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Centrifugal ultrafiltration preconcentration for studying the colloidal phase of a uranium-containing soil suspension

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Abstract

The objective of this work was to explore centrifugal ultrafiltration (UF) to separate and / or preconcentrate natural colloidal particles for their characterization. A soil suspension obtained by batch leaching was used as a laboratory reference sample. It was preconcentrated with concentration factors (CF) varying from 10 to 450. The dimensional analysis of the colloidal phase was carried out by Asymmetric Flow Field-Flow Fractionation (AF4)-multidetection. The colloidal masses were estimated by mass balance of the initial suspension, its concentrates and filtrates. The size-dependent distribution (expressed in gyration radius) and total colloidal mass (especially recovery), as well as chemical composition and concentration (including species partitioning between dissolved and colloidal phases) were determined to assess the effects of UF preconcentration on colloidal particles. The gyration radius of the colloidal particles recovered in these concentrated suspensions ranged from about 20 nm to over 150 nm. Neither de-agglomeration nor agglomeration was observed. However, only (64 ± 4) % (CF = 10) of the colloidal particles initially in the soil suspension were found in the recovered concentrated suspensions, and this percentage decreased as CF increased. The filter membrane trapped all other particles, mainly the larger ones. Whatever the CF, the centrifugal UF did not appear to change the dissolved-colloidal partitioning of certain species (Al, organic carbon); whereas it led to an enrichment of the colloidal phase for others (Fe, U). The enrichment rate was specific to each species (15% for Fe; 100% for U). By fitting the observed trends (i.e. conservation, depletion or enrichment of the colloidal phase in the concentrate) as a function of CF, the colloidal concentrations (total and species) were assessed without bias. This methodology offers a new perspective for determining physicochemical speciation in natural waters, with a methodology applicable for environmental survey or site remediation studies.

Key words: colloidal determination; mass balance; AF4; ICP-MS; nanoanalytics

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1. Introduction

The colloidal phase, i.e. all the submicron objects remaining in suspension in the water, is a key player in the environmental fate of trace elements and compounds [1-5]. This is mainly due to the ubiquity and mobility of the colloidal phase, as well as the large specific surface area of colloidal objects. Therefore, colloidal objects contribute significantly to the exchange between solid phases of soils and sediments and the aqueous phase; they have been shown to increase the mobilization and transport of elements and compounds [6–8]. The study of the colloidal phases present in natural waters remains a challenge in environmental and analytical technologies, mainly for two reasons:

(i) The characterization of the colloidal phase often cannot be carried out immediately after sampling. Isolating the colloidal phase is then necessary, as a whole or in fractions, for a subsequent determination [9];

(ii) The concentrations of colloidal objects are sometimes too low with regard to the sensitivity and limits of quantification of analytical methods [10, 11]. Preconcentrating the colloidal phase is then needed.

In any case, when morphological information and size distributions are desired, the physical and chemical preservation of the colloidal phase is also a challenge.

Membrane ultrafiltration methods are often used to separate and / or preconcentrate [12]. They have the advantage, compared to centrifugation, of being able to recover colloidal objects of very small sizes, i.e. typically of the order of a nanometer or a few nanometers; thus the natural polydispersity of the samples is better taken into account. They also better preserve the colloidal structures thanks to lower shear or aggregation forces on the analytes [13, 14]. However, little work has been devoted to comparing and / or evaluating these methods, in particular their impact according to the conditions of implementation. Recently, a comparative study of dead-end, tangential and centrifugal ultrafiltration methods for the preconcentration of the colloidal phase was published [15]. This investigation was carried out with a concentration factor (CF; expressed as the ratio between the mass of the initial suspension taken for preconcentration and the mass recovered after preconcentration) of 10 corresponding to a weak physical constraint. Using this CF, only centrifugal UF appeared capable of preserving the colloidal arrangement, no physical denaturation being observed by dynamic light scattering (DLS) and transmission electron microscopy (TEM). On the contrary, dead-end and tangential ultrafiltrations induced aggregation phenomena modifying the size

distribution, with, in addition, an enrichment of the concentrate in trace elements and organic carbon (OC) coming from dissolved phase, modifying the initial dissolved-colloidal chemical partitioning.

The present work was carried out with the aim of deepening knowledge on centrifugal ultrafiltration to separate and / or preconcentrate natural colloidal particles. The associated idea was to propose a methodology adapted to the characterization of natural colloidal particles that must be conditioned after sampling and until analysis. To carry out this study, a reference suspension was ideally desired. In previous work, it was observed that reproducible and repeatable suspensions (from the point of view of the chemical composition and partition between dissolved and colloidal phases, and of the size characteristics of the colloidal phase) could be obtained from the batch leaching of a natural soil (which was physico-chemically known as it was previously characterized). The soil and the soil suspension were thus used as laboratory reference samples [15, 16]. This soil was therefore considered in the present work to generate suspensions, from which the effects of centrifugal UF applied with different concentration factors were evaluated. Characterizing a colloidal phase and monitoring its evolution involves determining the size, structure and chemical composition of the colloidal particles [17]. In particular, the knowledge of size distribution and chemical species concentrations in dissolved and colloidal phases enables the possible phenomena of agglomeration / de-agglomeration, solubilisation / precipitation and sorption / desorption to be evaluated. For such investigation, the analytical method implemented was based on Asymmetric Flow Field-Flow Fractionation (AF4)- UltraViolet-Visible (UV-Vis)- Multi-Angle Light Scattering (MALS) coupled or associated with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Total Organic Carbon (TOC) analyser.

2. Materials and methods

2.1. Soil sample

A podzolic soil (upper horizon E) from the wetlands of the Landes de Gascogne (France) was sampled on a previously studied site, which is well known regarding the physico-chemical composition of water and soil [18–20]. This soil contains 0.6 wt% of organic matter and about 1 wt% of clay. It was observed that colloidal clay and organic matter can form aggregates and coatings on quartz grain surfaces. Uranium is of natural origin and also present as the result of ancient deposits of solid fragments from anthropogenic activities on

the surface of the soil [19]. Uranium was found in the dissolved and colloidal phases of the interstitial waters of the soil horizon sampled at this site. Colloidal particles were found to play a significant role in the uranium transport in (sub) surface waters. For example, the colloidal phase associated respectively around 10–30% and 70–90% of the uranium in the (sub) surface waters and the water resulting from the soil leaching. In addition, 85 % of colloidal uranium was in the size fraction less than about 500 nm [16, 21]. Therefore this soil was chosen to generate a soil suspension that could serve as a reference sample throughout the study, also considering trace elements (such as uranium) to track any change in physico-chemical properties that may be induced in the colloidal phase by the preconcentration procedure. After sampling, the soil was dried, homogenized, sieved to 1 mm and stored at room temperature in the dark until use as laboratory reference material.

2.2. Chemicals

Ammonium nitrate (NH_4NO_3, 99.999%, Sigma-Aldrich, Steinheim, Germany) was dissolved in ultra-pure milli-Q water (18.2 MΩ cm^{-1}, Elix 3 Millipore system, Bedford, MA, USA). The resulting solution at 10^{-5} mol L^{-1} was then filtered at 100 nm to be used as mobile phase for the AF4 separation system [22]. Nitric acid (HNO_3, 69% ultra-high quality, GT Baker) was used for sample acidification before ICP-MS element determination. Sodium hydrogen carbonate and potassium hydrogen phthalate solutions were used for the calibration of the Total Organic Carbon (TOC) analyzer (Nacalai Tesque, Kyoto, Japan) for the determination of inorganic carbon and total carbon respectively.

2.3. Soil suspension preparation

The soil was leached using the same protocol in static mode as that used in the comparative study of ultrafiltration methods [15]. Briefly, an aliquot of soil and milli-Q water taken in a 1/50 ratio were stirred with a rotary shaker (Stuart Equipment, EW-51600 series). After 24 hours of shaking, the soil/water mixture was let to settle at (4.0 ± 0.5) °C in the dark for 48 hours. Then, the supernatant was collected by frontal filtration at 0.45 µm (polyethersulfone (PES) membrane, rinsed several times with ultrapure water before use). Under these conditions, the soil colloidal suspensions obtained are repeatable (as illustrated in Figure 1A) and stable over several weeks (in chemical composition and partition between dissolved and colloidal phases, checked by elemental analysis, as well as size distribution of the colloidal

phase checked by DLS and AF4-UV-Vis-MALS). They were composed of hydroxides of Al (III) and Fe (III), and humic compounds (from a few hundred to a few thousand g / mol) in dissolved and/or colloidal phases, as well as colloidal clay and quartz particles [19–21]. According to the preparation protocol described above, the expected range of colloidal particles was between a few nm and approximately 450 nm in diameter. The soil suspension was generated in triplicate, each taken as an independent replicate of the reference soil suspension throughout the study (which involved characterization before and after preconcentration for each replicated soil suspension).

2.4. Centrifugal ultrafiltration

The devices used for the separation/ preconcentration experiments were Pierce™ Protein Concentrators (Thermo Scientific) with a polyethersulfone (PES) membrane at 10kDa. These membrane material and cut-off were selected from several available, after preliminary tests carried out on natural colloidal particles like those of the present study, and with CF = 10: less than 0.5% in mass of particles were stuck to the membrane and an effective cut-off of (2.06 ± 0.15) nm in hydrodynamic diameter was determined in accordance with the expected colloidal range [15]. The centrifugal UF was investigated by applying it with different durations so that the concentration factor varied between about 10 and 450 (nominal values). This CF concentration range is usually used when the extraction of the colloidal phase is needed to assess the dissolved-colloidal partitioning. It was expected that centrifugal UF leads to a concentrate (or retentate) and a filtrate. Ideally, and in accordance with the operating conditions previously selected and investigated (i.e. polyethersulfone (PES) membrane at 10kDa [15]), the concentrate should contain the colloidal phase with particles larger than about 2 nm (simply called "colloidal phase" below) and, depending on the applied CF, a part of the initial dissolved phase. This part corresponds to a mass which is all the lower as CF is high. The filtrate should contain the rest of the dissolved phase (most of it) and objects smaller than about 2 nm (simply called "dissolved phase" below). The implementation of the UF led not only to obtain a concentrate and a filtrate, but also to observe material trapped in the upper part of the filter membrane. Depending on the concentration factor applied, this trapped material was in greater or lesser quantity and therefore more or less observable. Before ultrafiltration, the devices were thoroughly rinsed 3 times with ultrapure water. Then, ultrafiltration was replicated (n=2) for each replicate of the reference soil suspension and for each level of concentration (i.e. for each CF). Finally, all the concentrates and filtrates obtained were analyzed, as well as the filter membranes. From the mass balance deducted (i.e.

by comparing the total mass of the initial suspension taken for UF to the sum of the masses of the concentrate, the filtrate and of the matter found in the filter), the overall recovery after UF was also calculated in order to validate the mass distribution determined for each CF. The overall recovery was between (97±3) and (102±3) % for all the UF performed regardless of the CF applied. For each concentrate, the actual concentration factor with which it was obtained was calculated (i.e. the ratio between the mass of the reference soil suspension taken for the centrifugal UF and the mass of this concentrate).

2.5. Analytical techniques

Fractionation of the colloidal particles was performed with the Asymmetric Flow Field-Flow Fractionation system "Eclipse 2" (Wyatt Technology, Dernbach, Germany). The channel was 26.5 cm long and 0.6 to 2.1 cm wide. The spacer was 350 µm in thickness. The membranes were in PES with a 10-kDa cut-off (Wyatt Technology). Flows were controlled using an Agilent 1100 series isocratic pump equipped with a micro vacuum degasser. Injections were made using an autosampler (Agilent Technologies 1100 series). The flow rates were the following: injection at 0.2 mL min^{-1} with a cross flow rate of 2.5 mL min^{-1}; then elution at 1 mL min^{-1} with a cross flow rate decreasing in 5 min from 2.5 (1 min) to 0.5 mL min^{-1} (30 min). AF4 recovery was evaluated for each sample considered (i.e. the reference soil suspension and its concentrates; the recovery was calculated as the ratio of the areas of the peaks (detected by the MALS detector presented below) of the fractionated and unfractionated sample expressed as a percentage); the mean value was found to be (98 ± 3%).

A variable wavelength UltraViolet-Visible detector (Agilent Technologies 1100 series from Agilent, Tokyo, Japan) was used at 254 nm to monitor organic matter. Indeed, it was showed previously that this detector responded proportionally to the organic carbon (OC) contained in the suspensions of this soil [16]. A MALS (DAWN DSP-F, Wyatt technology, Santa Barbara, USA) was used to determine the size of the colloidal particles. Data from UV-Vis and MALS detectors were collected and analyzed using Astra 5.3.4.18 software (Wyatt technology). Prior to the analytical run, accuracy and precision of the determination of the size information over 10 – 200 nm (*Rg*) were verified by AF4-UV-Vis-MALS analysis of size standards (polystyrene nanospheres from NIST, Gaithersburg, USA): 97–105% recovery were found from the certified sizes, with 2–7% Relative Standard Deviation (RSD) depending on the size

194 standards. Size and concentration information of the colloidal particles were determined in the
195 reference soil suspension (bulk) and the concentrates (see details below).

196 An Agilent 7900ce model inductively coupled plasma- mass spectrometer (Agilent
197 Technology, Tokyo, Japan) was used. It was equipped with a concentric nebulizer
198 (MicroMist), an ultra-high matrix introduction (UHMI) spray chamber model (cooled to 2°C),
199 Ni sampler and skimmer cones, and a Collision Reaction Cell (CRC) in hydrogen mode. The
200 operating conditions were as follows: carrier gas flow rate: 0.90 L min^{-1}; makeup/dilution gas
201 flow rate: 0.20 L min^{-1}; collision cell gas flow rate (H_2): 6 mL min^{-1}; dwell time: 0.1 s; tuning
202 solutions: 1 µg L^{-1} Li, Y, Tl, Ce in 2wt% HNO_3; isotopes monitored: ^{27}Al, ^{57}Fe and ^{238}U.

203 For elemental analysis in the entire sample (direct analysis), a nitric acid solution containing
204 yttrium and bismuth as internal standards was added to the sample (final acid content of 5%)
205 before the analytical run to correct for any variation in the instrumental response. For
206 elemental analysis in the colloidal phase, a 2-pump based coupling was used according to
207 Dubascoux et al. [23]: the first pump (AF4) for the mobile phase carrying the fractionated
208 colloidal particles to ICP-MS, the second one for the introduction of element standards into
209 the ICP-MS. Elemental determination was focused on Al and Fe as major elements of
210 colloidal and dissolved phases, and on U as trace element of interest in soil suspension.
211 External calibration was carried out based on individual Al, Fe and U standard solutions.
212 Limits of detection (LOD) were 0.012, 0.24 and 0.26 µg L^{-1} for U, Fe and Al respectively.
213 Limits of quantification (LOQ) were 0.036, 0.76 and 0.78 µg L^{-1} for U, Fe and Al respectively.
214 Prior to the experiments, accuracy and precision of the determination of the elemental
215 concentrations (direct analysis) were verified using TM-25.4 certified reference material (lot
216 0914): concentrations were found to correspond to the certified values within a bilateral
217 confidence interval of 95%, and with 2–4% RSD according to the element. In addition,
218 accuracy and precision of the elemental determination in the colloidal phase of the reference
219 soil suspension after fractionation (AF4-ICP-MS) was verified. For this, the elemental
220 concentrations were also determined after mineralization of the colloidal phase collected after
221 AF4, and the equality of the concentrations found in the non-mineralized sample (suspension)
222 and in the mineralized sample (solution) was verified [23]. Subsequently, elemental
223 concentrations were determined in the reference soil suspension (i.e. bulk) (ICP-MS for the
224 mass balances, and AF4-ICP-MS to characterize this suspension in order to validate the
225 method for determining elemental colloidal concentrations), concentrates, filtrates, filter
226 membranes, and blanks (including blank membrane) (ICP-MS for mass balances).

The TOC concentrations were measured using a Shimadzu TOC – V CSN. Detection and quantification limits were 0.04 and 0.12 µg mL^{-1} (TOC) respectively. OC concentrations were determined in reference soil suspension (bulk), concentrates, filtrates, filter membranes, and blanks (including blank membrane) respectively, for the mass balances.

2.6. Signal and data processing

Calculation of the gyration radii. The radius of gyration (*Rg*) is given as an estimate of the physical size of a particle accounting the internal mass distribution inside the particle relative to its center of gravity. Therefore, the radius of gyration inherently contains information about the shape and structure of the particles [24]. *Rg* turns out to be a parameter that is fundamentally independent and complementary to the hydrodynamic radius (*Rh*), the latter being related to the particle diffusion properties. When coupling typically between AF4 and MALS, *Rh* can be deduced from the elution time, and *Rg* is calculated from the different MALS measurements at different angles of the scattered light. For this, the amount of light scattered at different angles is used in order to extrapolate to the zero angle (incident light) to deduce the gyration radius of the particles by fitting (Debye formalism; first order extrapolation). In addition to Debye's formalism, several others derived from that of Debye are proposed by the Astra software in order to experimentally fit the MALS data (Zimm; Berry) [24–26]. In the present study, Berry's first-order formalism was used because it fitted the data significantly, accurately, and without any bias. To complete, note that at a similar chemical composition, MALS is sensitive to both gyration size and concentration; thus, when differences in MALS signals are observed in different samples with similar gyration radius evolutions, these differences can be attributed to differences in concentration [27].

Calculation of size distributions. Detector signals were numerically filtered (low-pass digital filtering). The size distribution expressed in gyration radius can be obtained from each fractogram by considering this fractogram as a set of n signal values S (t_i) recorded at a time t_i and with a regular interval dt; and using the following equation:

$$f(Rg_i) = [S(t_i)/\Sigma S_i] \times [dt/dRg] \quad \text{(I)}$$

where $f(Rg_i)$ is the normalized signal of the i^{th} recorded value corresponding to the gyration radius Rg_i, $S(t_i)$ the concentration detector signal at the time t_i, ΣS_i the sum of the n signal values $S(t_i)$, and dt/dRg a factor taking account of the selectivity variation along the fractogram (with dRg the difference in gyration radius over the recorded interval dt) [28].

Calculation of colloidal and dissolved masses. The physico-chemical investigation carried out enabled a complete mass balance to be obtained. It was assumed that it is possible to deduce the mass of the colloidal phase in the reference soil suspension (M (coll)) as well as the masses of colloidal (m_i (coll)) and dissolved (m_i (diss)) species i using the following equations (details are presented in paragraph 1 in Supplementary Material). These equations were obtained by focusing on the concentrate, which contains the colloidal phase, and from the mass balance (i.e. M (bulk) = M (conc) + M (filtrate) + M (filter) and M (bulk) = M (coll) + M (diss), with M (bulk) the total mass of the reference soil suspension (bulk) taken for centrifugal UF; similar relationships for the masses of species (element or organic carbon)). Thus when the concentration factor is such that the dissolved phase present in the concentrate becomes negligible compared to the colloidal phase:

$$M\,(conc) \rightarrow M\,(coll) \qquad (II)$$

$$m_i\,(conc) \rightarrow m_i\,(coll) \qquad (III)$$

In addition, whatever the concentration factor, it can be calculated:

$$m_i\,(coll) = m_i\,(conc) - [M\,(conc)/M\,(filtrate)] \times m_i\,(filtrate) \qquad (IV)$$

$$m_i\,(diss) = [M\,(bulk\ net)/M\,(filtrate)] \times m_i\,(filtrate) \qquad (V)$$

where m_i (conc) and m_i (filtrate) are the masses of the species determined in the concentrate and filtrate, M (conc) and M (filtrate) the measured masses of the concentrate and filtrate, and M (bulk net) the net mass of the reference soil suspension taken for centrifugal UF and subsequently recovered above and below the filter of the concentrator device.

These equations were obtained assumed that ideally there was neither loss nor exchange of matter between colloidal and dissolved phases, i.e. the centrifugal UF was a conservative process of extraction of the water initially present in the reference soil suspension without any physical and chemical redistribution of elements between the colloidal and the dissolved phases.

In addition, to enable the masses of concentrate and of the colloidal entities from different centrifugal UF to be used together, after each centrifugal UF, the relative masses of the concentrate, of the species i, of the colloidal phase and of the colloidal species i were calculated by dividing the corresponding absolute mass (i.e. M (conc), m_i (conc), M (coll) and m_i (coll) respectively) by the total mass of the aliquot of the reference soil suspension taken for the centrifugal UF (M (bulk)). The dimension of the relative masses thus obtained was

292 similar to a concentration (mass/mass of reference soil suspension, simply expressed as
293 mass/mass of suspension thereafter).
294
295 *Processing of values below limits of quantification*. When concentration values are found
296 between the detection limits and the quantification limits, it is possible to convert them to
297 numerical results according to the equation [29]:

298 $1/2 \times LOQ \times (1+U)$ (VI)

299 where U is the uncertainty of the analytical method used to determine the values.

300 Such a treatment enables, for example, the results to be graphically represented.

301

302 3. Results and discussion

303 In order to deepen knowledge about centrifugal ultrafiltration, mass balances were carried out
304 using both the concentrates and filtrates, thus taking into account both the colloidal and
305 dissolved phases. The final objective being to acquire a methodology for characterizing
306 natural colloidal particles, the results presented are therefore focused on the concentrates and
307 their colloidal phases. The characterization of colloidal particles requires their basic intrinsic
308 parameters to be determined, i.e. size, size dependent distribution, chemical composition and
309 concentration. These different parameters are examined hereafter.

310

311 *3.1. Size dependent distributions and masses of the colloidal phases*

312 *Size dependent distributions.* Figure 1B shows the variations in the radius of gyration as a
313 function of the elution time and the corresponding AF4-UV-Vis-MALS fractograms obtained
314 for the reference soil suspension and for the various concentrates generated by centrifugal UF.
315 In order to compare the results directly, for each concentrate obtained with a concentration
316 factor CF, the mass injected was CF times smaller than the mass of the reference soil
317 suspension injected. Up to a concentration factor of 100, the concentrates recovered were
318 directly analysable suspensions; beyond, they were too concentrated, i.e. in a physical state
319 that did not enable these concentrates to be injected in AF4 as they were. Therefore, only the
320 results for concentrates with CF between 10 and 100 are shown for comparison in Figure 1B.
321 As the UV signals were found to be close to the baseline (i.e. in limits of detection /

quantification) during fractionation, in the following only MALS signals are considered in the discussion.

The variation curves of the radius of gyration as a function of the elution time (top of Figure 1B) were found to be similar, even overlapped, for the reference soil suspension and its concentrates from about 20 nm to 150 nm. Beyond 150 nm, the *Rg* values seemed to decrease regardless of the sample considered, i.e. reference soil suspension (REF on Figure 1B; i.e. CF=1) or concentrates (CF=10, 50 and 100). This decrease occurred all the earlier during the elution and therefore for a gyration radius all the smaller as the concentration factor was high: typically around 29 min and 210 nm for CF=1; 28 min and 175 nm for CF=10; 27 min and 160 nm for CF=50; and 23 min and 150 nm for CF=100 (these elution times, taken as upper limits, are identified by vertical dotted lines in Figure 1B). These decreases in *Rg* also corresponded to a noisy signal; both are typically the result of too low colloidal concentrations to calculate *Rg* reliably. The observation of both the peak height and the shape of the fractograms confirms this: the end of peak occurred earlier for CF = 100 and the peak height decreased as a function of CF according to 100< 50 <10 <1. In other words, the variations in *Rg* observed beyond about 150 to 200 nm depending on the sample are not representative of the change in the size of the eluted colloidal particles, but a loss of colloidal particles. Furthermore, by reporting the values in elution times above quoted (23, 27 and 28 min respectively) as upper limits on each of their peak, the corresponding peak area can be calculated (as illustrated by the peak areas in grey on the fractograms in Figure 1B). These areas were (95±2)% (average for CF=10, 50 and 100 respectively). Knowing that MALS signal is sensitive to both size and concentration, this means that at least (95±2)% of the populations of colloidal particles was fractionated as expected (which corresponds to an increasing variation of *Rg*) regardless of the CF. Given all of this, the similar variation curves of the radius of gyration observed for at least 95% of the fractionated colloidal particles suggest that there was no observable change in this particle size range due to centrifugal UF. In particular, these curves all presented a continuous variation, without discontinuity in slope. This also suggests that neither agglomeration nor de-agglomeration occurred [16, 30]. As mentioned above, the size of gyration depends on the size and the internal mass distribution in the object analysed; and the internal mass distribution depends on the shape and / or the structure of the object. Therefore, from similar variation curves of the radius of gyration as a function of the elution time in different samples, it is possible to deduce that the size, shape and / or structure of colloidal objects are similar in these samples [16, 17, 30]. In addition, when the variations in radius of gyration are similar and overlap (i.e. same shape of the *Rg*

variation curve as a function of the elution time), it means that shape and / or structure of colloidal objects are similar. This is observed in Figure 1B. Moreover, the colloidal particles in the reference soil suspension and in the concentrates were previously imaged by electron microscopy (see [15] and Figure 1S in Supplementary Material). The images obtained confirmed that the colloidal particles are very predominantly polyhedral with aspect factors (i.e. ratio between the largest dimension is the smallest dimension) of approximately 1−1.5, of fairly homogeneous shape. No agglomerate was observed. Therefore, the MALS signal can be viewed as providing information on the concentration of the fractionated particles. Given equation (I) and the fact that at least 95% of the particles are fractionated without significant change in the shape of the *Rg* variation curve, the MALS fractograms in Figure 1B can also be taken as representative of the size dependent distributions. In addition to the above observations on the peak ends and signal heights, the ends of the fractograms shifted to smaller retention times, which correspond to smaller sizes; while the beginnings of peaks (8 min elution time for all the fractograms) and their shape remained the same. All this is characteristic of the loss of mainly the larger colloidal particles, with no apparent change in the distribution and / or size of the smaller ones (i.e. $Rg < 50$ nm according to Figure 1B).

Total and colloidal masses. The difference in intensity of the MALS signals (i.e. the difference in peak height of the fractograms in Figure 1B) can also give quantitative information relating to the total mass of the colloidal particles injected in AF4. However, the AF4 injection conditions were set so that the (expected) same mass of colloidal particles be injected, fractionated and therefore detected. Therefore, the colloidal recovery can serve to compare the total mass of colloidal particles present in different samples. In the present study, the colloidal recovery (values given in the legend of Figure 1B) decreased in the concentrates compared to the reference soil suspension. This indicates that the total mass of colloidal particles in the concentrates has actually also decreased, and this as CF increased. In addition to the information from the fractograms, the mass balances also provided total and colloidal mass information in the concentrates according to the CF. The determination of the masses also has the advantage of being simpler than AF4 implementation. In Figure 2, the evolution of the relative mass of concentrate as a function of the concentration factor is presented. The relative mass of concentrate decreased when the concentration factor increased. Such a trend was expected (as illustrated in Figure 2S in Supplementary Material). Figure 2A shows the relative masses of concentrate obtained directly by mass balance (after having recovered them

in the centrifugal UF devices, then weighed). The colloidal mass associated to each of these relative masses was estimated directly using equation (II) (*Estimate (a)*). The result is represented on the zoom of graph A (Aa). The colloidal concentration in the reference soil suspension (corresponding to CF =1) was thus deduced (around 2 mg g^{-1} of suspension) assuming an ideal conservative behaviour (therefore without loss or exchange between phases). However, on the one hand, as observed and discussed above, losses of colloidal particles occurred during UF. Such losses could be due to dissolution, de-agglomeration, aggregation / agglomeration and then trapping by the filter membrane, or direct trapping. On the other hand, a solid material was observed stuck on the membrane, suggesting that some colloidal particles were lost on the filter membrane during UF. The losses could also come from the difficulty of recovering the entire mass of concentrate, especially when the concentration factor was ≥ 100. The mass of these particles found on the filter membrane represented less than 1% of the total mass of the reference soil suspension for all CF. However, if this mass seemed negligible, it was not compared to the mass of concentrate recovered (and therefore compared to the colloidal particles recovered), in particular from CF = 50 (the mass of material on the filter membrane compared to the mass of concentrate recovered then exceeding 20%). The value of colloidal concentration was therefore likely underestimated. In view of these observations, two other estimates of the colloidal mass were made:

- *Estimate (b)*: It was based on taking into account the colloidal recovery values (i.e. masses of the concentrates recovered after AF4 fractionation compared to the mass of reference soil suspension also recovered after AF4). The curve of the colloidal evolution was thus deduced (Figure 2, curve Ab; white circles). This curve is typical of depletion behaviour (as shown schematically in Figure 2S C in Supplementary Material), with colloidal mass in the concentrate decreasing from approximately 5 to 2 mg g^{-1} of suspension for CF = 10 to 450. The estimate of the colloidal concentration in the reference soil suspension (corresponding to CF=1) deduced from this curve was at around 8 mg g^{-1} of suspension.

- *Estimate (c)*: It was performed taking into account the mass of material stuck on the filter membrane (M (filter); determined by weighing the dry filter before and after UF). It was therefore assumed that 100% of the material "lost on the filter" consisted of initially colloidal particles, i.e. that the actual mass of concentrate = M (conc) + M (filter). Figure 2B presents the relative masses of concentrates corrected according to this assumption for each concentration factor applied. The colloidal mass estimated using equation (II) (and

422 corresponding to CF = 1) was then deduced and corresponds to a colloidal concentration in
423 the reference soil of approximately 8 mg g^{-1} of suspension (Figure 2B, curve Bc).
424 The relevance of these estimates is discussed in the methodological section hereafter. In
425 addition to these observations, it can be noted that the colloidal losses evaluated by the
426 colloidal recovery calculated from AF4 analyses remained far below the concentration rates.
427 The net mass balance (i.e. preconcentration minus loss; illustrated by Figure 3S in
428 Supplementary Material) confirms that the preconcentration of the colloidal phase was
429 effective. This is in agreement with what was previously observed [15].
430 In summary, centrifugal UF did not induce any observable phenomenon of agglomeration or
431 de-agglomeration. For at least 95% of the fractionated particles, the sizes (*Rg*) remained the
432 same after centrifugal UF at least up to CF = 100. However, the centrifugal UF induced a
433 partial loss of the colloidal particles, mainly the larger ones (> 50 nm *Rg*). This loss was due
434 on the one hand to the sticking of the particles on the membrane, and on the other hand to the
435 difficulty of recovering the concentrate for large concentration factors (CF>100). Despite this
436 loss, the decrease in the volume of solution was such that the colloidal mass relative to this
437 volume, i.e. the final colloidal concentration, was significantly higher than the initial colloidal
438 concentration in the reference soil suspension. The concentration of the colloidal phase was
439 therefore effective.
440
441 *3.2. Chemical composition in concentrates*

442 Figure 3 shows the evolution of the relative masses of Al, Fe, U and OC in the concentrates
443 and the colloidal phase of the concentrates as a function of the concentration factor. Their
444 evolution differed from that observed for the entire colloidal phase where a partial loss was
445 observed (Figure 2). Indeed, for the elements in the colloidal phase, there were:

446 - A conservative behaviour for Al: the variations of its colloidal masses as a function of CF
447 were not significant (less than 4%) compared to the experimental repeatability;
448 - A global enrichment for Fe and U.

449 According to the knowledge acquired about the waters of the study site and their components, the
450 colloidal phase consists mainly of particles on which clays and oxides of Al and Fe are sorbed [19,
451 21]. From Figure 3, the colloidal particles contained Al and Fe with relative masses of the order of
452 98–107 and 18–21 ng g^{-1} of suspension respectively. These relative masses are relevant given the
453 colloidal concentrations previously found [16]. The colloidal enrichment in Fe and U was
454 observable from low CF. It appeared limited for Fe (15% between CF = 10 and CF = 450), and

455 more important for U (its relative mass doubled from 0.09 to 0.18 ng g^{-1} of suspension depending
456 on the CF applied). A similar evolution was obtained for Fe and U, the relative colloidal masses
457 of these two elements significantly correlating (r = 0.9904). This correlation was also observed on
458 the colloidal continuum (Figure 1C, Fe / U ratio). All of this suggests a similar behaviour of these
459 elements probably because they were involved in the same compounds; thus, part of these
460 compounds could sorb onto particles in the colloidal phase [16]. The behaviour appeared to
461 remain the same whatever the CF since the relative masses varied continuously as a function
462 of CF (no discontinuity in slope according to the dotted curves on the zooms of Figure 3).
463 Element concentrations in the material trapped by the filter membrane were lower than those
464 in the recovered concentrates. The masses of Al, Fe and U found in the filter membrane
465 decreased as the CF increased (<3% for CF> 250); this trend was opposite to that of the total
466 mass of material on the filter membrane (section 3.1). This suggests a "spinning effect" with
467 desorption from particles trapped by the filter membrane, then passage through the filter of
468 compounds containing Al, Fe and U. The lower elemental masses in the material trapped by
469 the filter membrane also explain why, contrary to what was proposed to evaluate the total
470 colloidal mass (Figure 2), it was not relevant to take into account the material trapped by the
471 filter membrane and very depleted in elements to estimate the colloidal masses of elements.

472 The behaviour of organic carbon appeared to be conservative (similar to Figure 2S A,
473 Supplementary Material). However, the colloidal concentration was found to be lower than
474 the LOQ (of TOC analyser) whatever CF, OC being present essentially in the dissolved phase.
475 This result was confirmed by AF4-UV-Vis, where no peak was recorded. This is in agreement
476 with the previous observations [15, 20]. In particular, it was found by Maria et al [15] that the
477 organic matter of the leaching waters from this soil was mainly composed of molecules with
478 low molar masses, around 200 Da, therefore being in the dissolved phase, or possibly being
479 initially sorbed on the colloidal particles and desorbed during UF process.

480 In summary, centrifugal UF induced a redistribution of certain species (Fe and U) from the
481 dissolved phase towards the colloidal particles on which they were then very probably sorbed;
482 while other species behaved conservatively: Al, present both in the dissolved and colloidal
483 phases, and organic carbon mainly in the dissolved phase and which remained there whatever
484 CF.

485

486

3.3. Methodological aspect of the colloidal evaluation

Based on the above results and observations, the objective of this part was to propose a methodology for determining the concentration of the colloidal phase and associated trace elements of interest.

The results presented above have shown that the physicochemical phenomena occurring during centrifugal UF followed a continuing trend as a function of the concentration factor. It was therefore interesting to empirically fit the overall trends observed (see the dotted curves in Figures 2Ab and 3). The aim of such a fit was to extrapolate to CF = 1 the colloidal masses (either total or of chemical species) obtained for the different concentrates (i.e. for different CF> 1); CF = 1 corresponding to the reference soil suspension before any centrifugal UF. Thus, it was expected to estimate the total colloidal concentrations and the colloidal concentrations of chemical species (as shown schematically in Figure 2S of the Supplementary Material).

As colloidal OC was found to be lower than the LOQ for the reference soil suspension, the approach detailed below was applied to the total and elemental (Al, Fe and U) colloidal concentrations.

The fitting of the curve (Figure 2Ab) enabling the initial concentration of colloidal particles in the reference soil suspension ($[P]_{coll}$) to be evaluated has the general expression:

$$[P]_{coll} = [P]'_{coll}\, CF^{B} \qquad \text{(VII)}$$

With B a constant reflecting the behaviour of colloidal particles during UF (conservative: B = 0; enrichment B> 0; depletion B <0), CF being the concentration factor of the suspension, and $[P]'_{coll}$ the concentration of colloidal particles estimated from equation (II) taking into account the colloidal recovery (*estimate (b)* presented above).

In the fitting obtained, the constant B = (- 0.211 ± 0.016) clearly reflects the loss of colloidal particles as discussed above. The colloidal concentration obtained with this fitting, as well as that obtained in the hypothesis where 100% of the material trapped by the filter membrane were colloidal particles (*estimate (c)*) are presented in Table 1, second column. They are in agreement (statistically equal in a 95% bilateral confidence interval) and much higher than the first estimated concentration (*estimate (a)*). These results give relevance to the hypothesis of part of colloidal material not recovered because trapped the filter membrane. The consistency

of the independent estimates *(b)* and *(c)* of the concentration of colloidal particles shows that it is possible to obtain an unbiased estimate by a complete mass balance.

Note that, in general, the evaluation of the total colloidal concentration in natural suspensions remains critical due to the low concentrations. It can be carried out from the initial suspension, by weighing [31]. However, this measurement sometimes lacks precision. The estimates *(b)* and *(c)* presented here have the advantage of being based on several independent mass balances carried out on concentrates. This provides precision and reliability.

The same approach was applied to evaluate the element concentrations $[x]_{coll}$ (the colloidal OC concentration was found to be lower than the LOQ as discussed above) on the basis of the same type of fitting:

$$[x]_{coll} = [x]'_{coll}\, CF^{B} \qquad \text{(VIII)}$$

With B representing the fitting constant reflecting the behaviour, and $[x]'_{coll}$ being the colloidal concentration calculated with equation (IV) for a given CF. The fitting curves are shown in Figure 3 (dotted curves).

The coefficients B are equal to 0.0065 ± 0.0056 (Al); 0.0315 ± 0.0029 (Fe); and 0.1856 ± 0.046 (U) respectively. For aluminum, this coefficient reflects its conservative behaviour. For iron and uranium, the coefficients B quantify the enrichment in these elements in the colloidal phase during centrifugal UF.

The concentrations obtained with these fittings are presented in Table 1 (last 3 columns), together with the element colloidal concentrations in the reference suspension determined independently by AF4-MALS-ICPMS. The concentrations of each of the elements obtained by the two methods are statistically identical (no significant difference in an interval of 95%). Their accuracies are comparable (around 5 to 9% relative standard deviation). The two estimates (by fitting the colloidal masses determined in each concentrate from expression (IV), and by AF4) were carried out independently. Knowing that the accuracy of the method by AF4 has previously been verified, the fitting method proposed here therefore provided an accurate estimate of the colloidal concentration in the reference soil suspension. It is therefore interesting to note that this method, by fitting several colloidal masses, appears capable of overcoming the possible bias of an individual determination of the colloidal mass by equation (IV).

4. Conclusion

A previous study had shown that centrifugal UF is the least impacting method in dimensional and chemical terms for the preconcentration of natural suspensions. Centrifugal UF has therefore been investigated in this work in order to deepen the knowledge to separate and / or preconcentrate colloidal particles. The multicriteria characterization offered by AF4 associated with several detectors contributed to an exhaustive understanding of the centrifugal UF process. It also enabled the methodology for evaluating colloidal concentrations to be validated. The present work therefore illustrates the interest of using AF4-multidetection to develop analytical methods.

It is confirmed that centrifugal UF had no observable impact on the size range (from about 20 to at least 150 nm in *Rg*) representing 95% of the colloidal phase of the concentrate recovered as suspension not too concentrated, i.e. for a CF up to 100. But the filter membrane trapped part of particles. However, by adding the mass of particles trapped by the filter membrane to the mass of particles in the recovered concentrate, 100% of the mass of the colloidal particles initially present in the soil suspension were found. The elements have either a conservative (Al) or non-conservative (Fe and U) behaviour with enrichment of the recovered colloidal particles from the dissolved phase where these elements were also initially present. The organic carbon very mainly present in the dissolved phase of the reference soil suspension remained in this phase after centrifugal UF.

Depletion or enrichment phenomena appear to depend proportionally on the applied concentration factor and vary continuously as a function of it. From this observation, the colloidal total and trace-component concentrations in the initial soil suspension were estimated. This estimation is based on mass balances (total mass determination, elemental and TOC analyses) carried out on the concentrates after preconcentration with different CF, determination of the colloidal masses, graphic representation of these masses as a function of CF, fitting of these curves, and finally extrapolation for CF=1. Such a methodology constitutes (i) an interesting alternative to an investigation strategy based on sophisticated analytical methods such as coupling methods, (ii) a solution enabling the problems of preservation of samples to be overcome. These two aspects are particularly important when the sampling site is far from the place of analysis (or when analysis immediately after sampling is not possible). Indeed, centrifugal UF can be used on site, and analysis performed later since the sample thus treated will retain the dissolved-colloidal partitioning. This work therefore brings interesting perspectives for the determination of the colloidal concentrations

and the dissolved-colloidal partitions of natural waters, and also for approaching the physico-chemical speciation within these waters. Work on other natural suspensions will have to be carried out to confirm the potential of such a methodology.

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References

[1] J. Buffle, H.P. van Leeuwen (Eds) Environmental particles. Environmental Analytical and Physical Chemistry Series, CRC Press (1992)

[2] A.B. Kersting, D.W. Efurd, D.L. Finnegan, G.J. Rokop, D.K. Smith, J.L. Thompson, Migration of plutonium in ground water at the Nevada Test Site. Nature (1999) 397, 56–59. https://doi.org/10.1039/C5EM00223K.

[3] R. Kretzschmar, M. Borkovec, D. Grolimund, M. Elimelech, Mobile Subsurface Colloids and Their Role in Contaminant Transport. Advances in Agronomy (Ed. Sparks, D.L.). Academic Press (1999) pp 121–193.

[4] J.F. McCarthy, J.M. Zachara, Subsurface transport of contaminants. Environ. Sci. Technol. 23 (1989) 496–502. https://doi.org/10.1021/es00063a001.

[5] J.C. Seaman, P.M. Bertsch, W.P. Miller, Chemical Controls on Colloid Generation and Transport in a Sandy Aquifer. Environ. Sci. Technol. 29 (1995) 1808–1815. https://doi.org/10.1021/es00007a018.

[6] G. Bin, X. Cao, Y. Dong, Y. Luo, L.Q. Ma, Colloid Deposition and Release in Soils and Their Association With Heavy Metals, Crit. Rev. Env. Sci. Tec. 41 (2011) 336–372. https://doi.org/10.1080/10643380902871464.

[7] Y. Ouyang, D. Shinde, R.S. Mansell, W. Harris, Colloid-enhanced transport of chemicals in subsurface environments: A review, Crit. Rev. Environ. Sci. Technol. 26 (1996) 189–204. https://doi.org/10.1080/10643389609388490.

609 [8] J.N. Ryan, M. Elimelech, Colloid mobilization and transport in groundwater, Colloids and
610 Surf. A Physicochem. Eng. Aspects 107 (1996) 1–56. https://doi.org/10.1016/0927-
611 7757(95)03384-X.

612 [9] J.R. Lead, K.J. Wilkinson, Aquatic colloids and nanoparticles: Current knowledge and
613 future trends. Environ. Chem. 3 (2006) 159–171. https://doi.org/10.1071/EN06025.

614 [10] T. Saito, T. Hamamoto, T. Mizuno, T. Iwatsuki, S. Tanaka, Comparative study of
615 granitic and sedimentary groundwater colloids by flow-field flow fractionation coupled
616 with ICP-MS. Journal of Analytical Atomic Spectrometry 30 (2015) 1229–1236.
617 https://doi.org/10.1039/c5ja00088b.

618 [11] B. Stolpe, M. Hassellöv, K. Andersson, D.R. Turner, High resolution ICPMS as an on-
619 line detector for flow field-flow fractionation; multi-element determination of colloidal
620 size distributions in a natural water sample. Analytica Chimica Acta 535 (2005) 109–121.
621 https://doi.org/10.1016/j.aca.2004.11.067.

622 [12] S. Sandron, A. Rojas, R. Wilson, N.W. Davies, P.R. Haddad, R.A. Shellie, P.N.
623 Nesterenko, B.P. Kelleher, B. Paull, Chromatographic methods for the isolation,
624 separation and characterisation of dissolved organic matter. Environmental Science:
625 Processes & Impacts 17 (2015) 1531–1567. https://doi.org/10.1039/C5EM00223K.

626 [13] K.L. Planken, H. Coelfen, Analytical ultracentrifugation of colloids, Nanoscale 2 (2010)
627 1849–1869. https://doi.org/10.1039/c0nr00215a.

628 [14] R. Salim, B. Cooksey, The Effect of Centrifugation on the Suspended Particles of River
629 Waters, Water Res. 15 (1981) 835–839. https://doi.org/10.1016/0043-1354(81)90137-8.

630 [15] E. Maria, P. Crançon, P. Le Coustumer, M. Bridoux, G. Lespes, Comparison of
631 preconcentration methods of the colloidal phase of a uranium-containing soil suspension,
632 Talanta 208 (2020) 120383. https://doi.org/10.1016/j.talanta.2019.120383.

633 [16] S. Harguindeguy, P. Crançon, M. Potin Gautier, F. Pointurier, G. Lespes, Colloidal
634 mobilization from soil and transport of uranium in (sub)-surface waters, Environ. Sci.
635 Pollut. Res. 26(6) (2019) 5294-5304. https://doi.org/10.1007/s11356-018-2732-5.

636 [17] S. Faucher, P. Le Coustumer, G. Lespes, Nanoanalytics: History, concepts and
637 specificities, Environ. Sci. Pollut. Res. 26(6) (2019) 5267-5281.
638 https://doi.org/10.1007/s11356-018-1646-6.

639 [18] P. Crançon, J. Van der Lee, Speciation and mobility of uranium(VI) in humic-containing
640 soils, Radiochim. Acta 91 (2009) 673–679. https://doi.org/10.1524/ract.91.11.673.23470.

641 [19] P. Crançon, E. Pili, L. Charlet, Uranium facilitated transport by water-dispersible
642 colloids in field and soil columns, Sci. Total Environ. 408 (2010) 2118–2128.
643 https://doi.org/10.1016/j.scitotenv.2010.01.061.

644 [20] E. Maria, P. Crançon, G. Lespes, M. Bridoux, Spatial Variation in the Molecular
645 Composition of Dissolved Organic Matter from the Podzol Soils of a Temperate Pine
646 Forest, ACS Earth Space Chem. 3(8) (2019) 1685-1696.
647 https://doi.org/10.1021/acsearthspacechem.9b00164.

648 [21] S. Harguindeguy, P. Crançon, F. Pointurier, M. Potin-Gautier, G. Lespes, Isotopic
649 investigation of the colloidal mobility of depleted uranium in a podzolic soil, Chemosphere
650 103 (2014) 343–348. https://doi.org/10.1016/j.chemosphere.2013.12.033.

651 [22] S. Dubascoux, F. v.d. Kammer, I. Le Hecho, M. Potin-Gautier, G. Lespes, Optimisation
652 of asymmetrical Field Flow Fractionation for environmental nanoparticles separation. J
653 Chromatogr A 1206 (2008) 160-165. doi:10.1016/j.chroma.2008.07.032.

654 [23] S. Dubascoux, I. Le Hecho, M. Potin-Gautier, G. Lespes, On line and off-line
655 quantification of trace elements associated to colloids by As-Fl-FFF and ICP-MS, Talanta
656 77 (2008) 60-65. https://doi.org/10.1016/j.talanta.2008.05.050.

657 [24] P. Schurtenberger and M.E. Newman In Environmental particles, J. Buffle and H.P. van
658 Leeuwen, IUPAC, environmental analytical and physical chemistry series, (1993) Chapter
659 2, vol. 2, 37- 115.

660 [25] P. Wyatt, Submicrometer particle sizing by multiangle light scattering following
661 fractionation, J. Coll and Interf. Sci. 197 (1998) 9-20. doi.org/10.1006/jcis.1997.5215.

662 [26] M. Andersson, B. Wittgren, K-G. Wahlund, Accuracy in Multiangle Light Scattering
663 Measurements for Molar Mass and Radius Estimations. Model Calculations and
664 Experiments, Anal. Chem. 75 (2003) 4279. doi: 10.1021/ac030128+.

665 [27] J. Gigault, B. Grassl, G. Lespes, Size characterization of the associations between carbon
666 nanotubes and humic acids in aqueous media by asymmetrical flow field-flow
667 fractionation combined with multi-angle light scattering, Chemosphere 86(2) (2012) 177-
668 182. https://doi.org/10.1016/j.chemosphere.2011.10.009.

669 [28] M.E. Schimpf, K. Caldwell, J.C. Giddings, Field-Flow Fractionation Handbook. John
670 Wiley & Sons (2000)

671 [29] I.M. Farnham, A.K. Singh, K.J. Stetzenbach, K.H. Johannesson, Treatment of nondetects
672 in multivariate analysis of groundwater geochemistry data, Chemom. Intell. Lab. Syst. 60
673 (2002) 265–281. https://doi.org/10.1016/S0169-7439(01)00201-5.

674 [30] J. Gigault, B. Grassl, G. Lespes, Size characterization of the associations between carbon
675 nanotubes and humic acids in aqueous media by asymmetrical flow field-flow
676 fractionation combined with multi-angle light scattering, Chemosphere 86 (2012) 177-182.
677 doi:10.1016/j.chemosphere.2011.10.009.

678 [31] H. El Hadri, J. Gigault, P. Chéry, M. Potin-Gautier, G. Lespes, Optimisation of flow
679 field-flow fractionation for the characterization of natural colloids, Anal. Bioanal. Chem.
680 406(6) (2014) 1639-1649. https://doi.org/10.1007/s00216-013-7369-0.

681

682

683

684 **Captions of Figures**

685

686 Figure 1: Typical variations in the radius of gyration as a function of the elution time (dotted
687 lines) and corresponding AF4 fractograms, for: A) 2 replicates of the reference soil
688 suspension generated by batch leaching; B) the reference soil suspension and its concentrates
689 recovered after applying different concentration factors (for fractograms: semi-solid line (UV)
690 and solid (MALS) line); C) the reference soil suspension, its monitored elements and their
691 ratios

692 For 1B: The colloidal recoveries was calculated relative to the reference soil suspension as
693 100×S/Sref, with S the MALS surface area obtained for a given suspension of concentrate and
694 Sref the MALS surface area for the reference soil suspension. The colloidal recoveries found
695 were (64 ± 4)% (CF = 10), (43 ± 3)% (CF = 50) and (36 ± 3)% (CF = 100), respectively.

696

697

698 Figure 2: Evolution of the relative mass of the concentrate according to the concentration
699 factor (left), with associated zoomed view (right). The different colloidal mass estimates are
700 presented in the zoomed views: A: direct (a), corrected for recovery (b); B: corrected for
701 colloidal particles on the filter (c). See explanations and calculation details in the text.

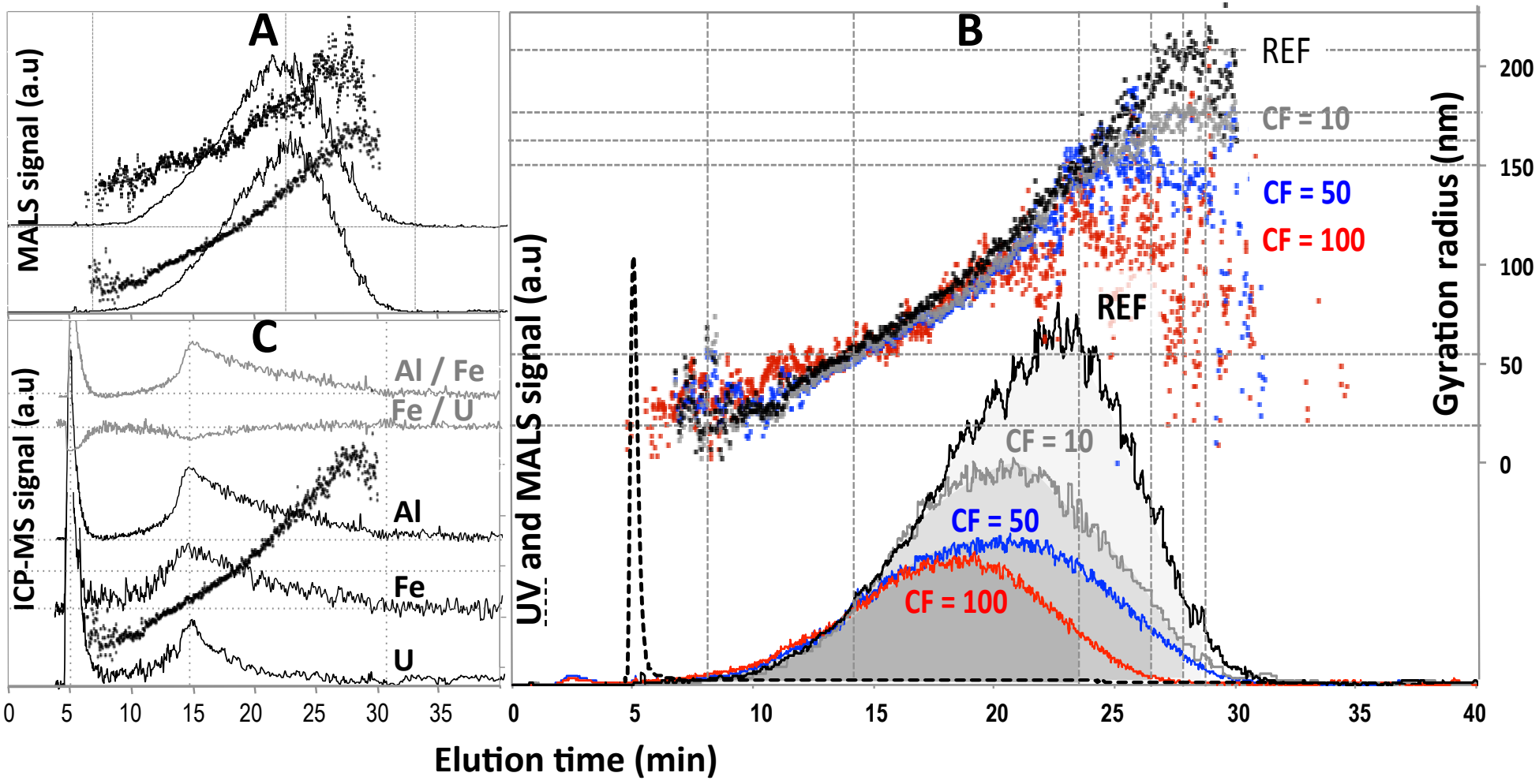
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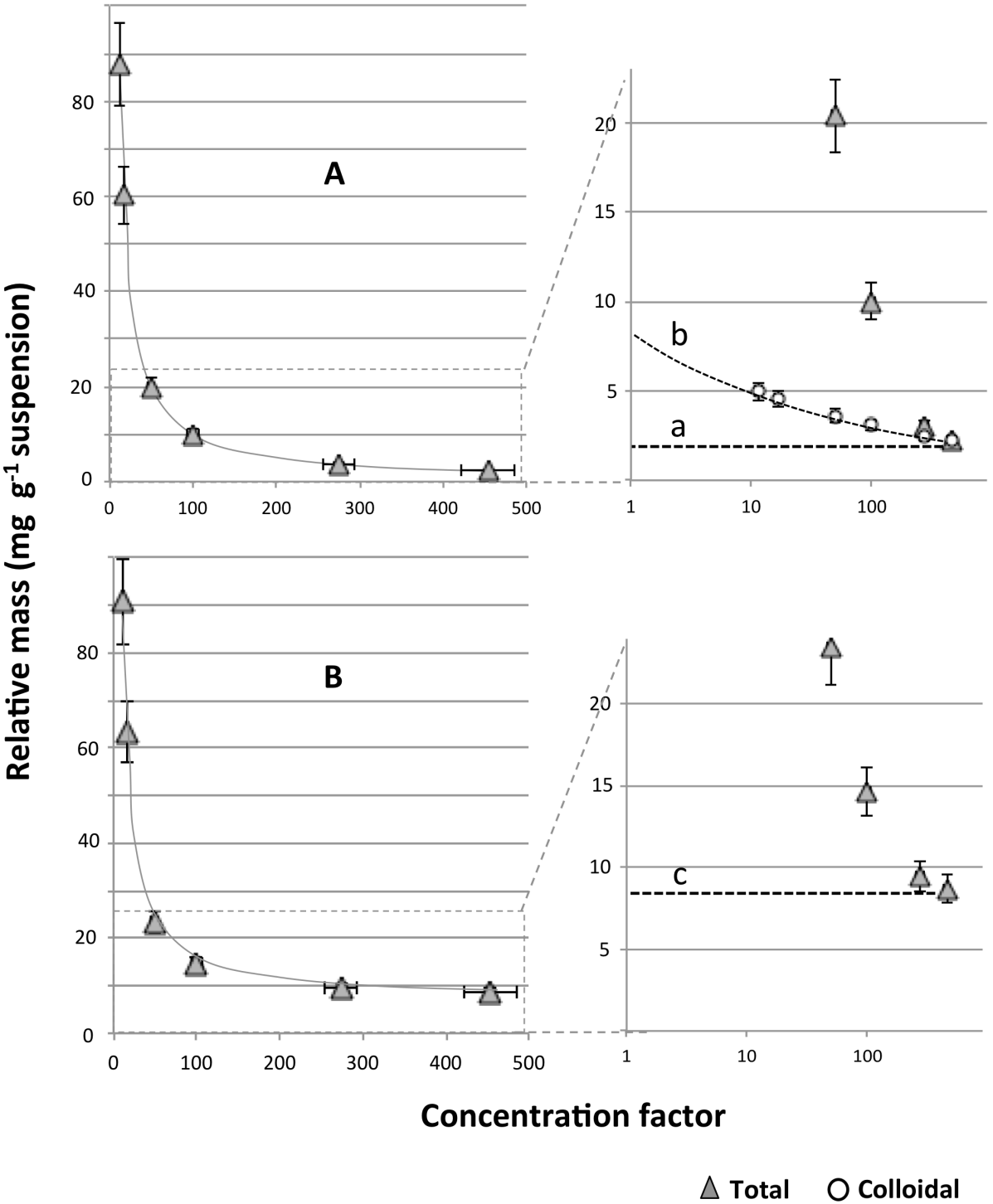
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704 Figure 3: Evolution of the relative masses of elements and organic carbon (OC) in the
705 concentrate (total; determined by ICP-MS and TOC analyser) and the colloidal phase
706 (determined using equation (IV)) according to the concentration factor.

707 When values were <LOQ, they were processed according to equation (VI).

708


A
MALS signal (a.u)
B
REF
CF = 10
CF = 50
CF = 100
UV and MALS signal (a.u)
Gyration radius (nm)
C
ICP-MS signal (a.u)
Al / Fe
Fe / U
Al
Fe
U
Elution time (min)


Relative mass (mg g-1 suspension)
A
B
a
b
c
0
20
40
60
80
100
200
300
400
500
1
5
10
15
20
Concentration factor
Total
Colloidal

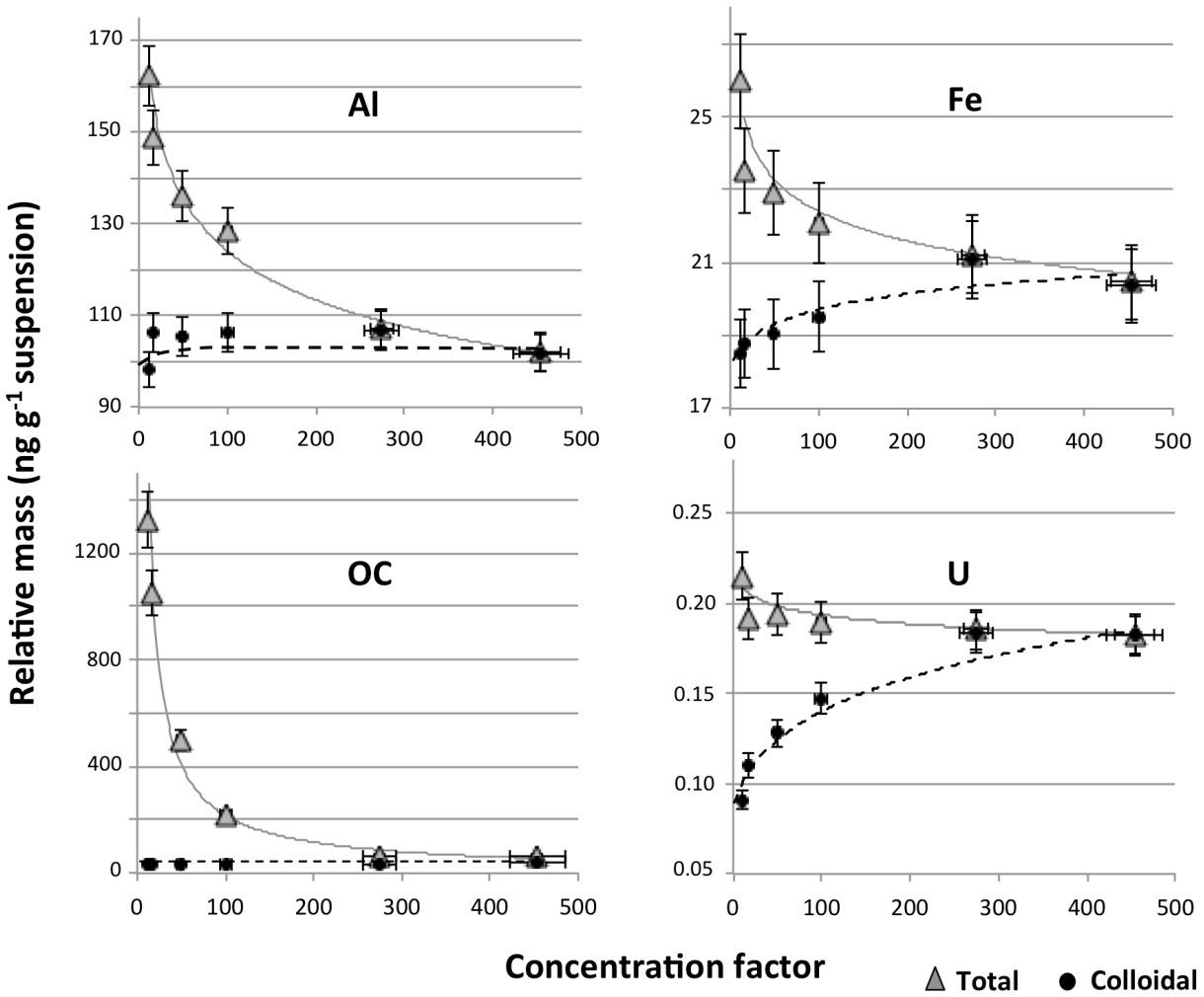

Al
Fe
OC
U
Relative mass (ng g-1 suspension)
Concentration factor
Total
Colloidal
170
150
130
110
90
25
21
17
1200
800
400
0
0.25
0.20
0.15
0.10
0.05
0
100
200
300
400
500

Table 1: Colloidal concentrations in the reference soil suspension, determined either after AF4-UV-Vis-MALS-ICP-MS or mass fitting from the set of concentrates and filtrates obtained by centrifugal ultrafiltration with different concentration factors and analysed by ICP-MS and TOC analyser

Concentrations	Total ($mg\ g^{-1}$)	Al	Fe	U	OC
		($ng\ g^{-1}$)			
AF4-UV-Vis-MALS-ICP-MS	n.d.	97.7 ± 8.3	17.65 ± 0.94	0.0530 ± 0.0050	< LOQ
Centrifugal UF and mass fitting	2.20 ± 0.18 [(a)] 8.72 ± 0.92 [(b)] 8.48 ± 0.98 [(c)]	101.2 ± 6.8	17.1 ± 1.0	0.0600 ± 0.0054	< LOQ

n.d. not determinable

(a) Directly deduced from equation (II); (b) corrected for the colloidal recovery; (c) corrected for colloidal particles on the filter; see the text for details