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Detection of Nanocolloids with Flow-Field Flow Fractionation and Laser-Induced Breakdown Detection

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A new nanosize colloid detection method comprised of flow-field flow fractionation (FFFF) with laser-induced breakdown detection (LIBD) is presented, which is capable of characterizing the colloid size distribution as well as determining the number density of each size fraction in very low concentrations. The method facilitates the detection of aquatic colloids particularly in the lower range of nanometer size (<50 nm) with the sensitivity much higher than a laser light-scattering method (LLS), i.e., the lower ppb range. The method is tested with a mixture of polystyrene colloids in three different nominal sizes, 19, 50, and 102 nm, and the results are compared with those of the LLS method. For colloids of 19-nm diameter, the present method demonstrates the detection sensitivity over 3 orders of magnitude better than that of the LLS method. The limitation of the detection sensitivity arises from the surface bleeding of a ceramic frit overlying the separation channel of the used FFFF instrument.

Colloids are ubiquituous in natural water in different concentrations depending on surrounding geo-matrixes[1,2] and even found in laboratory pure water owing to the surface bleeding of container materials in contact with these.[3] Aquatic colloids are known to play a carrier role for the migration of water-soluble contaminants of organic and inorganic nature,[4,5] particularly radionuclides of higher ionic charge ($Z \geq 2+$).[4] For this reason, a growing interest has developed in the detection of aquatic colloids and the appraisal of their size distribution for the environmental monitoring and the assessment of the migration behavior of hazardous contaminants, including radionuclides, in various aquifer systems.[6] In deep groundwater, aquatic colloids of either inorganic or organic composition may be chemically stable due to the extended time for equilibration with given geo-matrixes and thus can play a significant role for the subsurface migration of radionuclides.[7] Such colloids are generally found to be predominantly small in size, i.e., <50 nm. Investigations on colloid populations in natural ground and surface waters mostly show that the relationship of the particle number relative to the respective particle size usually obeys a Pareto power law. The range for the exponent values required to describe the natural size distribution is from 3 to 5,[8,9] i.e., the particle number concentration in natural waters is higher by 3 or 5 orders of magnitude when the particle diameter decreases by a factor of 10. Small colloids, therefore, dominate natural aquatic colloid particle concentrations by orders of magnitude, but unfortunately, cannot be detected easily for low concentrations. In the part per billion range or even below, the laser light scattering method (LLS) cannot be applied without preconcentration. The characterization and quantification of aquatic colloids of this size range requires a noninvasive method, or one with less perturbation, to appraise their real size distribution

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and number densities.

The present paper deals with a new approach for the size characterization of aquatic colloids and on-line quantification of their number densities. For this purpose, the commercially available flow-field flow fractionation (FFFF)[10,11] is combined with the laser-induced breakdown detection (LIBD),[12] which presently represents the most sensitive particle detection method. The intensity of the LIBD signal depends on a variety of parameters such as size, concentration, and to some extent on the elemental composition of particles, which complicates the analysis of samples containing unknown colloids of multimodal size distribution.[12,13] There are efforts underway to extract information on particle concentration and size from the measuring signal.[12] Those investigations, however, are not yet completed. The aim of this study is to obtain quantitative data for trace particle concentrations by LIBD as a function of particle size after prior size fractionation. The applicability of the method is demonstrated with a mixture of polystyrene reference colloids of three different monodispersed sizes.

EXPERIMENTAL SECTION

Principle of the Flow-Field Flow Fractionation (FFFF). The flow-field flow fractionation (FFFF) represents a separation technique with no stationary phase required as, e.g., in case of size-exclusion chromatography. Thus, a lesser extent of effects of interaction of sample components with equipment surfaces is expected. The separation of analytes in FFFF is achieved in a laminar carrier flow under the action of an applied flow field, consisting of a flow perpendicular to the carrier flow.[10,11] The position of individual colloidal species in the laminar carrier profile corresponds to their diffusion coefficient. FFFF allows the determination of the diffusion coefficient of analytes and, thus, according to the Stokes equation, the hydrodynamic colloid diameter is accessible without separate calibration. The relation of the retardation volume V_R to the hydrodynamic diameter and the diffusion coefficent is given by the following equation where η is

$$V_R = \frac{vw^2}{6D} = \frac{\pi\eta w^2 v_c}{kT}\frac{d}{2} \qquad (1)$$

the medium viscosity, w the channel width, v_c the volumetric cross flow rate, d the hydrodynamic diameter of colloidal species, k the Boltzmann constant, T the absolute temperature, and D the diffusion coefficient.

Procedure. A solution of 0.01% Tween 20 (Polyethoxysorbitanlaurate, Merck, Darmstadt, Germany) at ionic strength of 10^{-3} mol/L ($NaClO_4$) is applied as a carrier. Channel- and cross-flow rates are maintained at 1 mL/min throughout this work. Solutions of latex standards (Polysciences, Mainz, Germany) in ultrapure water are first shaken in an ultrasonic bath for about 10 min and then filtered through a 450-nm syringe filter before injecting (injected sample volume, 20 μl). A stop-flow procedure is used for sample relaxation. The sample is injected into the top of the FFF channel, and only the cross-flow with no channel flow is applied to the sample for 2 min. During this period the individual species spread into zones of a specific thickness which is determined by their diffusion coefficient and hydrodynamic diameter and the applied cross-flow field. Following this step, the channel flow is delivered and the fractionation begins.

Instrumentation. The fractionator used is a model F-1000 from FFFractionation, Inc. (Salt Lake City, USA). The lower frit of the channel (27.7-cm "effective" length, 2.0-cm width) is covered with a membrane consisting of regenerated cellulose with C. O. 5 kD from Schleicher & Schuell (Dassel, Germany). The carrier is degassed by an 1100 Series Vacuum Degasser model G 1322A and delivered at constant rates to the fractionator by a 1100 HPLC Iso-pump model G 1310A from Hewlett-Packard (Waldbronn, Germany). From the channel, the effluent is directed through an LLS detector and to the flow-through cell of LIBD. The cross-flow is provided by a double piston precision pump P-500 from Pharmacia Biotech AB (Sweden) in a recirculating cross-flow loop.

Laser Light-Scattering Detection (LLS). LLS detection is performed by a commercial DAWN-DSP-F light-scattering photometer from Wyatt Technology Corp. (Santa Barbara, USA). A 5 mW HeNe laser provides the incident light beam ($\lambda_{em.} = 632$ nm) and is directed through the detector cell of 70-μl volume. Scattered light is detected by an array of 15 photodiodes arranged at different angles relative to the incoming laser beam. Only the signal detected by the 90° detector is taken for the present study.

Laser-Induced Breakdown Detection (LIBD). The measuring principle of the LIBD is described in detail elsewhere.[12,13,16] Roughly, it is based on the plasma generation on colloids in the focal volume of a focused, pulsed laser beam. The needed power density to induce the breakdown of the dielectric properties of a medium, resulting in plasma formation, is significantly lower for solid matter than for liquids. Therefore, the laser energy is adjusted to a value so that in the focal volume the power density is higher than the threshold for inducing a breakdown in the solid matter but lower than that required for one in the solvent. Under these conditions, breakdown events in colloidal dispersions are selectively induced by colloids. The signal that is primarily obtained is the breakdown probability, i.e., the number of breakdown events per laser shot, which depends on both particle concentration and diameter. In principle, there are several methods described in the literature for obtaining information on both parameters independently. Size information can be derived from the time and spatial distribution or intensity of individual breakdown events.[14,15] Analyzing the dependency of the breakdown probability on the energy of the excitation laser pulse has also been found to provide size information.[12] At low laser pulse energy, only large particles generate breakdown events. Upon increasing the laser energy, smaller particles are also counted. A more recently reported approach is based on the observed dependency of particle diameter and length of the breakdown ignition along the laser beam axis, which turns out to be

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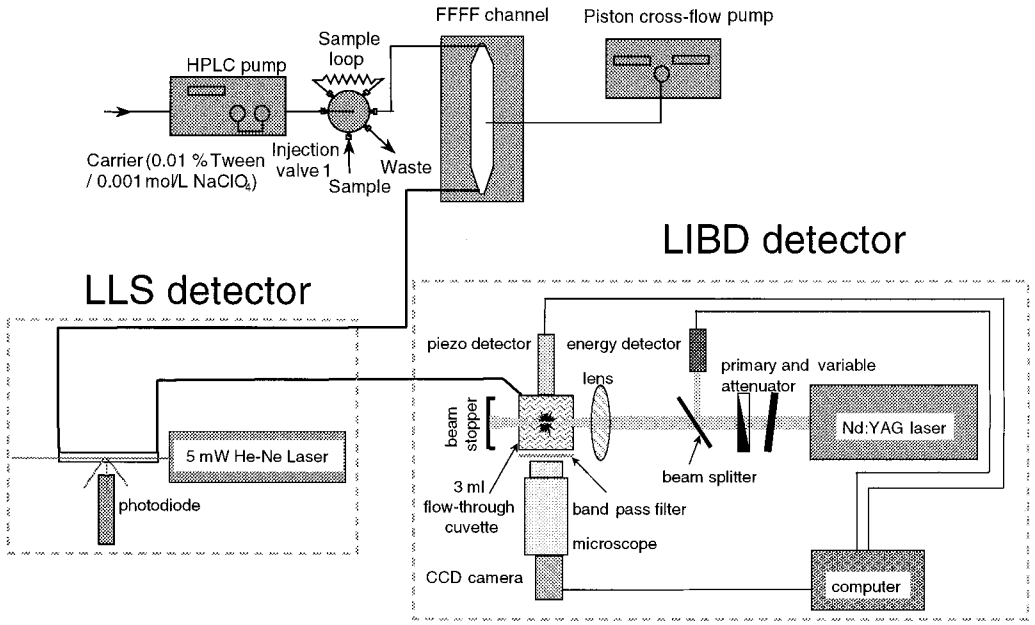

Figure 1. Schematic plot of the FFFF-LLS-LIBD arrangement.

independent of the particle concentration.[16] After a size calibration using particle standards with certified diameter, an average particle size can be attributed to a specific sample. Then, the colloid concentration is obtained for particles of a given size from a concentration calibration. All of these methods, presently, are highly affected by instrumental constraints, e.g., instability of the laser or a nonideal laser beam profile, or provide only average particle sizes instead of size distributions.

The experimental setup (Figure 1) is based on a pulsed Nd:YAG laser (Continuum; Surelite I) with frequency-doubled wavelength at $\lambda_{em.} = 532$ nm. Moderated by a variable attenuator, the laser beam is focused by one plano convex lens of 40-mm focal length into the sample dispersion placed in a rectangular flow-through cell with a 3-mL volume. Energy of incident laser pulse is monitored by a pyroelectric energy detector. The breakdown-induced acoustic wave is measured by a piezo electric detector (PZT). In additional to the acoustic wave, the plasma light emission is detected by a Macro-Microscope combined with a triggered Charge-Coupled-Device (CCD) Camera.

For measurement of the effluent coming from the FFFF, the laser runs constantly at a repetition rate of 20 Hz. The laser pulse energy, piezo electric signal, and images of the light emission of all plasma events are digitized and computed by a PC. Processing of the data from the CCD camera goes via a frame grabber card by way of a special software. After the measurement for every 300 laser pulses (15 s), an average breakdown probability is calculated. The results are plotted versus the elution volume (1 mL/min). The resulting curves show the elution peaks of polystyrene colloids and can be compared with the peaks found by LLS measurements.

RESULTS AND DISCUSSION

The FFFF arrangement with LLS and LIBD detectors in sequence is shown schematically in Figure 1. Both detector cells are connected via PEEK tubings of 0.508-mm diameter in order to reduce dead volumes. Fractograms of a mixture of polystyrene particles of 19, 50, and 102 nm are shown in Figure 2. The particle concentration is varied, and fractograms for both detectors are compared directly. FFFF parameters are adjusted so that the 19-nm particles appear at an effluent volume of about 5.1 mL, which is well separated from the void volume corresponding to the dead volume of the FFFF channel. Under the given conditions, the particles can be separated at constant flow rates (channel- and cross-flow: 1 mL/min).

According to eq 1, it is possible to calculate the average size of given particles directly from the retardation volume, once the channel width is known. Depending on the type of an inserted ultrafiltration membrane, the channel width can vary from the supplier's nominal value, which is then determined experimentally by injecting NaN_3 (20 μl 0.01% in ultrapure water). The eluting peak is detected by using a UV−vis detector ($\lambda = 254$ nm) at the identical channel flow rate (1 mL/min) but at a cross-flow rate of zero. From the known channel dimensions, a real channel width of 203 ± 0.7 μm is calculated from the experimental channel volume (1.112 ± 0.004 mL), which is found to be somewhat smaller than the supplier's nominal value (channel width = 254 μm). Using eq 1, particle diameters are then calculated from the retardation volume ($T = 298.15$ K, $\eta = 0.0089$ g cm^{-1} s^{-1}). Resulting diameters listed in Table 1 are in good agreement with the supplier's data.

A comparison of the full width at half-maximum for the two detectors (Table 1) shows that the peaks are broader in the case of using LIBD as the detector. This is due to the use of a 3-mL flow-through cuvette for the LIBD detection which causes a partial mixing of the eluted sample zones, resulting in a peak broadening as compared with the LLS signals. On the other hand, the LLS detector cell has a volume of 70 μl and the effective scattering volume amounts to only 0.5 μl. Nevertheless, LIBD peaks for the polystyrene standards are resolved. Only for the two smaller particle sizes a baseline separation is not achieved. The surprisingly low peak broadening might be due to the fact that (1) the

Table 1. Particle Diameters Determined from Elution Peak Maxima as Compared with Supplier's Nominal Values and Peak Widths at Half Maximum for the Corresponding FFFF-LLS and FFFF-LIBD Fractograms for Individual Particle Sizes

particle diameter (nominal) (nm)	19 ± 1.5	50 ± 2.0	102 ± 3
particle diameter determined by FFFF-LIBD (nm)	19.9 ± 0.6	49.5 ± 1.9	90.5 ± 1.5
FWHM (LLS) (mL)	2.5	3.0	3.9
FWHM (LIBD) (mL)	3.6	4.6	5.1

laminar flow through the LIBD cuvette remains rather undisturbed and (2) the real detection volume in the case of LIBD is restricted to the effective focus volume of 10^{-6}–10^{-4} μl which is determined by the instrumental parameters such as focal length of lenses, laser beam geometry, and size of colloids etc.[12] A flow-through cell of smaller volume will be further tested in order to improve the fractogram resolution.

From the fractograms obtained for different colloid concentrations, a large difference in the detector sensitivity becomes obvious. For the lowest concentration of particle mixture, the 19- and 50-nm particles cannot be detected any more by LLS, while at the highest concentration the LIBD signal is at saturation. As mentioned above, the measuring signal of the LIBD consists of a breakdown probability. As the breakdown probability increases, the linear relationship of the LIBD signal to the colloid concentration is no longer fulfilled, and an asymptotic approach to 100% is observed.[16] This behavior is responsible for the flattening of peaks observed in the fractogram at higher particle concentrations as reflected in Figure 3 where peak heights are plotted versus particle concentrations. In each case, a nonlinear dependency is observed. In Figures 2 and 3 only net signals are plotted after subtracting

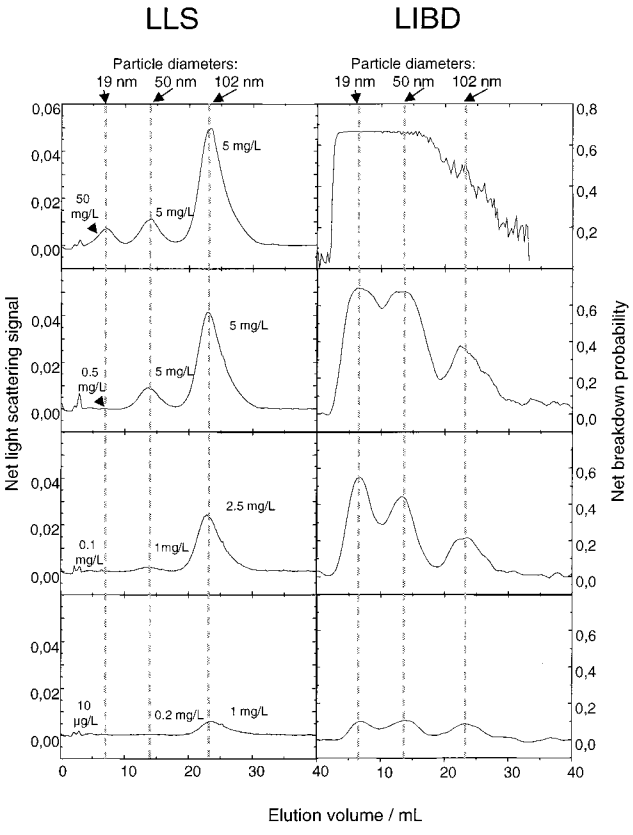

Figure 2. Fractograms obtained for a mixture of polystyrene standard colloids of 19-, 50-, and 102-nm diameter (nominal sizes).

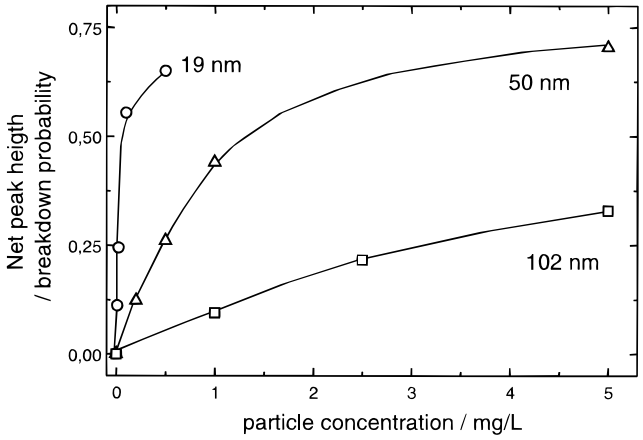

Figure 3. Plot of the LIBD signal versus particle concentration.

background signals. The background LIBD signal for the carrier solution is quite high, due to particle impurities from the carrier solution itself and surface bleeding from the channel, and amounts to a breakdown probability of about 0.3. The LIBD saturation in Figures 2 and 3, therefore, is attained at the values of about 0.7 for the breakdown probability. However, the LIBD parameters can be adjusted for the determination of higher particle concentrations by decreasing the laser-pulse energy.

From Figures 2 and 3, it becomes noticeable that the sensitivity for both detectors clearly depends on the particle size. According to the theory[17] and provided that the particle diameter $d \leq \lambda/10$ (λ, laser-light wavelength), the light intensity due to Rayleigh scattering is proportional to d^6. LLS fractograms reflect the relationship between light-scattering intensity and particle size. Consequently, the LLS detection becomes quite insensitive for small particles. For the LIBD, the relation between breakdown probability and particle size is determined by the probability of the particle being present in the focus of the laser beam[16] and the breakdown probability is found to be approximately proportional to d^1.

An estimation of the limits of detection (LOD) for the three particle sizes is made by calculating the 3-fold standard deviation (3σ) of the corresponding background noise. The LOD values for both detectors are given in Table 2. The dependency of LOD on particle diameter is plotted in Figure 4 for the FFFF-LIBD and FFFF-LLS. Data are compared with those reported in the literature for direct LIBD,[16] for the flow-through-LLS detector without prior fractionation, and data published recently in a review article for the photon correlation spectroscopy (PCS).[18] LOD values for the PCS method obtained with a commercial apparatus equipped with a 1 W Ar laser operated at $\lambda = 488$ nm are more than 1 order of

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Table 2. Limits of Detection for the FFFF-LLS and FFFF-LIBD Detection of Nanocolloids Determined from 3σ of the Background

particle diameter (nm)	19	32	41	50	102
LLS (mg/L)	5.3	1.9	1.3	0.54	0.05
LIBD	2 μg/L	8.4 μg/L	14 μg/L	0.04 mg/L	0.24 mg/L

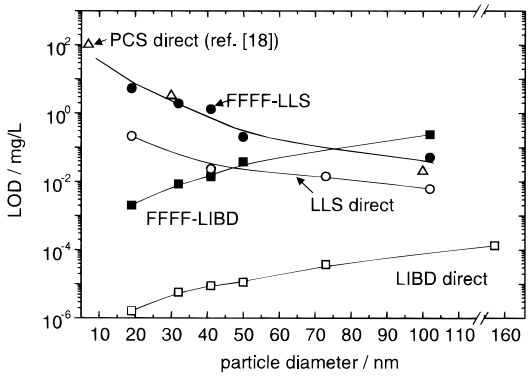

Figure 4. Limits of detection for different colloid sizes determined for FFFF-LIBD and FFFF-LLS determination compared with direct LLS and direct LIBD measurements; (values for the direct LIBD measurements are taken from ref 16[16]).

magnitude higher than found in this work for the direct injection into the flow-through-LLS detector. It might be possible to improve LLS sensitivity by using an optimized research goniometer and analyzing the scattered light at a more appropriate angle than 90°. However, scattered light becomes isotropic for particles with diameters less than 1/10 of the incident light wavelength. The detection of scattered light at angles different than 90° provides no advantage for the nanosized colloids with diameters < 100 nm investigated in this work.

Because of the different dependency of LIBD and LLS signals on the particle diameter, the breakdown detection of the FFFF effluent becomes more sensitive for particles <70 nm. The difference in sensitivity for the smallest investigated particles amounts to more than 3 orders of magnitude. LOD values for direct LIBD are 3 orders of magnitude superior to those observed for FFFF-LIBD. Three reasons are responsible for that: (1) For the FFFF separation a quite high sample dilution is made. 20 μl of the injected sample are eluted into ~10 mL, corresponding to a dilution factor of 500. The decrease of the LOD values by a factor of ~10 for the LLS, in the case of injecting the sample directly into the detector cell, is due to the dilution effect by dispersion during fractionation. (2) There is a quite high continuous background signal observed by LIBD partially induced by particle impurities in the carrier. It is assumed that the Tween used as detergent is not pure enough. Additionally, it was found in preliminary experiments that, with increasing cross-flow rate and increasing pH of the carrier solvent, the background particles generated by the FFFF channel are increased. ICP mass spectrometric analysis shows a direct correlation to the Al and Si concentration in the effluent. Therefore, it appears certain that a continuous leaching of the ceramic frit overlying the separation channel by the cross-flow is responsible for the background and, thus, for limiting the sensitivity of LIBD detection. The impurity colloids are small-sized and, thus, contribute much more to the LIBD than to the LLS background, due to the pronounced relationship of particle diameter and scattering light intensity. (3) The on-line LIBD measurement suffers from the relatively small number of laser shots used to calculate a breakdown probability. For the LOD determination of direct LIBD, 3000 laser shots are accumulated and, consequently, sensitivity is further improved by the better statistics.

CONCLUSIONS AND OUTLOOK

The on-line coupling of FFFF with LIBD provides a very sensitive method for the determination of colloidal species and their size distribution down to the microgram per liter range, for spherical particles without prior size calibration. For small-sized particles < 70 nm, the LIBD detection is much more sensitive than the LLS detection. A further increase of sensitivity can be possible for the detection of colloids in the part per trillion [nanogram per liter] range as being attained by the direct LIBD analysis. The outcome of the present study shows that an important limitation regarding the sensitivity of the FFFF-LIBD method is due to the high dilution and the colloid bleeding of channel components. In the meantime, equipment for the asymmetric FFFF are commercially available, which might be able to overcome both limitations. Asymmetric FFFF channels do not contain a ceramic frit any more. It is replaced by a glass or plexiglass plate which is expected to be a much smaller source of colloids. Furthermore, the asymmetric FFFF allows the inclusion of a preconcentration step prior to the particle fractionation much more easily than is possible in the case of symmetric FFFF.[19] It is expected that the combination of LIBD detection with the asymmetric FFFF may provide a further clear increase in sensitivity.

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