Journal of Colloid and Interface Science 368 (2012) 231–240

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Contribution of capillary electrophoresis to an integrated vision of humic substances size and charge characterizations

Fanny d'Orlyé [1], Pascal E. Reiller *

Commissariat à l'Energie Atomique et aux énergies alternatives, CE Saclay, CEA/DEN/DANS/DPC/SECR/LSRM, Bâtiment 391 PC 33, F-91191 Gif-sur-Yvette Cedex, France

ARTICLE INFO

Article history:
Received 8 September 2011
Accepted 19 November 2011
Available online 1 December 2011

Keywords:
Humic acids
Fulvic acids
Capillary electrophoresis
Size
Charge
Alkali-metal ion

ABSTRACT

The physicochemical properties of three different humic substances (HS) are probed using capillary zone electrophoresis in alkaline carbonate buffers, pH 10. Special attention is drawn to the impact of the electrolyte ionic strength and counter-ion nature, chosen within the alkali-metal series, on HS electrophoretic mobility. Taylor–Aris dispersion analysis provides insights into the hydrodynamic radius (R_H) distributions of HS. The smallest characterized entities are of nanometric dimensions, showing neither ionic strength– nor alkali-metal-induced aggregation. These results are compared with the entities evidenced in dynamic light scattering measurements, the size of which is two order of magnitude higher, *ca.* 100 nm. The extended Onsager model provides a reasonable description of measured electrophoretic mobilities in the ionic strength range 1–50 mM, thus allowing the estimation of limiting mobilities and ionic charge numbers for the different HS samples. An unexpected HS electrophoretic mobility increase (in absolute value) is observed in the order $Li^+ < Na^+ < K^+ < Cs^+$ and discussed either in terms of retarding forces or in terms of ion–ion interactions.

1. Introduction

The structure evolution of humic substances (HS), alkaline extracts of natural organic matter (NOM), with physical and chemical properties of solution has been a matter of debate for decades, even though more and more experimental evidence is pointing to an aggregation of rather small entities [1–3] in fractal organization [4–8]. The smallest objects evidenced up to now are of some nanometers in hydrodynamic radius [2,3]. Nevertheless, objects of some hundreds of nanometers have been commonly observed in dynamic light scattering [9–12]. Ionic strength has been identified as an influencing parameter on size and viscosity [12–14], acid–base properties [15,16], and affinity to metal ions [17,18]. But the extent of the ionic strength influence on size is not really clearly established. Some questions are still open: to which size scale does ionic strength actually affects the aggregate(s) size(s) of HS? are the smallest objects affected, or does it only affect the already known larger aggregates seen in dynamic light scattering [12]? and what is the actual influence of the alkaline cation?

The alkaline cations are weakly sorbed to HS [19], mostly non-specifically [20]. Influence of NOM on the cesium sorption onto minerals occurs mostly through the "blocking" of mineral sorption sites by NOM more than through the direct Cs–NOM interaction [21]. Nevertheless, the influence of alkaline metals through ionic strength on acid–base properties induces an acidification [15,16], which depends on the nature of the cation [19]. Some data are existing that compare Na^+ and K^+ [19], but to our knowledge, no systematic study was performed throughout the alkaline series.

To provide a deeper insight into NOM structure, a combination of complementary characterization techniques based on different physicochemical properties should be used, such as high-performance size-exclusion chromatography (HPSEC) [1], electrospray ionization mass spectrometry (ESI-MS) [22–25], atomic force microscopy (AFM) [2], or small angle neutron or X-ray scattering (SANS/SAXS) [5,6].

Over the last two decades, many authors have focused on the fingerprint characterization of HS using capillary-based electromigration techniques such as capillary zone electrophoresis (CZE) [26], capillary gel electrophoresis (CGE) [27], capillary isoelectric focussing (CIEF) [27,28] as well as micellar electrokinetic chromatography (MEKC) [27]. Special attention was paid to CZE as it combines the unique possibility to separate and detect NOM in aqueous solution within wide pH and ionic strength ranges that allow mimicking environmental conditions. Characterizations were

---

Abbreviations: HS, Humic substances; NOM, Natural organic matter; SRHA, Suwannee River Humic Acid; SRFA, Suwannee River Fulvic Acid; PAHA, Purified Aldrich Humic Acid; CZE, Capillary zone electrophoresis; BGE, Background electrolyte; EOF, Electroosmotic flow; TDA, Taylor dispersion analysis; DLS, Dynamic light scattering.

* Corresponding author. Fax: +33 1 6908 5411.
E-mail address: pascal.reiller@cea.fr (P.E. Reiller).

[1] Present address: Laboratoire de Physicochimie des Electrolytes, Colloïdes et Sciences Analytiques – UMR 7195, Chimie ParisTech, Ecole Nationale Supérieure de Chimie de Paris, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France.

0021-9797/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2011.11.046

performed mainly using uncoated silica capillaries except in few cases where the authors aimed to minimize the electroosmotic flow (EOF) [29] or HS sorption onto the capillary wall at low pH [30,31]. Among all separation electrolytes mentioned in the literature, the most frequently used are based on acetate [29,32], borate [29,32,33], phosphate [29,32–34], or phosphate–borate [33,35] buffer systems. More detailed information on the composition of the detected fractions was obtained using either diode array (DAD) or laser-induced fluorescence (LIF) detectors compared with that obtained using single-wavelength UV detection [33,36]. Commonly, NOM behave as anionic species in aqueous solutions, and the injection of rather concentrated sample solutions (0.1–1 g L^{-1}) produces typical fingerprints that are composed of a series of sharp peaks superimposed to a very broad hump sometimes followed by an asymmetrical peak on the higher mobility side. These electrophoretic patterns were interpreted as different HS subfractions co-migrating with a large supramolecular assembly consisting in an unresolved distribution of aggregate size and charge states. Nevertheless, only a mass-selective detector could allow differentiating the charge and mass distributions within the polydisperse humic samples. Thus, Schmitt-Kopplin and Kettrup [37] evaluated the use of CZE-ESI-MS for the characterization of Suwannee River NOM. They evidenced that the changes in the m/z ratio distributions with mobility show a decrease in the m/z ratio with increasing electrophoretic mobility in the humic hump at alkaline pH; superimposed on this hump, a low-molecular-weight fraction migrates at lower mobility. Schmitt-Kopplin and Junkers [26] gave an in-depth overview of the use of CZE in the characterization of NOM and looked at different pitfalls and artifacts that could come either from the instrumental setup or from separation buffer solution chemistry. In few words, they cautioned against the presence of a number of system peaks, which are inherent to the buffer or to different ionic strength distributions within the capillary (stacking effects). They also emphasized on artifacts caused by the potential interaction of buffer ions with HS samples, for instance in phosphate or borate buffers, which may improve the signal reproducibility but become a problem when interpreting the data in relation to the size and charge distribution of native samples.

Because of problems in the interpretation of the complex electropherograms, CZE has been scarcely used for HS mobility measurements, and only few attempts have been made to evaluate the dependence of electrophoretic mobility on electrolyte composition (pH, ionic strength, and chemical nature) as well as HS acid–base and complexation properties [34,38,39]. Schmitt-Kopplin and Junkers [26] related these problems to the choice of the separation electrolyte, especially reminding that borate ions compete with the same humic binding site as metals [40,41]. In this context, Norden and Dabek-Zlotorzynska [42] used capillary electrophoresis to study the humic–metal interactions in borate-based buffer systems and ranked the investigated metal ions (M^{z+}) in order of decreasing complex stability: $Al^{3+} > Hg^{2+} > Cu^{2+} > Pb^{2+} > Sr^{2+}$, in agreement with the Irving–Williams series generally stated for HS–metal interactions [43]. This is also consistent with the work of Lubal et al. [44] who reported that humic acids bind strongly with heavy metal ions and the stability constant of HA-metal ion complexes was found to decrease in the order: $Cu^{2+} > Ba^{2+} > Pb^{2+} > Cd^{2+} > Ca^{2+}$. Lately, there has been an effort to develop the applications of capillary electrophoresis hyphenated with inductively coupled plasma mass spectrometry (CE-ICP-MS) to the study of HS–metal interactions, mainly lanthanides [45–53].

Besides electrokinetic measurements, the capillary electrophoresis instrument has proved to be well suited to perform Taylor dispersion analysis (TDA) as an alternative technique to determine the diffusion coefficients, D, of solute molecules [54]. It relies on the specific dispersion profile of a solute plug in a laminar Poiseuille flow, subjected to the combined effect of diffusion and convection. This method is attractive as it offers a possibility for a fast evaluation of D, based on the variance associated with the solute peak broadening or boundary sharpness, and thus estimation of the effective dimension of a given analyte in solution: the Stokes radius, R_H. Besides electrophoretic measurements, CE instrumentation has proved to be particularly well suited for performing TDA as it offers all the functions of injection, detection, and data acquisition in a single automated apparatus. Recently characterized systems were as varied as phenylalanine, proteins, polymers, DNA fragments, colloidal particles, or magnetic beads [55–64].

The aims of the present work are (i) to study the applicability of CZE for HS characterization in terms of size and charge distributions, which are the main properties that affect HS reactivity in the environment, and (ii) to estimate the influence of the electrolyte ionic strength (I) and alkaline counter-ion nature ($M^+ = Li^+$, Na^+, K^+, Cs^+) on HS electrophoretic behavior.

2. Materials and methods

2.1. Chemicals and reagents

Standard 1 M and 0.1 M NaOH solution Normadoses were purchased from VWR (Strasbourg, France). Li_2CO_3 and K_2CO_3 were obtained from Merck (Fontenay-sous-Bois, France). Na_2CO_3 and *N*,*N*-dimethylformamide were provided by Fisher Scientific (Illkirch, France). LiOH, CsOH (50 wt.% in H_2O), standard 0.1 M KOH solution Fixanal, Cs_2CO_3, and humic acid were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). CO_2 in gas cylinder was provided by Messer (Puteaux, France). Suwannee River Humic Acid (SRHA) standard II and Suwannee River Fulvic Acid (SRFA) standard II were purchased from the International Humic Substances Society (IHSS, St. Paul, MN, USA) and used as received. Water used throughout was produced by a Barnstead Easypure II UV water purification system (Thermo Scientific, Dubuque, IA, USA).

2.2. Separation electrolyte solutions

The 0.1 M LiOH stock solution was prepared by dissolving 0.24 g of LiOH in 100 mL of deionized water. The 0.1 M CsOH stock solution was prepared by diluting 0.857 mL of the commercial CsOH (50 wt.% in H_2O) solution in deionized water to a final volume of 100 mL. All 5 and 0.1 mM alkali hydroxide solutions were prepared from the corresponding 0.1 M stock solution by applying successive dilutions in deionized water at 1:19 and 1:49, respectively. The 1 M alkali carbonate stock solutions were prepared by dissolving 7.4 g, 10.6 g, 13.8 g, and 32.6 g of Li_2CO_3, Na_2CO_3, K_2CO_3, and Cs_2CO_3 in deionized water to a final volume of 100 mL, respectively. All 0.1 M alkali carbonate solutions were prepared from the corresponding 1 M stock solution by applying a 1:9 dilution in deionized water. Carbonate buffer solutions of various ionic strengths (1–500 mM) were prepared by (i) the dilution of predefined volumes V_{M2CO3} of alkali carbonate stock solutions ($[M_2CO_3]_0$ = 1 or 0.1 M) in deionized water according to the desired BGE final volume V_f and (ii) $CO_2(g)$ bubbling until pH stabilization at 10. The composition of the resulting separation electrolyte solutions is summarized in Table 1.

N,*N*-dimethylformamide was used as EOF marker at 0.01% v/v in buffer solution.

2.3. HS samples

Aldrich Humic Acid was purified following the procedure described by Kim et al. [65]. Stock solutions of HS were prepared as follows: 10 mg of each solid sample was weighed and solubilized