Critical aspects of sample handling for direct nanoparticle analysis and analytical challenges using asymmetric field flow fractionation in a multi-detector approach

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The analysis of engineered nanomaterials (ENMs), especially engineered nanoparticles (ENPs), is a fast growing analytical research field. New trends in plasma spectrometry such as direct single particle inductively coupled plasma mass spectrometry (spICPMS) or the coupling of asymmetric flow field flow fractionation to ICPMS (A4F-ICPMS) allow direct analysis of ENPs by getting not only chemical but also size information simultaneously. However, sample handling of nanoparticles could be challenging and change the ENP properties. For most of the analysing techniques, dilution of ENP samples is needed as minimum sample preparation. The colloidal stability and the agglomeration behaviour depend on the ENP-type, the coating or functionalization agent and on the surrounding media. The stability of charge stabilized ENPs is especially sensitive to changes of pH or ionic strength, and sometimes even to dilution. Although the stability of sterically stabilized ENPs is typically less affected by the above-mentioned factors, agglomeration can still occur in certain environments. Thus, storage, handling and sample preparation are big challenges in ENP analysis. Kinetic studies of different ENPs, representative of typical nanoparticle types and coatings, point out that the behaviour is dependent on various influencing factors pertaining to the chemical environment (pH, ionic strength, dilution). In this study polyvinyl alcohol (Ag@PVA) and citrate (Ag@citrate) stabilized silver nanoparticles, as well as titanium oxide ENPs functionalized with polyacrylate (TiO2@PA) have been studied. A simple analytical approach using batch dynamic light scattering (DLS) is proposed for a fast assessment of samples containing unknown ENP types or structures. Besides aspects of sample handling, unwanted particle–membrane interactions, which often lead to inappropriate recovery rates in A4F fractionation, are investigated here. These interactions are caused by the electrostatic charges carried by different membrane materials and the resulting interaction with the ENPs’ charge. This is critically discussed for membrane materials typical for A4F analysis: polyethersulfone (PES), regenerated cellulose (RC), and polyvinylidene difluoride (PVDF).

Introduction

The interest in engineered nanomaterials (ENMs) increased constantly during the past decade. Nanomaterials exhibit rich physical and chemical phenomena, and their fascinating and unusual properties have opened up a myriad of applications in industry, medicine and many other fields and several products based on nanotechnology have already been introduced to the market. This is also visible in the increasing number of patents and publications related to nanotechnology. Several studies have shown that products containing ENM can release particles, some of them in the nano-range. Hence, powerful and sensitive analysing techniques are required to characterize nanoobjects such as engineered nanoparticles (ENPs) originating from ENM synthesis or application studies and for the investigation of safety aspects related to nanotechnology. Size information and chemical composition are probably the most frequently required parameters, but also surface functionality and shape are quite important as they significantly influence the
behaviour of ENPs. Several well established analytical techniques are available, among which electron microscopy and light scattering are predominately applied for size determination. Typical techniques and their application range are summarized in the review article of Hassellöv et al. For chemical characterization of nanoparticles, plasma spectrometry is one of the most powerful techniques. Krystek et al. recently reviewed the application of plasma spectrometry for the analysis of ENPs in suspensions and consumer products. The applicability of straightforward analytical methods such as sequential filtration or centrifugation, but also lately, more sophisticated analytical trends are discussed.

Techniques which allow direct online analysis of ENPs, by obtaining several parameters (e.g. size and chemical information) simultaneously, are preferred and therefore of growing interest in analytical research. Direct single particle mode analysis with inductively coupled plasma mass spectrometry (spICPMS) as well as the coupling of asymmetric flow field flow fractionation with inductively coupled plasma mass spectrometry (A4F-ICPMS) are currently the two most discussed innovation trends for nanoparticle analysis in the literature. A more detailed comparison of prospects and limitations of these two techniques are outlined below.

However, nanoparticle analysis in general can be challenging. Besides storage conditions (e.g. temperature or UV light), handling and sample preparation of ENPs might be critical. Changes in the chemical environment (e.g. dilution, or addition of surfactants) occurring during sample preparation or analysis can result in significant changes of the samples. Nanoparticle stability and agglomeration behaviour strongly depend on the particle type and structure, the chemical composition as well as the coating or functionalization. For example silver or gold nanoparticles can be synthesized in a bottom-up or top-down approach with a large number of different coatings/functionalization. These coatings or functionalization can range from inorganic components like sodium bicarbonate as a capping agent, or covalently bonded SiO₂, to organic coatings like citrate, polyvinylpyrrolidone (PVP) or polysaccharide. Composites are also possible. The bonding mechanisms between the coating/functionalization agents and the particle cores are yet not fully understood. However, they can be mainly divided into covalent and non-covalent. While non-covalently bound coatings/functionalizations are more likely to desorb and detach from the surface for example upon dilution, covalently bound coatings can provide better stabilization for a broad range of conditions. Irrespective of the bonding mechanism between the coating/functionalization agent and particle core, two types of stabilization mechanisms are distinguished, charge and steric stabilization. Fig. 1 displays examples for possible structures of various silver nanoparticles with examples for possible coatings/functionalization. Steric stabilizations for nanoparticles are often assumed to be relatively unsusceptible against changes in the chemical environment, whereas charge stabilized particles are especially sensitive to changes, e.g., of ionic strength or pH. However, also sterically stabilizing polymer coatings like polyvinyl alcohol (PVA) can show sensitivity to changes in specific the chemical environment due to chemical reactions like shifts in the cross-linking rate of the coating or functionalization components by hydrolysis equilibrium reactions.

Even a dilution might cause a size change due to variations of the concentration ratio of coating or functionalization compounds bound to the core. Moreover, dilution can accelerate the dissolution of nanoparticles which could lead to a larger ionic fraction in the dissolved suspension. The differentiation between ionic and particulate fractions is still a challenging task. Determination of the total fraction by bulk analysis and the ionic fraction by e.g. ion-sensitive electrodes or ultracentrifugation with subsequent analysis are proposed as suitable methods. The particulate fraction can be calculated by subtraction of the ionic fraction from the total amount. However, ion-sensitive

![Fig. 1 Possible silver nanoparticle structures with different coatings/functionalization as well as composite materials.](image-url)
electrodes show only moderate sensitivity with detection limits around 0.1 to 0.01 mg L\(^{-1}\). Techniques like ultrafiltration or ultracentrifugation methods are time consuming and costly, but these two-step methods provide better detection limits. Nevertheless, a centrifugation force which ensures a removal of nanoparticles down to about 1 nm might still not always ensure that the supernatant contains only ionic forms. Additionally, dissolved small metal complexes might also be present.

Besides composition, also structural details can influence the dissolution behaviour. Even ceramic nano-composite particles, which are usually supposed to be chemically stable, can show a preferential leaching. Metal doped titanium oxide-based nanoparticles for example can form completely different structures, depending on the doping metal and the way of synthesis. Elements like chromium or tungsten are often embedded in the TiO\(_2\) lattice replacing Ti,\(^{47-49}\) when the nanoparticles are synthesized by flame synthesis, whereas elements like silver or platinum appear as discrete knobs on the top of the TiO\(_2\) particles\(^ {50-52}\) and might be preferentially released in contact with water.

Next to sample preparation, also the analytical setup itself can influence analysis of nanoparticles. Hence, a basic knowledge about particle chemistry and possible behaviour of nanoparticles is essential to avoid false measurements caused by either sample handling or a non-optimum analytical setup. Since this knowledge is often not directly accessible, a fast and straightforward method to determine the influence of solution changes, e.g. on the agglomeration behaviour of nanoparticle samples based on kinetic studies with batch dynamic light scattering (DLS), is proposed. Three nanoparticle dispersions, representative of typical types of nanoparticle and coating or functionalization have been investigated: polyvinyl alcohol (Ag@PVA) and citrate (Ag@citrate) stabilized silver ENPs and titanium oxide ENPs with poly-acrylate (TiO\(_2\)@PA). Kinetic studies of these differently coated or functionalized ENPs were performed using batch analysis with dynamic light scattering (DLS) to demonstrate the dependency e.g. of agglomeration on various influencing factors of the chemical environment such as dilution, or the change of pH, or ionic strength. This straightforward analytical approach allows a fast assessment of the behaviour of unknown ENP types or structures.

The second part of this study focuses on the investigation of unspecific nanoparticle–membrane interactions which can have a negative influence on the nanoparticle fractionation efficiency and the recovery rates due to unspecific deposition on the membrane’s surface in the field flow fractionation channel. Inadequate recovery rates of A4F for the analysis of nanoparticles were reported in previous studies,\(^ {18,32,40}\) but the causes are poorly investigated. A possible explanation for unspecific nanoparticle–membrane interactions based on zeta (ζ) potential investigations is being proposed. The investigation considers typically used A4F membrane materials as well as nanoparticle and coating or functionalization types to provide information for qualitative prediction of unwanted interactions and possible deposits of nanoparticles in the A4F channel.

**Prospects and limitations of spICPMS and A4F-ICPMS**

Degueldre et al. introduced spICPMS for size distribution determinations of Zr, Th and Au nanoparticles in colloidal suspensions.\(^ {20-21}\) Meanwhile the technique has been improved, e.g., by using micro-droplet sample introduction devices,\(^ {24}\) to achieve better detection limits. The principle of spICPMS is based on transient signal spike counting statistics of highly diluted samples which requires a fast data acquisition.\(^ {1}\) The major advantage of spICPMS is the relatively low instrumental effort, which makes the technique easily accessible in each ICPMS laboratory. However, spICPMS analyses are limited by the time resolution of the mass spectrometer used. Most of today’s commercially available ICPMS instruments are based on sequential mass filters which allow only single element analysis. Measurements of two or more isotopes during the very short transient signal events require much faster signal acquisition, e.g., by using instruments with continuous simultaneous mass analysers\(^ {53}\) like the newest development of a magnetic sector field mass spectrometer with a CCD array detector approach or a fast time-of-flight analyser.\(^ {1}\) Another critical point in spICPMS is the number of measurements required to achieve an appropriate counting statistic of the highly diluted samples which are representative of the nanoparticle size distribution. Moreover, depending on the element the minimum detectable nanoparticle size in spICPMS mode ranges from 20 to 30 nm. The minimum detectable size limit is given by the relatively small number of atoms present in a single nanoparticle and the minimum element mass required to achieve ICPMS signals from the single particle event distinguishable from the background.\(^ {54,55}\) The upper size for direct particle analysis in ICPMS is mainly given by the sample introduction system, especially by the cut-off of the used spray chamber ranging typically between 1.5 and 2 μm, whereas the plasma power is usually sufficient to completely ionize even micrometre particles introduced as slurries.\(^ {1}\)

Field flow fractionation is more flexible in terms of the accessible lower size range down to about 1 nm. The injected sample volumes of typically 20 to 100 μL per run amount to a representative number of nanoparticles for each analysis. For example an injection volume of 50 μL of a 1 to 10 mg L\(^{-1}\) concentrated nanoparticle suspension amounts to 10\(^{14}\) to 10\(^{10}\) particles (1–100 nm) per replicate. For comparison it should be mentioned, that e.g. for image analysis in electron microscopy analysis typically only 150 to 2000 nanoparticles are counted for size determination. This nanoparticle fractionation allows exploiting also the multi-element capabilities of ICPMS. A further advantage of A4F is the possibility to couple the separation channel with several detectors in-line. Some recently published studies investigated the possibilities of A4F in a multi-detector approach coupled to UV/Vis, light scattering and ICPMS for size fractionation chemical analysis of gold and silver nanoparticles.\(^ {18,32,33,40}\) The flexibility to couple several different detectors and collect size-separated fractions via a fraction collector opens up a lot of new prospects including the possibility to achieve not only information on size and the chemical composition of the nanoparticle core material but also information about the coating or functionalization e.g. via ICPMS or a fluorescence detector. However, highly disadvantageous for the A4F fractionation principle are the unwanted losses of nanoparticles in the separation channel. The mechanism is still not fully explained, but unspecific particle–membrane interactions due to repulsion or attraction forces between charged particles and membrane surface are most likely. Table 1 gives an overview of the two methods.
Advantages

- Time for analysis: 10 min per run
- Size resolution: 10 nm
- Applicable size range: 20 nm to 8011, 8012, and 8013 nm

Disadvantages

- Usually only single element mode due to counting statistic
- Off-line (subsequent size analysis require time consuming calculation)
- Representative size analysis requires multiple runs to achieve appropriate statistics
- Required dilution might change particle properties (e.g. due to dissolution and change of size)
- Two-particle events cannot be distinguished from single-particle events
- Particle–membrane interactions due to different charges and resulting retention time shifts
- Impact on size calibration when size certified standard reference particles are used for size calibration
- Unpredictable particle losses due to depositions of particles on the membrane especially in the focus area and during the first injections after membrane exchange
- Dilution during size fractionation in the A4F channel might change particle properties (e.g. due to dissolution and change of size)

Table 1: Comparison of spICPMS versus A4F-ICPMS/MDA

<table>
<thead>
<tr>
<th>Analytical Method</th>
<th>spICPMS</th>
<th>A4F-ICPMS/MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallest detectable size in nm</td>
<td>ca. 20 nm, 24, 31</td>
<td>ca. 1 nm</td>
</tr>
<tr>
<td>Applicable size range</td>
<td>20 nm to ca. 1.5 μm</td>
<td>ca. 1 nm to 1 μm</td>
</tr>
<tr>
<td>Size resolution</td>
<td>10 nm</td>
<td>10 nm</td>
</tr>
<tr>
<td>Time for analysis</td>
<td>10 min per run</td>
<td>20–45 min per run</td>
</tr>
<tr>
<td>Advantages</td>
<td>Size and elemental information, Low additional costs, Fast analysing time per run</td>
<td>Size and elemental information, Multi-element mode, Online approach, Highly flexible due to possibility of coupling different detectors (e.g., UV, FLD, MALLS, DLS, RI, and ICPMS) in line</td>
</tr>
<tr>
<td></td>
<td>Flexible size range</td>
<td>Sampling via fraction collector for other analysis like electron microscopy, etc.</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Usually only single element mode due to counting statistic</td>
<td>Particle–membrane interactions due to different charges and resulting retention time shifts</td>
</tr>
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<td></td>
<td>Required dilution might change particle properties (e.g. due to dissolution and change of size)</td>
<td>Sometimes poor recovery rates</td>
</tr>
<tr>
<td></td>
<td>Two-particle events cannot be distinguished from single-particle events</td>
<td>Dilution during size fractionation in the A4F channel might change particle properties (e.g. due to dissolution and change of size)</td>
</tr>
<tr>
<td>Application for ENP types</td>
<td>Th, Zr, U, Au, Ag</td>
<td>Ag, Au, poly styrene</td>
</tr>
<tr>
<td>Other applications</td>
<td>Aquatic colloids, polymers, proteins</td>
<td>Macro-molecules, e.g. polymers, proteins</td>
</tr>
</tbody>
</table>

Experimental

Chemicals

Nitrile acid (65% w/w), hydrochloric acid (30% w/w) and 2-propanol were purchased in suprapur quality from Merck (Merck GmbH), sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium bromide (DTAB) from Sigma Aldrich (Sigma Aldrich Chemie GmbH). A Millipore, Milili-Q A-10 water unit (Millipore AG) was used for the production of 18 MΩ cm⁻¹ deionised water (DI-water). All solutions used as carrier for the A4F system were filtered prior to use through a 0.1 μm filter cartridge (PALL, Filtron) and degassed in an ultrasonic bath.

ICPMS standards were prepared from single element standards in ICP quality from Merck (Merck GmbH). For A4F method validation, certified reference material Au particles purchased from National Institute of Standards and Technology (NIST SRM® 8011, 8012 and 8013) were used. After opening, the standards were transferred to nitrogen flushed and pre-cleaned (ethanol p.a.) PE-LD vessels and then stored in the dark. Before measurement, the standards were always freshly prepared, diluting from the stock with carrier solution directly in the 2 mL HPLC (PE-LD) vials. Details concerning synthesis and characterization of Au nanoparticles can be found elsewhere, as well as details on the obtained size and concentration levels.

The silver nanoparticles with different coatings were provided by NatoSys GmbH – Fluids and Consulting, Switzerland. The titanium oxide nanoparticles originate from the European Research Project NanoHouse NMP-2009-1.3-1 & ENV.2009.3.1.3.2; Collaborative project no. 247810 (ref. 54) which focuses on the investigation of the release, environmental fate and toxicity of nanoparticles from paint products.

Batch DLS kinetic studies and ζ-potential for particles and A4F membranes

The influence of dilution, pH and ionic strength on the change in size distribution in a batch dynamic light scattering approach as well as the zeta (ζ) potentials for the above described titanium oxide and silver nanoparticles were monitored using a Zetasizer Nano ZS (Malvern Instruments Ltd., Germany). All samples were filtered prior to measurement using 0.22 μm PVDF syringe filters (BGB Analytik) and tempered for about 5 minutes to 20 °C in the batch cell before analysis.

The streaming potentials of typical types of membranes for the A4F have been analysed using an electro-kinetic flow-through zeta potential analyser Mütēk™ System Zeta Potential SZP-06 (BTG Instruments GmbH in Herrsching, Germany). Round membrane segments of 2 cm diameter (stationary phase) were
placed in the flow through cell of the zeta potential analyser. The
liquid phase streams through the membrane due to the applied
pulsating vacuum (mechanical force). The electric potential
(streaming potential) is measured from which the zeta potential is
calculated. Each condition was measured in multiple determina-
tions (5 times). Conductivity and pH were additionally
determined using separate sensors.

Membrane materials were provided by Wyatt Technology (RC
10 kD regenerated cellulose acetate, Alfa Laval, RC-70PP and
the PES 100 kD, Millipore, Biomax) and by GE Osmonics
(PVDF 30 kD, Sepa CF-PVDF-UF-JW). The streaming poten-
tials of these membranes were determined over a large pH range
and with different ionic strengths.

Instrumental setup: A4F-UV/Vis-LS-ICP-MS

The A4F system is composed of a separation channel and a flow
control unit (Eclipse®3) from Wyatt Technology Europe con-
ected to a metal-free HPLC system from Shimadzu. It consists of
a DGU-20A3 degasser, a LC-10Ai pump, a SIL-20AC auto-
sampler, a CBM-20A control unit and a FRC-10A fraction
collector. The A4F system was coupled directly to an UV/Vis
diode array detector (SPD-M20A, Shimadzu Germany GmbH)
and an 18 angle MALLS detector (DAWN Heleos® 2, Wyatt
Technology Corporation), which operates at a laser wavelength
of 658 nm. At an angle of 108° of the MALLS detector, a DLS
NanoStar® (Wyatt Technology Corporation) was connected via
a glass fibre cord. Two Eclipse®3 channels of different dimen-
sions had been used: the longer with 275 mm and the shorter with
145 mm length, and both of 50 mm width and with a channel
height of 350 µm given by a PEEK (polyaryletherketone) spacer.

DI-water with a pH of 6.5 was applied as carrier flow, if not
mentioned otherwise. The A4F separation consisted of 5
consecutive steps according to the description given in a previous
given paper. A4F elugrams show the separation from the pre-sepa-
ration step until beginning of the post-separation step.

For online introduction of the samples to the ICPMS (Element
2, Thermo), the flow coming from the previous detectors (first
UV/Vis, then MALLS/DLS) was split by a factor of 200. To
correct for non-spectral interferences an internal standard (flow
rate of 10 µL min⁻¹, 10 µg L⁻¹ Ir) in 10% aqua regia was added to
the remaining flow. The sample introduction system consisted of
a self-aspirating nebulizer (PFA-ST, Elemental Scientific) and
a water-cooled Scott-type quartz spray chamber. A detailed
description of the setup and the split design including operation
conditions of the peristaltic pump (Gilson, M312) and Tygon®
tubing diameters are given in ref. 18 and 32.

Data from the light scattering detector were processed with the
ASTRA software (Wyatt, Version 5.3.4.20), UV/Vis data using the
LC solution software (Shimadzu, Version 1.22 SP1), and
ICPMS transient signals were evaluated applying the Element 2
software (Thermo, Version 3.1.0.236).

Each membrane was pre-cleaned, following a 3-phase proce-
dure with a leaching, a cleaning, and an equilibration step. After
2 hours of leaching in 25% 2-propanol, the membrane was
thoroughly rinsed with DI water and equilibrated for 12 hours
with carrier solution in the A4F channel. Recovery rates for
different membranes were determined for a 30 kD PVDF- (GE
Osmonics, Sepa CF-PVDF-UF-JW), a 100 kD PES- (Millipore,
Biomax), a 10 kD regenerated cellulose acetate (Alfa Laval, RC-
70PP), and a series of ultra- and nanofiltration membranes
purchased by GE Osmonics (DESAL UF 3.5kD GK, DESAL
NF- DK, HL and 270). Recoveries for each membrane were
determined in triplicate with Au nanoparticles of different sizes
(20, 30 and 40 nm). Recovery rates were determined by a direct
flow injection analysis of particles, followed by an A4F separa-
tion and comparison of the peak areas obtained by UV/Vis for
both runs.

Results and discussion

Selection of the nanoparticles for the study

Electrostatically and sterically stabilized nanoparticles as the two
predominant ENP functionalization types are displayed in Fig. 2.
Also the most interesting parameters as well as the influence
factors of the chemical environment are summarized. Silver
nanoparticles represent a wide spread type of nanoparticles, of
which the citrate functionalization investigated here shall repre-
sent the electrostatic stabilization type and the polyvinyl alcohol
(PVA) functionalization the steric type. Whereas Ag@citrate is
easily accessible or self-synthesized, the polymeric functionalized
Ag@PVA nanoparticles allow embedding e.g. in polymer
materials proposed as dielectric elastomers. The third ENP class
investigated here is a photo-catalytic active titanium oxide with
a poly-acrylate functionalization (TiO2@PA). Whereas non-
coated or functionalized titanium particles usually form aggre-
gates, the poly-acrylate functionalization supports the homoge-
neous dispersion e.g. in paints. A dissolution of silver
nanoparticles is often discussed whereas titanium oxide
nanoparticles are supposed to remain unaffected in aqueous
environments.

Effects of dilution, pH and ionic strength

As mentioned above, changes in the environment can have
a significant impact on the analysis of nanoparticles. Dilution is
required for several analysing procedures, especially for
spICPMS. However, also during A4F-ICPMS fractionation the
sample is diluted due to the addition of channel and cross-flow.
Dilution is supposed to have an influence on electrostatic forces
between coating or functionalization agent and nanoparticle and
hence on the agglomeration state. Both silver nanoparticle types
showed a slight increase of the size when the sample was diluted,
whereas for TiO2@PA nanoparticles no significant change in size
was observed. Fig. 3 presents exemplarily the influence of dilu-
tion for the Ag@PVA nanoparticles. The small changes in size
might be caused by a change of the ratio between nanoparticle
counting and comparison of the peak areas obtained by UV/Vis for
both runs.

Effect of dilution for Ag@PVA nanoparticles

In the case of Ag@PVA, the changes due to dilution were relatively
small and in comparison to a given size resolution of about 10 nm
e.g. for A4F the differences are small. However, the significance
of possible changes for size-dependent analysis should be
assessed for each specific case. Therefore, it is recommended to
control for specific nanoparticle types, if a dilution effect occurs.
A fast screening test using batch DLS prior to time consuming
analysis (e.g. by A4F-ICPMS/MDA or spICPMS) is therefore recommended.

In A4F-ICPMS analysis it is also sometimes recommended to add a surfactant or an electrolyte (e.g. Tween, SDS, etc.) to support the fractionation. However, an ionic surfactant or electrolyte can result in a change of ionic strength which might have a significant effect on nanoparticle properties. The agglomeration of the two silver nanoparticle types was significantly influenced by addition of sodium nitrate, whereas TiO2@PA showed no significant size increase (Fig. 4). It is supposed that the addition of ions might change the electrostatic forces between coating or functionalization agent and nanoparticle core, which leads to the assumption that sterically stabilized nanoparticles are less sensitive to ion strength changes than electrostatically stabilized nanoparticles. However, specific salt types can also influence the pH of the environmental medium or even buffer the pH (e.g. phosphates).

The change of pH is also supposed to have greater impact on electrostatically stabilizing coatings/fuctionalization than on steric ones. However, beside electrostatic forces there might be also additional chemical interactions, an influence on the cross-linking or binding between polymer coating/fuctionalization components of a steric type, e.g. due to hydrolysis or other chemical reactions like decomposition.

This was also observed for the three coating/fuctionalization types investigated here. Fig. 4 displays also the influence of a pH change on the size of the nanoparticles. The graph demonstrates exemplarily that also the sterically stabilizing types like the here investigated PA and PVA can be sensitive to pH changes. The time dependent curves give also hints on the kinetics of the agglomeration behaviour. For Ag@PVA a relatively steep increase was observed up to about 150 nm after 120 minutes, whereas Ag@citrate shows stabilization at about 120 nm after ca. 60 minutes. A possible reason might be a protonation of the citrate and a change in the PVA by cross-linking e.g. due to a shift in the hydrolysis balance equation which probably needs more time (slower kinetics).

It could be assumed, that for citrate a protonation of the functionalization agent occurs, for PVA possibly also a change in cross-linking of the polymeric stabilization agent. These changes probably lead to a significant change of the bonding strength between the nanoparticle core and the stabilizing agent. Therefore, fast batch-DLS measurements as screening tests prior to more sophisticated measurements are recommended to assess possible changes during sample preparation or analysis.

It could be concluded, that batch-DLS measurements represent a fast method (ca. 5 min per measurement) to detect significant changes in size of nanoparticles, even if the detection power is limited. It has to be taken into account that DLS shows more sensitivity for larger particles than for smaller ones, and provides average sizes only, which limits the applicability especially for samples with broad or multimodal size distributions. In these cases, the average size might be overestimated. However, batch DLS could be very helpful to study the behaviour of unknown nanoparticles as well as the influence of added chemicals before more time consuming analyses are started.

Effects of surface charge

The change in the binding or electrostatic forces between coating or functionalization agent and core also influences the charge of the nanoparticles, which affects the so-called zeta potential. However, especially the charge of the nanoparticles could have an influence on the fractionation efficiency in A4F. Therefore both the charge of the membrane-material and the interactions with the particle charge were studied thoroughly here. According to the fractionation theory of Giddings who is supposed to be the principal inventor of the field flow fractionation principle as
as Wahlund, the separation of components according to their sizes is based mainly on the cross-flow in the fractionation channel and the diffusion which depends on the component size. This means that the size fractionation is only dependent on the size of the component but independent of the chemistry. However, newer studies, especially for the fractionation of nanoparticles, showed slight retention time shifts depending on the membrane material and the particle type which needs to be investigated more thoroughly. A second phenomenon observed in A4F fractionation is a sometimes significant particle loss, especially directly after replacement of the membrane. The recovery rates usually improve after several injections. An example of 6 replicates for Ag@citrate injected one after another directly after PVDF membrane replacement is displayed in Fig. 5. Even a slight shift in retention time is visible from the first to the last injection, but since the fractionation efficiency and size resolution capabilities of A4F are limited to about 10 nm for A4F, this small shift probably makes no visible influence in size determination. Anyway the particle loss due to deposition of nanoparticles especially in the focus zone area is nicely visible in particular for coloured nanoparticles such as gold nanocrystals (red). The increase of the signal and therefore also improvement of the recovery rate occurring after a few injections are probably caused by a deposition of the nanoparticles in the focus zone which later prevent further deposition of particles due to increasing repulsion forces. However, this assumption needs to be further investigated. Nevertheless, the unpredictable losses make quantification in A4F often difficult, so that alternative quantification strategies, such as batch analysis with prior ultracentrifugation procedure, are sometimes recommended.

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**Fig. 3** Influence of dilution on the size of PVA stabilized silver nanoparticles for a 1000 mg L\(^{-1}\) nAg@PVA dispersion after dilution 1 : 50, 1 : 100, 1 : 500, 1 : 1000, and 1 : 5000 measured by batch DLS.

**Fig. 4** Time-dependent influence of a change in ionic strength (adjusted with NaNO\(_3\)) and pH (with HNO\(_3\) acidified to pH 3) on the size of (a) Ag@citrate, (b) Ag@PVA, and (c) TiO\(_2\)@PA nanoparticles in comparison to the primary nanoparticle size (red line) measured by batch DLS.

**Fig. 5** A4F fractograms of 6 successive injections for Ag@citrate measured directly after PVDF membrane replacement and the increase of the peak maxima of these replicates.
However, it was recently discussed how much the different charges of particles and membrane materials influence the particle–membrane interaction, but no investigations of the A4F membrane materials were available. Therefore, selected membrane materials are studied here. Regenerated cellulose (RC) and polyethersulfone (PES) are the mainly used membrane types in A4F. For nanoparticle analysis polyvinylidene di-fluoride (PVDF) is often recommended. The zeta-potentials of the three membrane materials were determined using a flow through zeta-potential device and are displayed in Fig. 6. The curves demonstrate that a pH change results in a larger zeta potential value change for membrane materials with pH-sensitive functional groups such as OH, or COOH, whereas a PVDF membrane with covalent fluor-carbon bonds shows less sensitivity against pH change.

Fig. 7 displays the A4F fractograms of Ag@citrate with a diameter of ca. 15 nm and Ag@PVA with a diameter of ca. 12 nm using a PVDF membrane.

In Ag@citrate the first peak appears after about 9 minutes and a second peak occurs after about 25 minutes. Since the A4F elugrams cover the separation from the pre-separation step until beginning of the post-separation step, it can be assumed that the second peak might be caused by retained particles which finally elute after cross-flow stops. The Ag@PVA fractogram shows only one slightly broader peak after about 10 minutes. The kink in the peak is not completely clear, but might be caused by unspecified interaction effects between the particle and the membrane or a slight polydispersity of the sample due to longer storage time. However, according to the DLS measurements and the polydispersity index (PDI) the Ag@PVA nanoparticles were less polydisperse than the Ag@citrate nanoparticles.

The zeta potentials were determined to be −2.3 mV for Ag@PVA and −19.5 mV for Ag@citrate at neutral pH. Would the fractionation depend on size only, the retention time for the slightly smaller Ag@PVA should be a bit shorter than for the Ag@citrate, but the retention time until the peak maximum appears is at 8.99 to 9.01 minutes for Ag@citrate a bit shorter than at 10.25 to 10.26 minutes for Ag@PVA. Therefore, it is proposed that the charge of the separated particles as well as the membrane charge could have some influence on the size fractionation capabilities and retention time, especially when the particle sizes are in a similar size range with differences smaller than about 10 nm. In the following section a theory is proposed which can explain these effects.

**Assumed theory of particle–particle and particle–membrane interaction**

In general, two types of stabilization mechanism for nanoparticles can be distinguished: charge and steric stabilization (see Fig. 2), which strongly depend on the type of the capping or coating/functionalization agent used. Charge stabilization is mainly dependent on surface charge, also called ζ-potential of a particle which can be described by a double layer model described elsewhere. Attractive forces between particles are usually calculated according to the microscopic theory of Hamaker, and depend on the material which the particles are made of. On the other hand, repulsive interactions, such as steric and electrostatic interactions, depend primarily on the surface properties of the nanoparticles, which are strongly affected by the presence of coating agents. Hence silver particles with different coatings show different zeta potentials.

Also membranes show different surface charges, which depend on the material type. It can be assumed that van der Waals and electrostatic forces are responsible for the particle–membrane interactions in the aqueous environment of an A4F channel. Electrostatic forces are active between charged particles and a charged membrane surface whereas van der Waals forces as short-distance intermolecular dipole–dipole interactions are always present. The attractive force between the membrane surface and the particle is determined by the membrane material,
especially of the upper surface layer, the particle diameter, the distance between particle and membrane surface and the medium environment. The electrostatic forces \( F_{el} \) between charged particles and charged membrane surfaces depend on the charge of the membrane surface in comparison to the particle charge, which can either result in an attraction (surface load is different to the particle) or repulsion (same surface charge). The electrostatic force can be described by the sphere-plate model \( ^{60,61} \)

\[
F_{el} = \pi d^2 k T \rho_a \delta e^{(a_0 + \delta)} d^{-1} \tanh \left( \frac{z e^2}{4 k T} \right) \tanh \left( \frac{z e^2}{4 k T} \right)
\]

where \( F_{el} \), electrostatic forces between charged particles and charged membrane surfaces; \( T \), absolute temperature; \( \rho_a \), density of the media; \( z \), ion valence; \( \zeta \), zeta potential of the substance; \( \delta \), thickness of the diffusive layer; \( a_0 \), adhesion distance; and \( a + a_0 \), distance between the two interacting partners.

The attractive van der Waals force can be described by the sphere-plane model as follows:

\[
F_{vdW} = - \frac{HR^3}{F(2R + l)^2}
\]

\( F_{vdW} \), van der Waals force between a particle and a membrane surface; \( H \), Hamaker constant for the solvent-mediated particle-membrane interactions; \( l \), surface-to-surface distance between particle and membrane; and \( R \), particle radius.

The attraction or repulsion force between particle and membrane, and hence a possible particle deposition (adhesion), is determined by the van der Waals force \( F_{vdW} \), and the electrostatic force \( F_{el} \). If the membrane surface and the particles are similarly charged, the resulting electrostatic repulsion between particle and surface can prevent the particle moving close enough to the membrane surface so that also van der Waals forces become active. However in the case of differently charged particle and membrane surface both electrostatic and van der Waals forces are attractive.\(^9\) In this case also depositions of particles on the membrane surface are possible, for example as observed for gold and silver nanoparticles.\(^{18,32,33,39} \) On the other hand, the stronger the repulsion forces between particle and membrane, the larger is their distance, making the depositions less probable. However, especially the focusing phase directly after injection is critical. During the focusing step flows from both sides of the channel are directed to the so-called focus point. A deposition in the focus point area is more likely for lower repulsion forces between a specific particle and the membrane surface. Hence, depositions of particles occur especially in the focus area, which is nicely visible e.g. for red coloured gold particles like the NIST certified standard reference nanoparticles. During the relaxation phase particles experiencing weaker repulsion can remain closer to the surface whereas particles experiencing stronger repulsion can reach faster field lines in the laminar channel flow, which results in a faster transport out of the channel and therefore shorter retention times. Fig. 8 displays these phenomena schematically.

The proposed qualitative picture can explain the above-mentioned results of Fig. 7. It should be taken into consideration that the size difference between PVA and citrate functionalized silver nanoparticles is relatively small (\( \Delta = 3 \) nm). The zeta potential of Ag@PVA is with \(-2.3\) mV less negative than \(-19.5\) mV for Ag@citrate. Both measurements were performed with exactly the same flow conditions and membrane in the A4F fractionation channel. The zeta potential of the PVDF membrane at neutral pH was determined to be \(-11.1\) mV. Since both, particles and membrane are negatively charged a repulsion force should be active between particles and membrane. However, the low zeta potential value of Ag@PVA indicates a weak repulsive force, whereas the repulsion force between Ag@citrate particles and PVDF membrane should be higher. Thus, the Ag@citrate particles probably reach slightly faster velocity lines in the laminar channel flow than the Ag@PVA nanoparticles which results in a slightly shorter retention time and faster elution. This hypothesis should be further evaluated with additional investigations.

### Conclusion

Sample handling or changes in the chemical environment during analysis can have a significant impact on nanoparticle properties especially on size distribution or chemical transformation (e.g. dissolution). For example dilution can accelerate dissolution e.g. for specific silver nanoparticle types, and can even have a certain influence on agglomeration behaviour depending on the coating/functionalization agent type. However, changes in pH or ionic strength usually have a stronger influence. They can have an influence not only on charge but also on sterically stabilized ENPs due to chemical reactions like changes in the cross-linking...
or binding between the polymer functionalization agent and the nanoparticle core. Therefore, it is recommended to check the behaviour of nanoparticles using a fast screening method like batch DLS prior to analysis for size-classified, chemical analysis like spICPMS or A4F-ICPMS/MDA. Such a screening test allows the assessment of possible effects due to dilution, or other media changes (e.g. a change in ionic strength due to addition of surfactants of electrolytes, pH change, etc.) during sample preparation or analysing procedure and prevents erroneous results.

The charge of nanoparticles can also have an influence on the separation efficiency and recovery rate in A4F-ICPMS/MDA analysis. The charge of the particles and the membrane materials used in A4F fractionation can result in a slight retention time shift. These shifts are especially detectable for smaller size differences <10 nm between two particle types as shown here for PVA and citrate functionalized silver nanoparticles with a primary size of 12 and 15 nm respectively. However, for larger size differences between two particle fractions the effect is probably of minor importance. The here presented first results give probably good hints on the particle–membrane interactions in A4F, but further investigations are needed to fully understand the mechanisms. Therefore, the charges of different membrane materials and the interaction with different particle types should be studied in more detail in a future project to understand the mechanism of specific particle membrane interactions.

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