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Electronic Supplementary Material

Application of the Asymmetric Flow Field-Flow fractionation (AsFIFFF) coupled to Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) technique to the characterization of natural colloids and synthetic nanoparticles: new on-line quantification method development.

M. Bouby, H. Geckeis, F.W. Geyer

The idea is to provide the interested reader a detailed description of the equipment and of its use in this study as it was never presented in our previous papers mainly due to the fact that the quantification process was only recently developed. This information is nevertheless better suited as supporting informations as it is not our first publication with this equipment which is in addition not the more recent one provided by the company.

Asymmetric Flow Field-Flow Fractionation (AsFIFFF) coupled to UV-Vis spectrophotometer and Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

The hyphenated equipment and the separation mechanism are presented schematically on Fig. S1 and described in more details hereafter. The AsFIFFF system (HRFFF 10.000 AF4) is from Postnova Analytics (Landsberg, Germany) and comprises laminar, injection and syringe pumps, an injection valve (model 9725i from Rheodyne (Postnova Analytics (Landsberg, Germany)), with a polyetheretherketone (PEEK) sample loop (Loop 1 in Fig.S1), the module and the channel support box. The module regulates the different flows under the control of a software (Postnova Analytics) allowing the user to select the desired outflow rate and the cross-flow strength, that means the cross-flow rate which has to be applied. A measurement comprises three phases: the injection one, the focusing one and the elution one where the particles fractionation really takes place. The cross-flow rate is defined as the flow applied perpendicularly to the membrane during the elution phase and it could vary, usually by an exponential decrease. When this is the case, one speaks about programmed cross-flow. The fractionation channel is delimited by the upper channel wall being composed of a plexiglass plate with holes and ports for the inlet (number 1 on Fig.S1) and the outlet of the channel flow (numbers 3 on Fig.S1) as well as for the sample injection (number 2 on Fig.S1). The lower channel wall is made of polymethylmethacrylate (PMMA) with a ceramic frit and a rubber joint integrated with a hole for the crossflow outlet (number 4 on Fig.S1). On top of the frit a membrane, namely the accumulation wall, and a spacer are located. The accumulation wall consists of an ultrafiltration membrane (regenerated cellulose; Cuttoff: 5 kDa; Postnova Analytics (Landsberg, Germany)). The polytetrafluoroethylene (PTFE) spacer is trapezoidal
and delimits the area where the fractionation takes place. 500 µm has been selected as a spacer thickness and has been
found in our previous works (see e.g [48] in the main text) to be efficient for the fractionation of very small colloids
such as humic colloids. The carrier solution (eluent) consists of ultra pure water adjusted to pH 9.3 by addition of ultra
pure NaOH 1 M (Merck, (Darmstadt,Germany)). The stability of the pH has been checked during the course of the
experiments. The carrier is degassed prior to use by a vacuum degasser (HP 1100, Model G 1322A, Agilent
(Waldbronn, Germany)). On-line polytetrafluoroethylene (PTFE) membrane filters with 100 nm pore size (Postnova
Analytics (Landsberg, Germany)) have been installed in tubings guiding the carrier to the injection pump, and to the
module via the laminar pump. Another filter is located between the module and the inlet port of the channel (see
Fig.S1). The injected sample volume (via the Loop 1 on Fig.S1) is 100 µL. After fractionation, the effluent is directed
through an UV detector (LambdaMax LC Modell 481, Waters (Milford, USA)). In this work, we have selected a
programmed cross-flow. The laminar outflow rate is adjusted nominally to 0.8 mL.min⁻¹ while the cross flow rate
decreases exponentially from 2.4 ml.min⁻¹ at the beginning down to 0 ml.min⁻¹ after 30 min. For the analysis of the
colloid element composition, the effluent is then mixed via a T-piece with 6 % nitric acid containing 50 µg.L⁻¹ Rh
taken as an internal reference and introduced into an ICP-Mass Spectrometer (ELAN 6000, Perkin-Elmer (Rodgau -
Jügesheim, Germany)) at a constant rate via a peristaltic pump (Minipuls3 ABIMED, Gilson (Villiers le Bel, France)).
A flow of 0.5 ml.min⁻¹ was added to the channel flow (0.8 ml.min⁻¹) when a cross-flow type nebuliser was used. An
additional injection valve (Rheodyne) with a 100 µL loop (Loop 2 on Fig. S1) is installed between the channel box
and the UV-Vis detector and is used to inject directly the whole sample (giving results as seen on Fig. S2b)). A last
injection valve is located in the line delivering the Rh containing HNO₃ (Loop 3 on Fig. S1) and is used with different
sample loop volumes: 130.4 µL (Omnifit, (Fridolfing, Germany) for the injection of the sample after its acidification
(and consequently its dilution) in HNO₃ 6 % (giving results as seen on Fig.S2b)) or ~ 2.7 mL (consisting of ~ 610
cms of Dupont TEFLON® FEP (fluorinated ethylene polypropylene) tubing with an 0.75 mm inner diameter) for the
injection of the acidified calibration solution S_{calib} (see details hereafter and on Fig. S2a)). Both loops are required to
perform sample recovery and calibration injections.
Fig. S1. a) Schematic arrangement of the AsFIFFF/UV-Vis./ICP-MS instrument. 1: inlet laminar flow, 2: port for sample injection, 3: outlet laminar flow, 4: outlet crossflow; b) Cross-section of a small part of the AsFIFFF-channel illustrating flow conditions and the effect on colloidal species during injection and focusing. The different valves inside the module are controlled via the software. The eluent is introduced in opposite directions via the port 1 and 3 while the sample, only during the injection phase, is injected via the port 2. The nanoparticles injected are concentrated into a small band near the injection port when the liquid flow passes though the membrane and goes to the wastes via the port 4. c) Cross-section of a small part of the AsFIFFF-channel illustrating the flow conditions during the elution phase which leads to the separation of a bidisperse colloidal sample. In this case the
inflow is introduced via the Port 1 and splitted into the laminar outflow and the cross-flow. The cross-flow is sucked through the Port 4, when the fractionated nanoparticles are eluted outsides from the channel via the Port 3 and directed towards differents detectors. Back diffusion of smaller colloids is more significant than that of larger ones; Smaller particles reach consequently higher speed flow stream lines leading to their faster elution.

Fig. S2. a) Determination of $R_{Elk}$ by injection of calibration solution (see text for more details). b) Typical sequence use for quantification (see Fig. S1 for the position of the Loops 2 and 3).