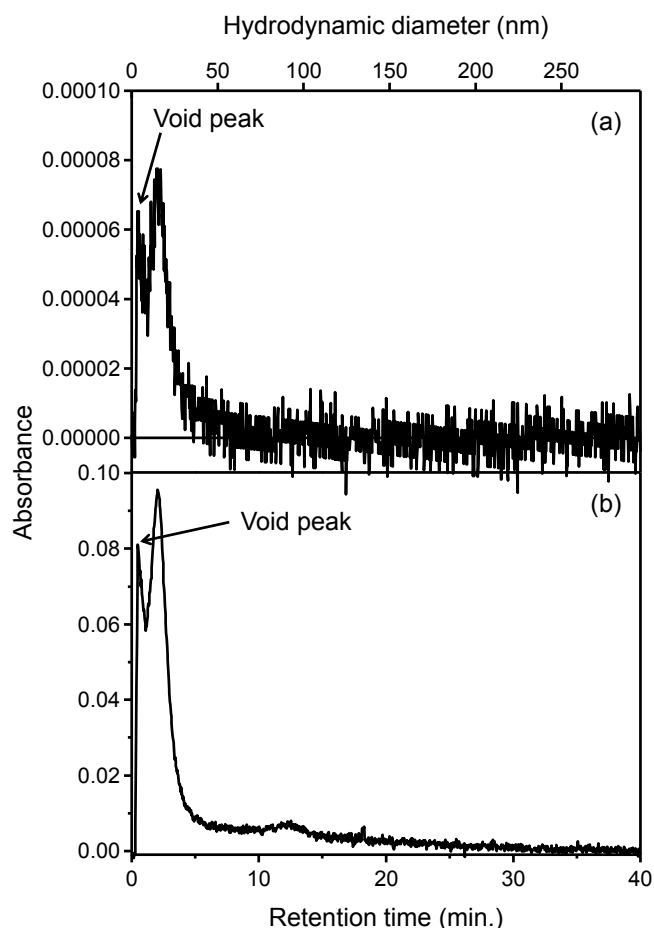


Fig. 3. AF4 fractograms of natural nanoparticles in granitic GW were obtained using flow-through cells with OPL of 0.05 cm (a), and 100 cm (b). 2 mL of GW sample was injected and concentrated using the on-line concentration method prior to separation.

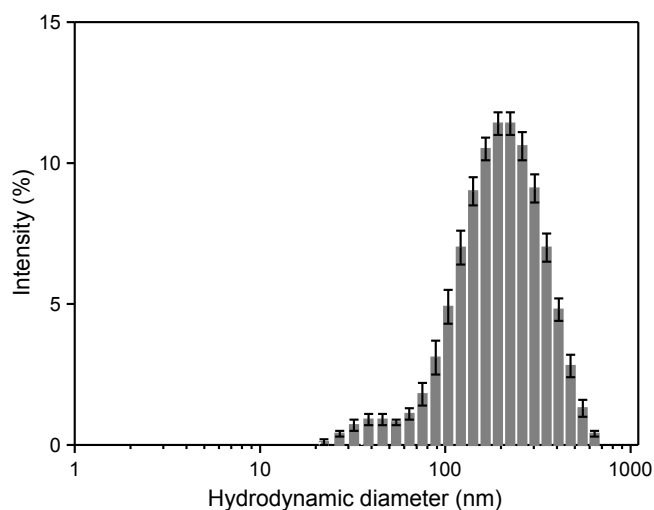


Fig. 4. Size distribution of natural nanoparticles in the granitic GW was investigated by DLS after $100 \times$ pre-concentration. 50 mL of the GW sample was concentrated to 0.5 mL using ultrafiltration before measurement.

Smaller nanoparticles than the pore size of the membrane are lost during the concentration process that is similar with the AF4-LWCC results. It cannot be excluded that natural nanoparticles adsorb on the filter membrane and aggregate during centrifugation. A portion of nanoparticles can be lost for recovering the concentrated solute. In

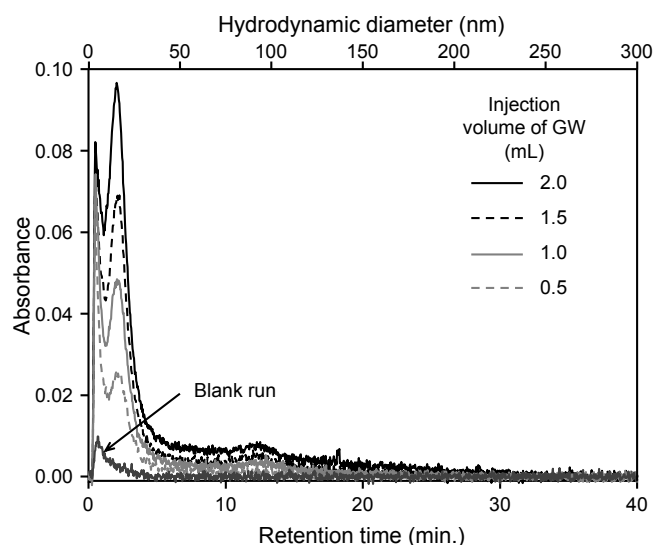


Fig. 5. AF4 fractograms of natural nanoparticles in the granitic GW were obtained using a 100-cm LWCC. The injected sample volume was varied from 0.5 to 2.0 mL and concentrated using the on-line concentration method prior to separation.

addition, the detection sensitivity of DLS measurement rapidly decreases as the particle size decreases below 100 nm due to the fact that the scattering intensity is proportional to the sixth power of the particle diameter (Filella et al., 1997). Thus, it cannot be confirmed whether particles smaller than 20 nm were absent or were present in the granitic GW sample in undetectable concentrations. Filella et al. reported the limitation of DLS for analysis of highly polydisperse samples such as GW because large particles which intensively scatter light, mask the presence of small ones in the sample or interfere with their size measurement. This emphasizes the necessity of fractionation prior to DLS measurement of natural samples (Filella et al., 1997).

Fig. 5 shows AF4-LWCC fractograms of the GW sample obtained with the sample injection volume varied from 2.0 (the same fractogram shown in Fig. 3 b) to 0.5 mL by the on-line concentration method (Lee et al., 2015). The upper x-axis shows the hydrodynamic diameter calculated by Eq. (1). As the injection volume decreased, the signal intensity decreased, and the signal for the second population eventually disappeared. In the GW sample investigated in this study, the size distribution of the natural nanoparticles exhibit a bimodal distribution with the main population being < 50 nm. This size is difficult to detect using either a commercial UV/Vis detector or a DLS combined with ultrafiltration. The AF4-LWCC system proposed in this study may be useful for determination of the size distribution of natural nanoparticles in low concentrations, which show a broad distribution of sizes.

4. Conclusions

The AF4-LWCC system has been successfully applied to improve detection sensitivity and analyze accurately the size of natural nanoparticles in trace-level concentrations. The detection sensitivity using flow-through cells with long OPLs improved without significant change in the retention time and peak broadening. The coupling of AF4 with LWCC provides enhanced sensitivity for detecting colloidal particles down to ppb level ($\mu\text{g L}^{-1}$) for AgNPs and natural nanoparticles in granitic GW. This AF4-LWCC system is suitable for the particle size determination of multi-modal samples and trace amounts of nanoparticles without an additional pre-concentration process. The newly proposed system is expected to be a promising tool for the detection of various types of nanoparticles, including polymers and proteins, as well as inorganic and organic colloids, at dilute concentrations.

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