

Factors Affecting the Surfactant Properties of Humic Acids

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ABSTRACT

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The surface tension (ST) of 30 mg L⁻¹ solutions of estuarine, lacustrine and Aldrich humic acids (HA) was monitored under varied conditions of pH (2 to 12), ionic strength (up to 0.3 with NaCl and MgCl₂) and in the presence of pyrene (0.1 mol L⁻¹). In general, ST decreases with an acidity increase, a minimum occurring between pH 3 and 5. This minimum results from the migration to the surface of the amphiphilic species developed by the protonation of the negatively charged sites. The presence of NaCl modifies slightly the pH profiles shifting the minimum to higher pH values (5 to 6). If the salt is MgCl₂ (identical I) the pH profiles become atypical. In this case, the lacustrine and the Aldrich HA behave as if they were completely solubilized through the entire pH range, such a behavior being attributed to their ability to produce intramolecular domains in the solution. For the estuarine HA, on the other hand, the presence of MgCl₂ causes precipitation above pH 9. Mg²⁺ hydrolysis followed by the accumulation of mixed Mg-OH-HA insoluble complexes is responsible for this effect. The addition of pyrene modifies drastically the pH profiles. In this case, a slight increase is observed in ST values between pH 6 and 7. Solutions containing both pyrene and salt also showed particular pH profiles that were dependent on the characteristics of each sample. Observed effects are explained in view of conformational arrangements of HA which tend to agglomerate in micelle-like domains in aqueous solution, assembling their aromatic moieties around the pyrene molecules.

ADDITIONAL INDEX WORDS: *Humic substances, surface tension, pyrene.*

INTRODUCTION

Dissolved humic substances (HS) are known to influence the uptake of hydrophobic organic compounds by aquatic organisms (HAITZER *et al.*, 1999). Evidence that the mode of interaction between HS and hydrophobic species in aqueous medium is similar to that observed for surfactants, has been reported by WANDRUSZKA and co-workers (1997, 1998, 1999). Briefly, from these studies it can be inferred that HS, mainly humic acids (HA) can aggregate inter and/or intramolecularly in aqueous solutions generating micelle-like agglomerates capable of sequestering hydrophobic pollutants of low molecular mass like pyrene.

In previous studies, using surface tension (ST) measurements, we monitored the amphiphilic behavior of HS from aquatic and terrestrial environments under varied conditions of concentration, pH and ionic strength (RAUEN *et al.*, 2002). At pH 7 HA presented a surface tension vs. concentration profile similar to that of regular surfactants. The ST value decreased with increasing HA contents and at a concentration value of approximately 1000-1200 mg L⁻¹ a decrease in the rate of the solute migration to the surface was observed for all samples.

This value was reduced to approximately 750 mg L⁻¹ and the break in the ST decrease sharpened, with an increase in the ionic strength of the solutions to 0.3, with NaCl. Surface tension also decreased with increasing acidity, showing a minimum between pH 3 and 5. These effects were explained in view of the conformational arrangements of HA which, under specific physical-chemical conditions, tend to agglomerate in aqueous solutions.

Following this approach, we present here the results concerning the variation in the ST of aqueous solutions of HA from aquatic and terrestrial sources, in the presence and absence of two different salts, NaCl and MgCl₂, and of pyrene, under varied conditions of pH and ionic strength. HA samples were obtained according to the IHSS procedure and the experimental conditions (HA concentration of 30 mg L⁻¹ and I = 0.3) were chosen in order to preserve the environmental validity of interpretations. From such data, the mode and conditions under which HA molecules can sequester non-polar constituents from

an aqueous medium is inferred.

METHODS

Origin, Extraction, Purification and Characterization of HA

Lacustrine and estuarine sediments were, respectively, collected from Peri Lagoon (HA1) and Ratones Mangrove (Gran1, Gran8 and Gran11) on the Santa Catarina Island, southern coastal zone of Brazil. Peri Lagoon is a freshwater lake located in a hydrographic basin surrounded by a dense and well-preserved sub-tropical rain forest. Ratones Mangrove is a well-preserved estuary. HA were obtained via the International Humic Substances Society recommended procedure (IHSS HOME PAGE, 2003).

The Aldrich HA sample was included as representing a material of terrestrial origin and also to make possible eventual comparisons with other studies. Information on the specific source and nature of this product is not usually given by the suppliers, but according to MALCOLM and MCCARTHY (1986) this HA sample appears to be similar to Leonardite and Wyoming dopplerite (IHSS HOME PAGE, 2003), being different from soil and water HS. The commercial sample was submitted to the same purification process applied to the other samples (GIOVANELA *et al.*, 2003).

Surface Tension (ST) Measurements

Surface tension measurements were carried out in a Krüss K8 Tensiometer, fitted with a 20-mm-diameter platinum-iridium ring, in a thermostated (22 °C) cell. 2000 mg L⁻¹ HA stock solutions were prepared by adding the appropriate mass of solute to volumetric flasks, adding Milli-Q water up to 50 % of the total volume, adjusting the solution pH to 7 with KOH, sonicating for 1 h and then adding Milli-Q water to volume. These solutions were further filtered through GF/F-47 mm, pre-combusted (500°C) Watman fiberglass filters.

To evaluate the pH effect, 50 mL of 30 mg L⁻¹ HA solutions were prepared. Each solution was subdivided into two 25 mL portions, one of them being acidified (HCl) for the measurements at pH 7 and the other basified (KOH) for the

measurements at pH 7.

For the ionic strength effect evaluation, NaCl or MgCl₂ (I = 0.3) aqueous solutions were employed instead of distilled water. These solutions were vigorously stirred and then left to equilibrate in the dark for at least two hours before analysis.

Pyrene stock aqueous solutions (1.0 mol L⁻¹) were prepared according to the procedure described in WANDRUSZKA *et al.* (1997).

All measurements were carried out in triplicate.

RESULTS AND DISCUSSION

Elemental Characteristics of the Studied HS

Table 1 shows the elemental composition of the studied HA. These values are within the ranges of those shown in the literature (RICE and MACCARTHY, 1991). Due to their complexity, individual information on HS elemental composition is not very conclusive and the atomic ratios (H/C and N/C) have been preferentially employed to establish the organic matter (OM) sources, the diagenetic transformations and the environmental conditions under which the HS were generated (STUERMER *et al.*, 1978; RICE and MACCARTHY, 1991; MEYERS and ISHIWATARI, 1993). Marine sediments being less aerated than superficial soils and having an aliphatic and nitrogen-rich source of OM, produce HS with high H/C and N/C ratios (STUERMER *et al.*, 1978; RACHID, 1985; RICE and MACCARTHY, 1991). Terrestrial samples, on the other hand, present low H/C ratios due to the higher plant inputs. These trends are confirmed here. The Aldrich HA, for example, being generated in terrestrial environments presents the lowest N/C and H/C values. All the other sites receive OM from both aquatic and terrestrial sources and the extent of the influence from each source is reflected in its H/C and N/C ratio values. The Gran1 HA sample, for example, was extracted from sediment collected beneath the water column within the bay, being, supposedly, strongly influenced by the marine OM contribution. Such influence is reflected in its relatively higher N/C value. The two other estuarine samples (Gran8 and Gran11) were collected in zones of alternated marine water inundation, receiving in addition, material from the surrounding terrestrial vegetation and present intermediate N/C values. The Peri Lagoon (HA1) sample was extracted from a sediment sample collected at the bottom of the lake being influenced by both terrestrial and algal inputs. These variables together contribute to the slight differences observed between the elemental compositions of studied samples.

Surface Tension vs. pH Profiles

Figure 1 shows the effect of pH on 30 mg L⁻¹ HA solutions at six different conditions. This HA concentration was chosen because it is within the range of OM contents that can be found in natural aquatic systems like estuaries (SIERRA, 1992). The choice of a 0.3 ionic strength had two reasons. First, an experiment on which the salt concentration was increased steeply from I = 0 to I = 1.0 with both, NaCl and MgCl₂ was carried out for each HA solution. In all cases, and for the two salts, only at I = 0.3 some kind of effect was observed on the ST of the solutions. Second, this level of ionic strength may be found in estuarine environments where the usually salinity varies from 0 to 35 g kg⁻¹ (i.e., from I ~ 0 to I ~ 0.7).

Table 1. Elemental composition and atomic ratios* of the studied HS.

Sample	C (%)	H (%)	N (%)	H/C	N/C
Aldrich	51.1	4.6	1.0	1.09	0.02
HA1	48.7	5.3	3.2	1.31	0.06
Gran1	44.5	4.8	3.9	1.30	0.08
Gran8	47.9	5.5	3.9	1.38	0.07
Gran11	46.5	5.2	3.3	1.34	0.06

*H/C = [(%H/%C)*12.011]/1.00794

*N/C = [(%N/%C)*12.011]/14.0067

Concerning the pH effect alone (Figure 1a) the behaviors of the samples were similar, the only exception being the Gran1 sample. Profiles comparable to those shown here have been reported by YATES and WANDRUSZKA (1999). In general, the ST diminished with increasing acidity, reaching a minimum between pH 3 and 5 and increasing again thereafter. This behavior has been interpreted as follows. In basic conditions the carboxylic and phenolic groups are completely deprotonated and the repulsion between the negatively charged sites imparts an extended configuration to HA structures. Under such conditions of pH and concentration, the material might be very hydrophilic thus having no effect on, or even increasing slightly, the ST of the solutions by attracting the water molecules to the bulk of the solution as a consequence of the entropy increasing. The increasing acidity and consequent partial proton-neutralization produces amphiphilic species, which migrate to the surface. Such an effect is reflected in the ST diminution. At very low pH, the molecules, now completely neutralized, become more hydrophobic, with low surface activity and the ST is partially restored, in some cases. Also at acidic pH, HA tend to flocculate. In all cases, the 6 to 7 pH range is critical concerning the surface activity of HA. This pH range is usually found in coastal or estuarine waters, strengthening the environmental implications of such an observation. Differences among samples might be associated to the relative quantities of acidic groups neutralized at this pH range, modifying the percentage of amphiphilic structures from one sample to another. The Gran1 sample, for example, shows hydrophilic behavior over the entire pH range. Being mostly derived from marine OM this HA has peculiar characteristics like as lower molar mass and lower aromatic character than the other samples. According to STEVENSON (1982) the carboxyl content of HA is inversely related to their molar mass. The reduced response of the Gran1 sample to pH may be ascribed to the high carboxylic group density of these relatively small molecules, which allows extensive H bonding with aqueous solvent throughout the pH range, hindering the development of an amphiphilic character (YATES and WANDRUSZKA, 1999).

The presence of NaCl (Figure 1b) modifies the pH profiles but a similar interpretation can be made. In this case, since the negative sites are partially neutralized by the Na the amphiphilic species are developed earlier i.e., at higher pH values, except in the case of the Aldrich HA. Under these conditions the Gran1 HA develop a slightly amphiphilic character in the acidic pH range approaching the behavior of the other samples.

When the salt is MgCl₂ (Figure 1c) the pH profile is different to both the original (without salt) and the NaCl containing profiles. In this case the lake (HA1) and the Aldrich HA do not appear to be affected by the pH influence, the ST values remaining high and almost constant over the entire range of pH. No migration of amphiphilic species to the surface seems to occur and the structures are completely solubilized at any pH. These two samples also showed differentiated ST vs. HA concentration (with I = 0.3) profiles (RAUEN *et al.*, 2002). Here they behave as though they could coil up to protect their hydrophobic moieties producing micro-systems stable in aqueous media, independently of the pH. WANDRUSZKA and co-workers (1997 and 1999) also observed differentiated degrees of sensitivity to pH and salt variations for HA from distinct sources. Presently it is not very clear the reasons for this differentiated behavior relative to the other samples but as in the case of YATES *et al.* (1999) they might be related to special structural features. The Aldrich HA sample is rich in phenolic constituents and the lake HA sample, besides phenolic constituents, presents strong signals of protein moieties with high incidence of amide groups (GIOVANELA *et al.*, 2003). Both HA are also thought to be rich in aliphatic moieties and to present a high molar mass distribution (SIERRA *et al.*, submitted). The simultaneous presence of long aliphatic chains and aromatic moieties can impart to them the ability of develop intramolecular domains in aqueous media. Our data suggest that Mg²⁺ is more efficient in pulling the humic molecules into such a coiled configuration than Na⁺. ENGBRETSON and

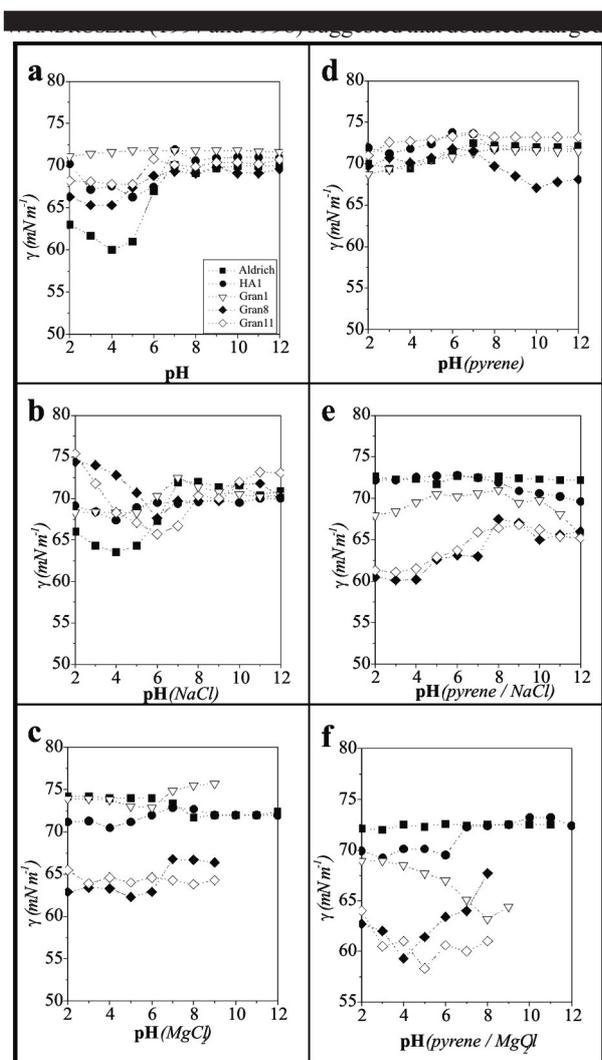


Figure 1. The effect of pH on ST of 30 mg L⁻¹ HA solutions under different conditions. a) HA alone; b) in the presence of NaCl (I = 0.3); c) in the presence of MgCl₂ (I = 0.3); d) in the presence of pyrene (0.1 μmol L⁻¹); e) in the presence of pyrene plus NaCl; f) in the presence of pyrene plus MgCl₂. T = 22 °C.

cations like Mg²⁺ can act as a bridge between different strands of HA macromolecules or between two different low molecular HA structures producing hydrophobic micro-systems in aqueous media. For the other HA two different effects are observed between pH 2 and 8: the Gran1 sample remains in solution, whereas the Gran8 and Gran11 samples develop amphiphilic character and migrate to the surface in this pH range. For these three estuarine HA samples, flocculation occurs above pH 9. Mg²⁺ hydrolysis followed by the accumulation of mixed Mg-OH-HA insoluble complexes might be responsible for this effect (BUFFLE, 1980). The formation of these complexes might be associated to a higher incidence of carboxylic groups in these estuarine samples since the lake (HA1) and the Aldrich HA samples do not show signs of precipitation. The low salt and acidity effects on the Gran1 HA sample may be explained by the same reasons stated above, i.e., its low molar mass and high content of acidic functional groups. The Gran8 and Gran11 HA samples, on the other hand, that had barely developed amphiphilic character in presence of NaCl, are more strongly influenced by the MgCl₂. Such an effect might be also associated with the “bridge” function of the magnesium ion as stated above (ENGBRETSON and WANDRUSZKA, 1997 and 1998).

The presence of pyrene modifies completely the ST vs. pH profiles (Figure 1d). In this case, the ST values are approximately constant over the entire profile, with a slight

increase around pH 6 to 7, in some cases. Such an increase is not substantial but might reflect a mechanistic effect. The decrease observed below pH 7, in the absence of pyrene (Figure 1a), is not seen here and the ST values remain high even at acidic pH values. Such a profile may be interpreted as follows. At high pH, the high density of negative sites maintains the HA molecules in the extended form which do not interact significantly with pyrene molecules. As the HA are hydrophilic and pyrene, in such a concentration, is entirely solubilized in water, the ST values remain close to that of pure water. As the pH is lowered and the negative charges are neutralized, the functional groups may come closer to each other and the flexibility of the structures, as well as the possibility of intra and intermolecular associations, increases. Hence, between pH 6 and 7 the partial neutralization of negative charges, associated with the hydrophobic-hydrophobic interactions between aromatic moieties of HA and pyrene molecules promotes the formation of agglomerates with micelle characteristics. In fact these profiles suggest that pyrene molecules act as nuclei for such arrangements. Such a configuration seems to be relatively stable in the aqueous environment since, even at acidic pH, the migration to the surface practically does not occur. The Gran1 sample, even being highly soluble in aqueous media at any pH (see Figure 1a), seems to be also slightly influenced by the presence of pyrene.

Figures 1e and 1f show the combined salt plus pyrene effect for NaCl and MgCl₂, respectively. Here again differentiated behaviors are observed for the samples. In the presence of NaCl plus pyrene (Figure 1e) the behavior is quite regular for individual samples over the entire pH range. For three samples (HA1, Aldrich and Gran1) the profiles are similar to those observed with pyrene alone. Practically no migration to the surface occurs indicating that the species are mostly dispersed into the bulk of the solution for the reasons stated above, i.e., for the first two HA samples due to the formation of micelle-like domains and for the Gran1 sample due to single solubilization. Contrary to individual experiments with NaCl and pyrene (Figures 1b and 1d), the simultaneous presence of these two components exerts some effect on surfactance of the Gran8 and Gran11 HA samples. Here the structures remain at the surface (i.e., they are more amphiphilic than under the previous conditions) in the acidic range of pH. Above pH 8 deprotonation diminishes their amphiphilic character and they partially “sink” into the solution, increasing the ST values. However, they are not as capable as the lake (HA1) and Aldrich HA samples of pulling into micelle-like configurations and such an inability might be a consequence of their relatively low molecular weight and poor aromatic content when compared to these samples.

With pyrene and MgCl₂ the surface tension values are quite irregular and show approximately the same trends as those for MgCl₂ alone. In the presence of MgCl₂ plus pyrene, at neutral pH, the Gran1 HA sample finally develops some amphiphilic character. Here again the bridge effect of the MgCl₂ seems to be present. As with the other estuarine HA sample, moreover, it flocculates in the alkaline range due to the hydrolysis of Mg²⁺ and the formation of mixed metal/OM hydroxides. As in the case of the MgCl₂ effect alone, the lake and Aldrich HA samples seem not to form such insoluble complexes, whose formation might be associated with a significant presence of carboxylic groups.

CONCLUSIONS

Measurements of the surface tension (ST) of aqueous solutions of a series of HA samples from varied sources under varied conditions of pH, ionic strength and in the presence of pyrene provided a convenient way to assess the relative ability of HA to fold down to produce intramolecular domains in aqueous solutions.

A pH decrease gave rise to an amphiphilic character for four of the five samples tested, with a minimum in ST values observed at around pH 4. The presence of NaCl and MgCl₂

produced differentiated effects on the samples, with the latter salt being the most effective in modifying the HA configurations.

The ability of HA to form hydrophobic domains is closely related to their structural configuration and, consequently, to the OM sources of the samples. The presence of long aliphatic chains combined with a significant presence of aromatic centers, for example, seems to be the most important condition for the formation of such intramolecular domains. Finally, the pyrene seems to act as a nucleus for HA micelle-like arrangements in aqueous environments.

The general interpretations are in line with WANDRUSZKA'S (1997, 1998 and 1999) studies.

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