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Journal of Chromatography A, 1093 (2005) 156–166
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Natural sample fractionation by FlFFF–MALLS–TEM: Sample stabilization, preparation, pre-concentration and fractionation

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Received 1 June 2005; received in revised form 21 July 2005; accepted 25 July 2005
Available online 16 August 2005

Abstract

Two flow field flow fractionation (FlFFF) systems: symmetrical (SFlFFF) and asymmetrical (ASFlFFF) were evaluated to fractionate river colloids. Samples stability during storage and colloids concentration are the main challenges limiting their fractionation and characterization by FlFFF. A pre-fractionation (<0.45 μm) and addition of a bactericide such as NaN_3 into river colloidal samples allowed obtaining stable samples without inducing any modification to their size. Stirred cell ultra-filtration allowed colloidal concentration enrichment of 25-folds. Scanning electron microscope (SEM) micrographs confirmed the gentle pre-concentration of river samples using the ultra-filtration stirred cell. Additionally, larger sample injection volume in the case of SFlFFF and on channel concentration in the case of ASFlFFF were applied to minimize the required pre-concentration. Multi angle laser light scattering (MALLS), and transmission electron microscope (TEM) techniques are used to evaluate FlFFF fractionation behavior and the possible artifacts during fractionation process. This study demonstrates that, FlFFF–MALLS–TEM coupling is a valuable method to fractionate and characterize colloids. Results prove an ideal fractionation behavior in case of Brugeilles sample and steric effect influencing the elution mode in case of Cézerat and Chatillon. Furthermore, comparison of SFlFFF and ASFlFFF fractograms for the same sample shows small differences in particle size distributions.

Keywords: FlFFF; MALLS; TEM; SEM; River colloids; Pre-concentration; Steric elution

1. Introduction

Many physicochemical processes taking place in natural aquatic systems such as: colloids settling, re-suspension, adsorption, and transport depend on their size distribution [1,2]. Different components of colloidal matter (i.e. humic substances, iron oxides, alumiosilicates, etc.) often occur in a characteristic size range, which may result in differential transport, deposition, or pollutant adsorption [3–8]. Thus, colloidal size fractionation and determination are indispensable, which implies the necessity for a high-performance fractionation technique and sensible detection systems. Flow field flow fractionation (FlFFF) was proved to be a valuable technique for colloidal matter fractionation [9,10]. However, several factors such as: sample stability during storage time and the low concentration of rivers colloids, often 1–100 mg l^{-1}, combine to make the characterization of rivers colloids extremely difficult [11,12].

A stable colloidal sample is a sample resistant to aggregation and removal by settling, or filtration. In order to optimize sample stability, minimize artifacts, minimize chemical, or physical modifications induced during sampling and storage time; Buffle and Leeuwen [12,13] suggested that: (i) all measurements (including fractionation and colloid structure studies) must be carried out within 2–3 days after sampling since significant changes by coagulation or bacterial activity mainly occur after this period, provided storage is done at 4 °C in the dark, (ii) physical and chemical changes of samples must be minimized, (iii) sampling vessels must be pre-equilibrated, and (iv) several techniques must be used in parallel, both to derive as much structural information as pos-

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doi:10.1016/j.chroma.2005.07.103

sible and to act as cross check on the possible artifacts, which may occur during sample preparation and analysis.

The typical low concentration of colloidal river matter can be overcome applying pre-concentration process prior to analysis. A pre-concentration of 1:10 to 1:1000 is then required before fractionation by FlFFF [14,15]. Several methods are available for colloidal sample concentration including ultra-filtration, centrifugation, coagulation, and on channel concentration [14,16]. The pre-concentration step is time consuming process and is a potential cause of sample aggregation and losses. Consequently, reducing the required degree of concentration would be a significant advance [11].

FlFFF has been used extensively in biological and polymers research, but it was much less used in environmental research. This is presumably due to the low stability and concentration of natural samples [17]. To date, two environmental applications of the FlFFF have been explored. The first is the characterization of river-borne colloids [2,11,16] and soil colloids [15]. The second is the determination of molecular weight distribution and size of humic substances [9,18,19].

The rigors of FlFFF theory has been extensively described elsewhere [9,17,20], and need not concern us here. FlFFF theory uses the Stokes formula for converting diffusion coefficient into particle size. Consequently, the size and size-distributions determined from FlFFF system differ from the theoretical values as soon as colloids deviate from homogeneous hard spheres and only an equivalent diameter is retrieved. Interferences inside the FlFFF channel such as: overload effects, steric/hyperlayer elution mode transition (i.e. elution of large and small particles at the same elution volume), particle-wall interactions, and shape selective retention are frequently observed for natural samples. These interferences may hamper the interpretation of FlFFF fractograms and limit its environmental applications. Therefore, FlFFF needs to be coupled to sensitive independent size measuring techniques such as spectroscopic or imaging techniques [10].

UV–vis spectrophotometers have long been used for the determination of the relative amount of mass in the separated fractions, simply assuming that absorbance, or turbidity is proportional to the mass concentration [10]. One possible uncertainty using the UV–vis detector arises from the underestimation of small particles size concentration (less than the radiation wavelength 254 nm) and the dependence of the signal on particle and other parameters [2]. Fluorescence detection (FLD) has also been used as a concentration detector in nephelometric mode as a simple light scattering detector [21]. Multi-angle laser light scattering (MALLS) is a powerful technique, permitting the determination of particles size by measuring scattered light intensity at a range of fixed angles. The light scattering theory has been extensively described in [22,23]. The main advantage of the MALLS technique lies in that, it is an absolute technique and FlFFF independent. Direct examination of colloidal particles by transmission electron microscope (TEM) is very useful as it provides an independent determination of particle size [14], which may then be used to verify the elution mode of particles and to determine their thickness, aspect ratio [24], geometric surface area [10], and qualitative elemental composition when coupled with energy dispersive X-ray spectroscopy (EDS) [10,16]. The FlFFF–MALLS–TEM coupling allows 3D calculation of colloids dimensions, i.e. thickness, aspect ration, and surface area [24,25].

Colloidal size determination suffers many limitations including: the limited size range covered, inaccuracies in theories, lack of resolution, and inability to fractionate and size samples. These deficiencies in the commonly used separation methods have hindered attempts to gain information on colloidal size distribution. This work aims to provide a sample processing strategy from sampling to fractionation process, which should account for: (i) stability of natural (river) colloids, (ii) concentration, separation, and size determination of river colloidal samples by FlFFF–UV-FLD–MALLS–TEM, and (iii) elucidate the different possible behaviors of natural colloidal samples during the fractionation process and how the MALLS and TEM can be used to discover these behaviors.

2. Materials and methods

2.1. Sampling and sampling locations

First, one sample was collected from the Loire river at Orleans to test its stability under storage conditions. The stability test will be described in Section 2.3. Then eight other samples were collected from the Loire River watershed. The Loire River watershed, sampling sites, and sample collection is extensively described in Baalousha et al. [26]. Briefly, the Loire River from its source in the Massif Central to the Atlantic Ocean, is 1010 km long. The total basin area is 117,800 km^2. The Loire is one of the principal European riverine inputs of water to the Atlantic Ocean. The Loire watershed is characterized by varied geological formations. The bedrock composition of the studied area comprises (i) older plutonic rocks granite, gneiss, and mica schist (500–300 My), and a large volcanic area, as wall as (ii) a sedimentary bedrock the (Paris Basin) consists primarily of sedimentary deposits (200–6 My).

2.2. Instruments

Two FlFFF systems have been used in this study: symmetrical (SFlFFF) and asymmetrical (ASFlFFF). The SFlFFF system used is F1000 model Universal FFFractionator (Postnova Analytics Europe, Landsberg, Germany). The channel dimensions are: 29 cm length, 2.5 cm width, and 254 μm thickness. A 10 kD regenerated cellulose membrane (Postnova Analytics Europe, Landsberg, Germany) was used as the accumulation wall. ‘Milli-Q’ water (Millipore, Bedford,