

HUMIC ACID-LIKE MATTER ISOLATED FROM GREEN URBAN WASTES. PART I: STRUCTURE AND SURFACTANT PROPERTIES

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A humic acid-like substance (cHAL2) isolated from urban green wastes before composting was compared to a humic acid-like substance (cHAL) isolated from a mix of urban organic humid waste fraction and green residues composted for 15 days. cHAL2 was found to contain more aliphatic and O-alkyl C atoms relative to aromatic, phenol, and carboxyl C atoms, and to yield higher critical micellar concentration ($\text{cmc} = 0.97 \text{ g L}^{-1}$) and surface tension at the cmc ($\gamma_{\text{cmc}} = 37.8 \text{ mN/m}$) in water than cHAL ($\text{cmc} = 0.40 \text{ g L}^{-1}$; $\gamma_{\text{cmc}} = 36.1 \text{ mN/m}$). The results point out that biomass wastes may be an interesting source of biosurfactants with diversified properties that depend on the nature of waste and on its process of treatment.

Keywords: Urban refuse; Compost; Biosurfactants; Humic acids; Biomass.

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INTRODUCTION

Separate collection of urban food and green wastes and their compost products are interesting low entropy sources of organic C. They are available from confined spaces, and may have relatively low water (35-55 %) and high organic matter (26-50 %) contents (Quagliotto 2006; Ozores-Hampton and Obreza 2007). Recycling of the organic matter of these wastes for further uses is a worthwhile scope to be pursued for economic and environmental reasons. Composting is carried out nowadays in public and private facilities throughout the world (Kraft 2006; Newman 2006; Ozores-Hampton and Obreza 2007; Stoffella 1997) at a processing tipping fee of about 70 €/ton, while the compost product is proposed as fertilizer at a current market value which is not above 15 €/ton. Therefore, composting urban humid and vegetable residues has a net cost of about 55 €/ton. Very recently we have shown that compost is a rich source of biosurfactants and suggested that these materials may have a range of potential applications in the chemical industry. Indeed a humic acid-like compound isolated from compost was found to have quite remarkable surfactant properties (Quagliotto 2006) and good performance as a chemical auxiliary for dyeing nylon 6 (Savarino 2007). For the sake of simplicity, we

refer to this compound as cHAL. Composting, however, yields a wide range of different products, depending on the compost wastes mix and the composting time. As these compounds might in principle offer a range of properties from which to choose those tailored for specific needs, we wish to report herewith the structure and surfactant properties of a new compost isolated humic acid-like substance (here and after named cHAL2). Compared to cHAL, isolated from a mix of urban organic humid waste fraction and green residues composted for 15 days, cHAL2 was isolated from green urban wastes only before composting. Our long range purpose is to build a data inventory which at some point will make it possible not only to understand how the parameters characterizing the waste nature and the composting process influence the structure and surfactant properties of the isolated humic-like substances, but also to assess how much the source and structural difference of these substances affect their performance as auxiliaries for chemical technological uses.

EXPERIMENTAL

All reagents were Aldrich products, unless otherwise indicated. Ground urban green wastes, collected from the Amiat municipal plant in Torino, Italy were extracted according to a known procedure (Quagliotto 2006) to yield, in 12 % w/w yield relative to the starting dry waste, the humic acid-like material (cHAL2) investigated in this work. This material was found to contain 7.47 % water, 91.60 % volatile solids and 0.93 % ash by the weight losses measured after heating first at 105 and then at 800 °C. Further characterization for cHAL2 was performed by elemental analysis, solid state ^{13}C and solution ^1H NMR spectroscopy, IR spectroscopy, and surface tension measurements as previously reported (Quagliotto 2006). The determination of free phenol and carboxylic acid groups (Table 1) was accomplished by potentiometric titration according to a previous procedure (Brunelot 1989). Under our experimental conditions, deionized water was boiled under nitrogen atmosphere to remove dissolved CO_2 . This water was used to make the required sample and reagents solutions. The cHAL2 sample was dissolved at 1.87 g/L concentration in 0.015 N NaOH. The total alkali content in the solution was in excess relative to the total cHAL2 carboxyl and phenoxide content (Table 1) determined by ^{13}C NMR spectroscopy. This solution with pH about 12 was titrated with standardized 0.01 N aqueous HCl. Similar titration was performed on a blank solution containing the same amount of alkali as the above sample solution, but no cHAL2. The titrations were performed at 25 °C using an automatic Cryson Compact Titrator with a resolution of 1 μl of titrant in a thermostated glass cell under nitrogen blanketing to prevent dissolution of atmospheric carbon dioxide in the sample.

Under these experimental conditions, it was possible to obtain a potentiometric curve of satisfactory quality (Fig. 1). The elaboration of the experimental data (pH versus titrant volume in Fig. 1a) obtained under this condition is based on the linearization functions proposed by Gran (1952). According to the above method, from the linearized data plotted in Fig. 1b it was possible to extrapolate 3 equivalence points. Two of these points were obtained from the sample (cHAL2) titration curve and correspond to the added titrant volumes V_a and V_b . The third equivalence point, corresponding to the

added titrant volume V_c , indicates the endpoint of the titration of the blank alkali solution. This solution contained the same amount of added NaOH as the above titrated cHAL2 solution, but did not contain any cHAL2. In addition to these equivalence points, other two points, corresponding to the added titrant volumes V_{e1} and V_{e2} , were extrapolated from the first derivative of the cHAL2 titration curve in Fig. 1a. Based on the pH values measured at each of the above titrant volumes, the following assignments are given: i.e., V_b is considered the HCl volume necessary to titrate the excess NaOH over the sample total acidity, $V_b - V_{e1}$ is the HCl volume necessary to convert all ArONa groups to ArOH, and $V_{e2} - V_{e1}$, $V_a - V_{e2}$ and $V_c - V_a$ correspond to the volumes of HCl necessary to convert the sodium salts of carboxylic acids with increasing acidity strength to their free acid $-COOH$ forms.

Table 1. Data^a Found for cHAL^b, cHAL2^c and Peat Humic acid (PHA)^{d,e}

	C w/w %	Empirical Formula				
		C	H	N	O	S
cHAL	59.9 ^b	10	13.4	0.86	3.4	0.036
cHAL2	57.9 ^c	10	12.6	0.63	3.0	0.018
PHA	50.4-58.8 ^{d,e}	10	7.3-12.7	0.25-0.52	4.6-5.8	0.052

	pH	COX ^f meq/g	COOH meq/g	ArOY ^f meq/g	ArOH meq/g	MW
cHAL	4.00	5.71	1.10 ^g	3.38	1.90 ^g	15610
cHAL2	3.96	3.56	2.91 ^g	2.46	0.87 ^g	217630 ^h
PHA			2.76 ^e		3.28 ^e	17000 ^e

^aC content (w/w %) in ash free dry matter, elements atoms in empirical formula, pH of a water suspension (12 mg/30 mL) containing 0.01 N NaCl, free carboxylic (COOH) and phenol (ArOH) groups, weight(MW) and number-(MN) averaged molecular weights defined as previously reported (22).

^bIsolated in previous work (Quagliotto 2006).

^cIsolated in this work..

^dPeat (Montoneri 2003).

^ePeat (Terashima 2004).

^fTotal carboxyl ($X = H$ or N) or phenoxide ($Y = H, R, Ar$) contents = $A/(1201.1 B)$, $A = \%$ band relative area from column 8 (carboxyl) or column 7 (phenoxide) in Table 2; $B = 10^{-2}$ C w/w % from Table 1.

^gBy potentiometric titration.

^hData obtained in this work by SEC-MALS.

Dynamic light scattering (DLS) measurements were obtained with a ZetaSizer® (Malvern, UK), which has a detection window included between about 0.6 nm and 5 μ m. The measurements were carried at pH 7.0 and 25 C after filtering the cHAL2 sample solution on a cellulose acetate disk (Schleicher & Schuell) with a size cut-off of 0.8 μ m. The CONTIN method (Provencher 1976) was used to analyze the DLS data for calculating the hydrodynamic diameter (D_h) of the molecules or aggregates in solution.

Electrospray ionization measurements (ESI) were obtained using a LTQ Orbitrap high mass (M) resolving power ($R = M/\Delta M$) spectrometer (Thermo, Rodano, Italy), with electrospray interface and ion trap as mass analyzer. The flow injection effluent was delivered into the ion source using nitrogen as sheath and auxiliary gas (flow rate 10 $\mu\text{L}/\text{min}$). The source voltage was set at 4.0 kV in the negative ion mode. The heated capillary was maintained at 270°C. The tuning parameters adopted for the ESI source were the following: source current 100 μA , capillary voltage -9 V, tube lens -120 V. Mass spectra were collected in full scan negative mode in different ranges between 200 and 1500 mass to charge (m/z) ratio.

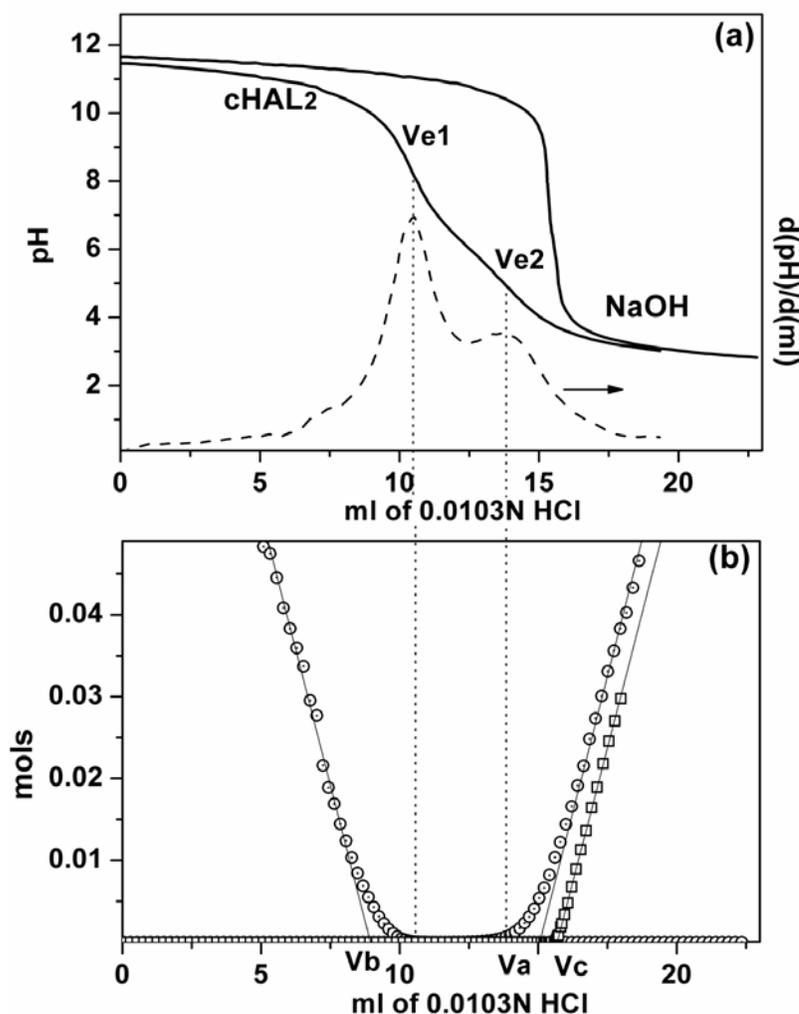


Fig. 1. Potentiometric titration of cHAL2. (a) Titration of 10 ml of a 0.0156 N NaOH solution dissolved in 30 ml of water and back-titration of 18.7 mg of cHAL2 dissolved in 10 ml of aqueous NaOH 0.0156 N and diluted in 30 ml of water. The dashed line indicates the first derivative (dpH/dml) of the cHAL2 back-titration. (b) Linearization of the NaOH titration curve (\square) and of the cHAL2 back-titration curve (\circ) according to the Gran method (Gran 1952).

Molecular investigation was performed also through fractionation and characterization by a multi-angle laser light scattering (MALS) detector on-line to a size exclusion chromatography (SEC) system. The SEC-MALS system consisted of an Alliance 2690 separation module, a 2414 differential refractometer (DRI) from Waters (Milford, MA, USA), and a MALS Dawn DSP-F photometer from Wyatt (Santa Barbara, CA, USA). This multi-detector SEC-MALS system was described in detail previously (Mendichi 2001). The wavelength of the MALS laser was 632.8 nm. The light scattering signal was detected simultaneously at fifteen scattering angles ranging from 14.5° to 151.3°. The calibration constant was calculated using toluene as standard, assuming a Rayleigh factor of $1.406 \cdot 10^{-5} \text{ cm}^{-1}$. The angular normalization was performed by measuring the scattering intensity of a concentrated solution of BSA globular protein in the mobile phase assumed to act as an isotropic scatterer. The refractive index increment, dn/dc , with respect to the solvent was measured by a KMX-16 differential refractometer from LDC Milton Roy (Riviera Beach, FL, USA). The dn/dc value for cHAL2 was 0.214 mL/g. The humic acid-like substance solubilization procedure and the SEC experimental conditions were quite similar to those previously reported (Kawahigashi 2005). The starting sample solution contained cHAL2 at 1 g L^{-1} concentration in 0.01M K_2HPO_4 -0.01M KH_2PO_4 aqueous buffer (pH 7.0) containing 10% methanol. The same solvent was used as SEC mobile phase under the following conditions: single aqueous Shodex OHpak KB805 column from Showa Denko (Tokyo, Japan), 35 °C temperature, 0.8 mL/min flow rate and 100 μL sample injection volume.

RESULTS AND DISCUSSION

To understand compositional and structural difference between materials isolated from composted wastes, one should first consider that composting is an aerobic biodegradation process of refuse biomass, leading to some mineralization of the original organic C and N and to chemical modifications of the remaining organic residue (Genevini 2002). Compared to the starting biomass waste, the composted waste is generally characterized by a lower content of polysaccharides, and by a relatively higher concentration of lignin-like material. Changes also involve chemical identities. Native lignin is modified to lignin-humus. The latter material, although not well defined, is usually characterized by its aliphatic/aromatic C ratio and by its content of carboxylic and phenolic functional groups. The humic acid-like organic fraction is separated (Montoneri 2003a) from the polysaccharide, protein, fats and other humic-like matter by extraction of the starting biomass with alkali and precipitation of the humic acid-like fraction at $\text{pH} < 1.5$. In our case, the humic acid-like material investigated in this work (cHAL2) was isolated from urban green wastes that were sampled right before composting. By comparison, our previously investigated (Quagliotto 2006) humic acid-like material (cHAL) was isolated from a 1:1 v/v mixture of food residues and public parks green wastes that had been composted for 15 days. To evidence the structural features of cHAL2, we like to refer to the above previous cHAL material for which the structure represented in Fig. 2 was proposed, based on the elemental analysis, acid groups

determination, and NMR data. Tables 1-3 report these characterization data for both cHAL and cHAL2.

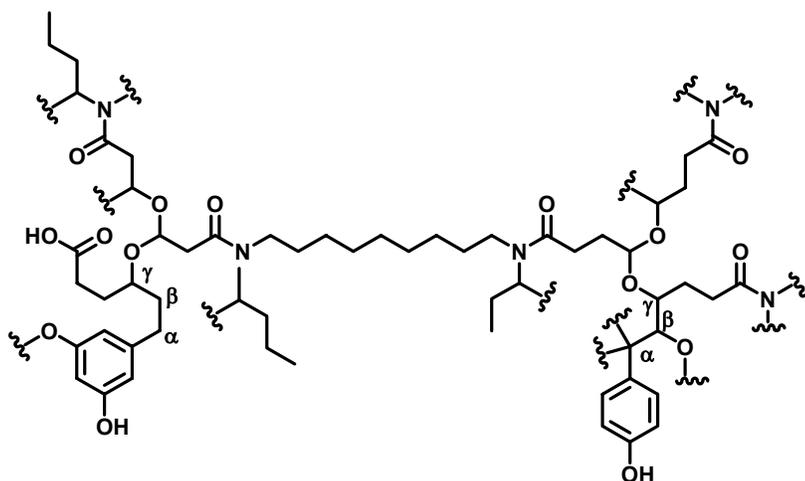


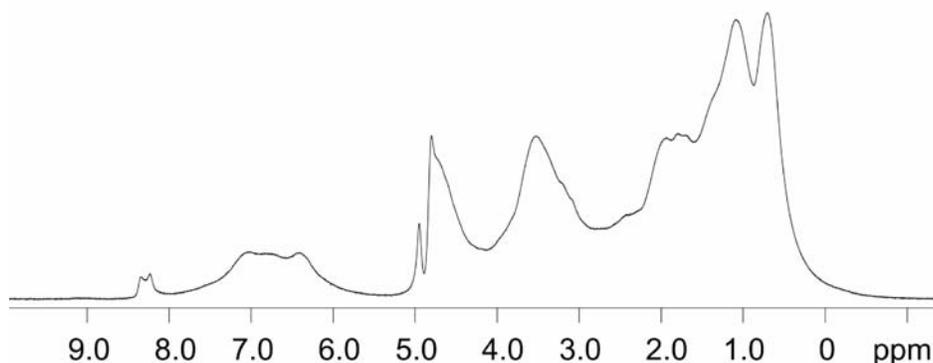
Fig. 2. Proposed molecular fragment for cHAL (Quagliotto 2006). H bonded to C omitted; sinusoidal bold lines indicate other fragments C, O, N atoms.

From Fig. 2 the cHAL structure may be visualized as an elongated hydrophobic portion made by a central 9 C long chain and by peripheral 2-3 C short chain aliphatic amide and di-O alkyl ether groups, to which two polar hydrophilic substituted propylphenol hydrocarbon residues are attached. The data obtained for cHAL2 (Tables 1-3) show that this material, compared to cHAL, is likely to have some significant structural differences: i.e., relative to the composition of the molecular fragment represented in Fig. 2 for cHAL, cHAL2 has 15 short chain aliphatic C, 5 O-alkyl C and 1 di-O-alkyl C more (Table 2). On an eq/g concentration basis, these features are accompanied by a relatively lower content of carboxyl and phenoxide functional groups (Table 1).

One aspect deserving particular attention in the characterization of humic acid-like substances is the determination of acid groups. It may be observed from the data in Table 1 that large fractions of the carboxyl and phenoxide groups consist of free carboxylic acids (-COOH) and phenolic (ArOH) groups. For the determination of these groups we have adopted the method suggested by Brunelot et al. (1989) for the potentiometric titration of humic and fulvic acids. As pointed out in the experimental section, these authors suggest that the potentiometric titration method may also yield the breakdown of carboxylic acids into functional groups with increasing acidity strength. We are convinced that this point would deserve deeper investigation in a specifically dedicated work. Within the more general issue addressed by our work, we are content with reporting the results in Table 1 as phenol (ArOH) and total carboxylic acid (COOH) groups. It may be observed that the values obtained for these groups by the above potentiometric titration method are consistent with the data obtained for total carboxyl

and total phenoxide groups by ^{13}C NMR spectroscopy (Fig. 3), inasmuch as the free acid groups turn out a fraction of the total phenoxide and carboxyl groups.

^1H NMR



^{13}C NMR

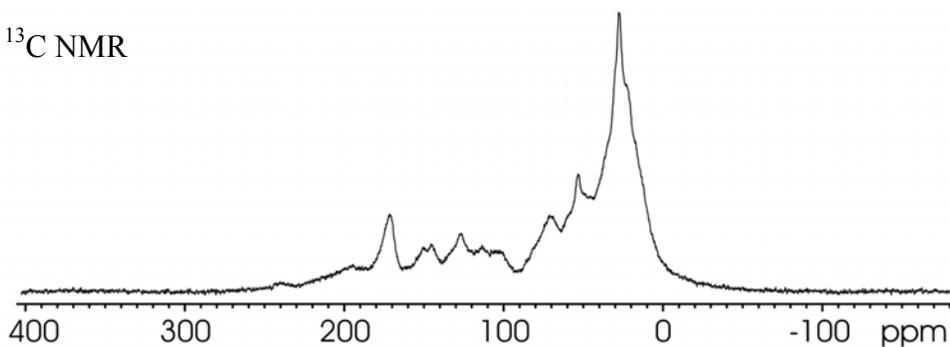


Fig. 3. Proton and carbon NMR spectra of cHAL2.

The IR spectra of the two humic acid-like compounds were also consistent with the presence of several functional groups and C bonds. IR peak absorbances were found at 1718 cm^{-1} for free carboxylic acid groups, at 1518 cm^{-1} for amide groups, at 1458 cm^{-1} for aromatic rings, at 1278 cm^{-1} for C-O bonds in phenol, phenyl ether, and COOH groups, in the $2000\text{-}3700\text{ cm}^{-1}$ range for O-H and/or N-H groups in COOH, phenol, amide groups, and carbohydrates, and at 2928 and 2855 cm^{-1} for protonated aliphatic C chains (Quagliotto 2006). In agreement with the NMR spectra the IR spectrum of cHAL2, compared to that of cHAL, showed higher intensity of the bands assigned to protonated aliphatic C chains and OH groups relatively to the bands assigned to C-O bonds in phenol, phenyl ether, and COOH groups. These features are likely to be memory of the main molecular constituents of the investigated green wastes such as polysaccharides, protein, lipids, and lignin. Particularly the higher content of O-alkyl and di-Oalkyl C in cHAL2, which is supported by the relatively higher intensities of both the ^{13}C NMR signals at 53-110 ppm (Table 2) and of the ^1H NMR signals at 2.5-4.2 ppm (Table 3), is equivalent to the presence of 1 carbohydrate hexose fragment per two aromatic rings.

Table 2. Assignments by Chemical Shift (δ , ppm) ranges and Relative Area of ^{13}C CPMAS NMR Bands, and C Distribution per Molecular Fragment Containing Two Aromatic Rings.

	Assignment band δ range (ppm)	1 Aliphatic C bonded to other aliphatic chain or to H		2	3	4	5	6	7	8	9	10
		Short chain 0-32	Long chain 32-53	Total aliphatic C 0-53	O-CH ₃ or N-alkyl C 53-63	O-alkyl C 63-95	di-O-alkyl C 95-110	Aro-matic C 110-140	Phenol or phenyl ether C 140-160	Carbox-yl C 160-185	Keto C 185-215	Other C 215-250
cHAL	band relative area (%)	32.3	12.9	45.2	8.4	9.2	3.5	14.8	6.8	11.5	0.6	-
	C distribution for two aromatic rings	17.9	7.2	25.1	4.7	5.1	1.9	8.2	3.8	6.4	0.33	-
cHAL2	band relative area (%)	36.0	13.6	49.5	6.5	11.5	3.6	9.1	5.1	7.4	4.6	2.6
	C distribution for two aromatic rings	30.4	9.5	39.9	5.5	9.7	3.0	7.7	4.3	6.2	3.9	2.2

Table 3. Assignments by Chemical Shift (δ , ppm) ranges and Relative Area of ^1H NMR Bands

	H in aliphatic C for hydrocarbon chains substituted at β or farther C, or in CH ₂ and CH ₃ bonded to aromatic C, or to carboxylic or amide groups		H in methine group bonded to aromatic C, or in aliphatic C bonded to O or to N	aromatic and olefinic H
band δ range (ppm)	0-1.2	1.2-2.9	2.9-4.3	5.9-8.1
cHAL2	23.6	39.0	26.5	10.9
cHAL	47.2	28.1	15.1	9.6

This molecular fragment is unlike to represent evidence of poly- or oligo-saccharide molecules simply mixed with the humic acid-like matter, since this material has been isolated by precipitation at $\text{pH} < 1.5$ (Montoneri 2003a). We rather favour the hypothesis that the above fragment was bonded covalently to the alkyl-phenoxy lignin-like part of the humic acid-like molecule. The higher content of fragments with carbohydrate structure in cHAL2 than in cHAL seems consistent with the different origin of these materials. Indeed, cHAL is a humic acid-like compound isolated from a refuse subjected to aerobic biodegradation for 15-d. Therefore, considering the longer biodegradation of cHAL relatively to cHAL2, a higher content of molecular fragments with carbohydrate structure is to be expected in the latter for thermodynamic and kinetic reasons (Montoneri 2003b). Also, it may be observed that the ^1H NMR spectra (Table 3) indicate a relatively higher content of aromatic H in cHAL2 than in cHAL. This fact, coupled with the relatively lower content of aromatic C indicated by the ^{13}C data (Table 2), suggests that the aromatic rings in cHAL2 are both fewer and less substituted. In turn, the lower substitution degree of the aromatic rings in cHAL2 seems consistent with the lower content of phenoxy groups reported in Table 1.

As most of the potential technological appeal of compost isolated humic acid-like substances have been inferred by us (Quagliotto 2006) to lie on their surface activity properties in solution, surface tension measurements constitute a basilar characterization for these compounds. Surfactants properties for cHAL2 were expected based on its structural analogies with cHAL and naturally occurring humic acids, i.e., presence of hydrophobic C chains and of polar groups. In principle, surfactants should give a surface tension (γ) versus concentration (C) plot where two clear linear regimes, premicellar and postmicellar, are evidenced. This is mostly found for simple surfactant molecules with one polar head. Oligomeric surfactants, such as the gemini surfactants (Quagliotto 2006) show a gradual transition between the two regimes, whose extension may be very large. These surfactants are made of molecules in which two or more polar groups are connected by lipophilic chains of variable length. Their oligomeric or polymeric nature is a key point in determining their capacity to micellize. The same could be expected for humic-like substance. When these materials are in solution as single molecules, these lie at the air-water interface to expose as the lowest possible hydrophobic surface to water. At higher concentration, when the air-water interface is saturated, the excess surfactant molecules aggregate forming micelles in the bulk water phase. In this form several molecules are held together by intermolecular forces to yield spherical or quasi-spherical clusters where hydrophobic surfaces stay in the inner micellar core and polar heads are directed toward the water phase. In a typical case, the premicellar and the postmicellar regimes are defined by two linear γ -C with different slopes. The intersection of the two

lines gives the cmc. For cHAL2, the results of our surface tension measurements against concentration are shown in Fig. 4. From the γ -C plot the slope change can easily be identified and the cHAL2 critical micellar concentration (cmc) and the surface tension at the cmc (γ_{cmc}) may be calculated 0.97 g L^{-1} and 37.8 mN/m . These values are higher than those reported for cHAL, i.e., $cmc = 0.40 \text{ g L}^{-1}$ and $\gamma_{cmc} = 36.1 \text{ mN/m}$ (Quagliotto 2006). Although the higher content of aliphatic C of cHAL2 usually would lead one to expect a lower cmc value than that of cHAL, the higher content of polar functional groups contributed by the O-alkyl and di-O-alkyl C in the former may introduce a higher degree of hydrophilicity that hinders micelles formation (Sulthana 2000). A similar effect has been reported for humic acids isolated from peat and soil upon increasing the relative content of carboxylic groups (Quagliotto 2006).

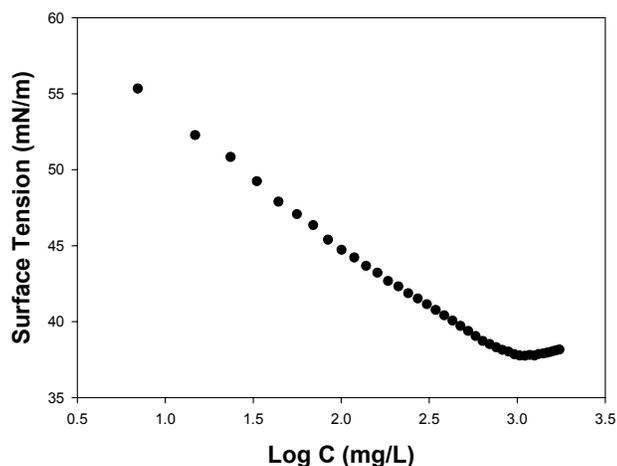


Fig. 4. Surface tension versus concentration (Log C) for cHAL2 water solution at pH 7

One structural feature of humic acid-like material which is currently a matter of dispute is their molecular weight (Sutton 2005). Measurements based on size exclusion chromatography (SEC) indicate values of the order of 10^4 - 10^5 D, whereas measurements performed by electrospray ionization (ESI) indicate values of the order of 10^3 Da. The ESI results, coupled with the fact that by gel permeation chromatography and high-pressure size-exclusion chromatography some workers have found that the apparent size of humic fragments changes drastically with addition of simple organic acids, has originated the belief that humic acid-like substances are aggregates of small molecules linked by hydrogen bonds and hydrophobic interactions, and not polymeric compounds containing covalent bonds only. The dispute over the molecular weight of humic acid-like substances is not only academic, but has relevance in relation to the performance of these materials as surfactants. With specific reference to our cHAL2 material, assessing the size of the molecules which give rise to the curve in Fig. 4 would make it possible to establish structure-properties relationships and then to use these in order to exploit the full potential of the material for specific technological applications. Thus, in this paper, we have address the molecular weight issue based on considerations and on experimental results which are described hereinafter.

In principle humic acid-like substances may yield two types of conformation in solution, which may be responsible for important interaction with other organic and inorganic species. These may be classified as micelles and pseudo-micelles (von

Wandruszka 2000). Whereas the former are aggregates of relatively small molecules held together by intermolecular forces, the latter may be visualized as macromolecules which, by virtue of the flexibility of the C chain, may fold and coil in a manner that directs hydrophilic (e.g. carboxy and hydroxy) groups outward and keeps more hydrophobic (e.g. hydrocarbon) moieties isolated in the center. This process produces an entity that is operationally similar to a conventional micelle, albeit more structurally constrained. Like a micelle, it has a hydrophobic interior and a more hydrophilic surface, giving it distinct solubilizing powers for nonpolar solutes. For our cHAL2 materials, we have carried out measurements of the molecular size and mass by three different techniques: i.e., ESI, SEC-MALS and DLS.

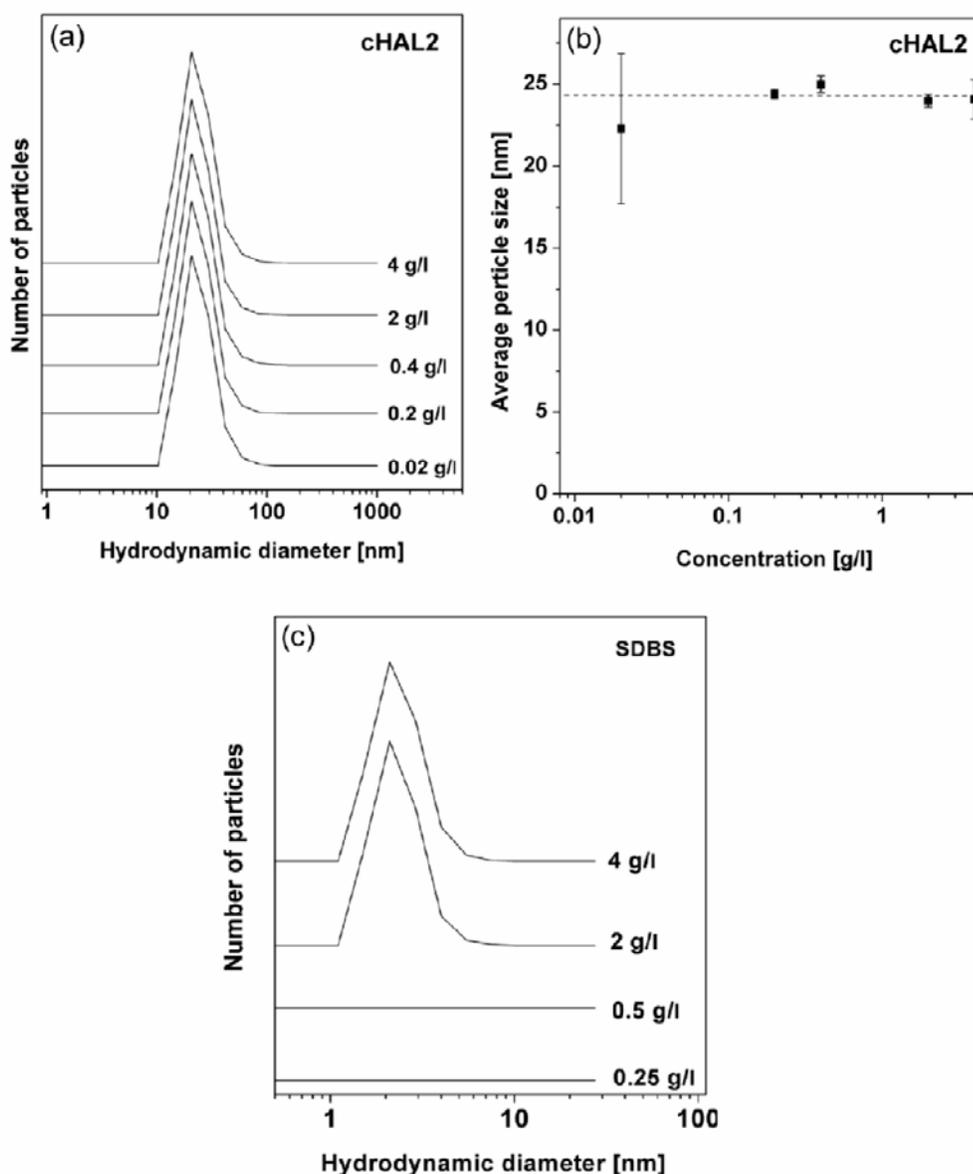


Fig. 5. Comparison between the particle size at different concentrations of cHAL2 (a and b) and the commercial additive SDBS (c). The particle size distribution of the two surfactants was measured at concentrations above and below the respective cmc values.

Dynamic light scattering (DLS) theory is a well established technique for measuring particle size over the 10^{-10} - 10^{-6} m range (Microtrac, Inc. 2007). DLS measures the velocity at which particles within a solvent diffuse due to Brownian motion. This is done by monitoring the fluctuation in intensity of the scattered light over the time. Indeed the scattered intensity due to the phase addition of the moving particles is constantly evolving, depending on the particle size. The Brownian diffusion velocity is inversely proportional to the particles size, expressed as hydrodynamic diameter (D_h). As this technique allows the determination of particle size distributions in solution at variable concentration, and therefore allows the calculation of the hydrodynamic diameter of the molecules or aggregates in solution, we performed DLS measurements at variable cHAL2 concentration (0.02-0.2 g L⁻¹). Based on our surface tension data (reported above) indicating formation of micelles for cHAL2 at 0.97 g L⁻¹, we expected by carrying DLS measurements below and above this concentration value to observe a change of D_h with cHAL2 concentration; i.e., a D_h increase above the 0.97 g L⁻¹ value due to the formation of molecular aggregates. Contrary to this expectation the plots in Fig. 5 do not show any significant change of D_h with concentration. The DLS measurements were repeated three times for each sample, and an average D_h value was calculated for every concentration. In Fig. 5b the average size, as calculated from three measurements, is reported as function of the concentration. The vertical bars indicate the standard deviation for every set of measurements. The data suggest that cHAL2 consists of particles with sizes included between 10 and 100 nm, and that this particle size distribution does not change upon changing the sample concentration. An average D_h of 24.4 nm was extrapolated from all the data here presented.

The same particle size was found by DLS measurements of a reference humic acid (HA) sample isolated from Suwannee River and supplied by the International Humic Substance Society (IHSS, St. Paul, USA) which was analyzed in solution at pH 7 and 0.04 g/L concentration (Baalousha 2006). The sizes measured for cHAL2 and the IHSS HA do not imply the absence of smaller particles. In fact, scattering intensity is inversely proportional to the sixth power of the radius for particles smaller than the wavelength of the laser; thus, larger particles scatter light more effectively than smaller ones. Therefore, large particles mask the presence of smaller ones in the sample. By comparison, we performed the same measurement on sodium dodecylbenzenesulfonate (SDBS). This well known synthetic commercial surfactant is reported to form micelles at 0.7-1.4 g L⁻¹ concentration in water and at pH 7 (Savarino 2007). With this molecule, our DLS measurements (Fig. 5c) showed signals at 2-4 g L⁻¹ concentration, whereas below the surfactant cmc value (0.25-0.50 g L⁻¹) the concentration the scattered intensity was extremely low and only the noise due to impurities bigger than 30 nm in the solution was detectable. In may be observed that the range of particle size for the SDBS small molecule is one order of magnitude lower than that observed for cHAL2. Also, contrary to the cHAL2 case, the data for SDBS indicate an increase of the particle size above the surfactant cmc value. The SDBS data therefore prove that DLS may show aggregate formation for small molecules, in the absence of large masking particles. By comparison, the lack of change of D_h with cHAL2 concentration, and therefore the absence of correlation between DLS and surface tension data, suggest that the large particles observed by DLS are not responsible for the surface tension dependence on concentration shown in Fig. 4, since their size seems unchanged over a very wide concentration range. Although the data do not make it possible to assess whether the large particles are aggregates or macromolecules, other small molecules must be present together with the larger particles. The small molecules would aggregate upon raising the cHAL2 concen-

tration and be responsible of the change of surface tension observed in Fig. 4. Evidence for the presence of both small molecules and large molecules was sought by the other two techniques, SEC-MALS and ESI.

In the SEC-MALS measurements the optimisation of the solubilization procedure and of the SEC experimental conditions for humic acid-like substances is not trivial. As reported in literature (Quagliotto 2006) and shown hereinafter, humic acid-like substances in aqueous solution are strongly aggregated and may yield micelles of various sizes. Furthermore, the aggregation extent may be significantly affected (Terashima 2004) by pH, ionic strength, and type of counterion of the used solvent. In addition to this, the SEC fractionation for these substances is a problem because column non-steric separation could occur. Often upon using non-optimized SEC conditions, multimodal and/or asymmetrical chromatograms with very long tails are obtained. With cHAL2 in the water- methanol solvent (see experimental), we have obtained a quasi-symmetrical chromatogram without a long tail, as opposed to asymmetrical long tail chromatograms obtained in the pure aqueous solvent. This fact suggested minimization of any presumable molecular aggregation due to the presence of methanol in the sample and elution solvent.

Figure 6 reports the experimental differential refractometer index (DRI) and light scattering (LS) signals, and the calculated molar mass (M) as a function of the elution volume (V). The $M=f(V)$ experimental function is the classical SEC calibration curve obtained directly from the on-line MALS detector. Using the $M=f(V)$ experimental function and the DRI curve, the molar mass distribution (MMD) for the cHAL2 sample reported in Fig. 7 was obtained. A summary of the molecular characterization of this sample is reported in Table 4. Basically, the cHAL2 sample shows an apparent molar mass centred around 150 kg/mol with a polydispersity of about 1.5-2.

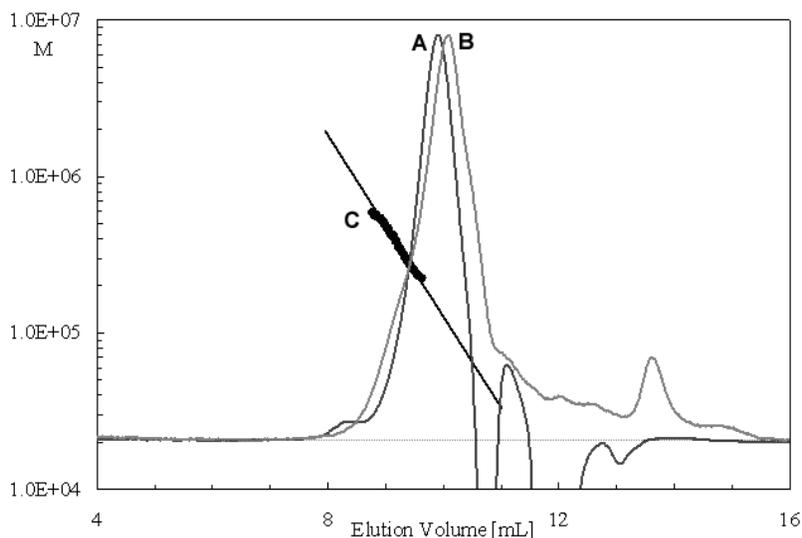


Fig. 6. Superimposition of concentration by DRI (curve A) and MALS with photodiode located at 90° (curve B) signals together with the calculated molar mass (M) as a function (line C) of the elution volume (V) for the cHAL2 sample.

Table 4. SEC-MALS Molecular Characterization^a of cHAL2.

M_p	M_n	M_w	M_z	M_w/M_n	M_z/M_w
g/mol	g/mol	g/mol	g/mol		
143,400	149,340	217,630	432,340	1.5	2.0

^aMolar mass of the chromatographic peak (M_p), number- (M_n), weight- (M_w) and z- (M_z) molar mass averages, and polydispersity indexes M_w/M_n and M_z/M_w .

The on-line MALS detector is also able to measure the molar mass (M) of the eluting fractions together with the dimension of the macromolecules, which is generally known as radius of gyration (R_g). This latter parameter is measured from the angular variation of the scattering intensity, when this is approximately higher than 8-10 nm. The $R_g = f(M)$ experimental function, (i.e., the conformation plot) of the cHAL2 sample is shown in Fig. 7. It is evident that the dimensional R_g parameter for the cHAL2 sample approximately ranges from 10 nm to 20 nm. Furthermore, from the estimated slope of the conformation plot (approximately 0.4) a compact quasi-spherical conformation of the cHAL2 aggregate or molecule could be inferred. The above 10-20 nm R_g value measured by SEC-MALS is consistent with the results obtained by DLS. By comparison, the molecular radius of naturally occurring humic acids has been reported to increase from 9.8 to 17.8 Å as the molecular weight increases from 5000 to 30000 (Tan 2003). Similarly to the DLS results, the SEC-MALS do not provide evidence of the presence of small molecules.

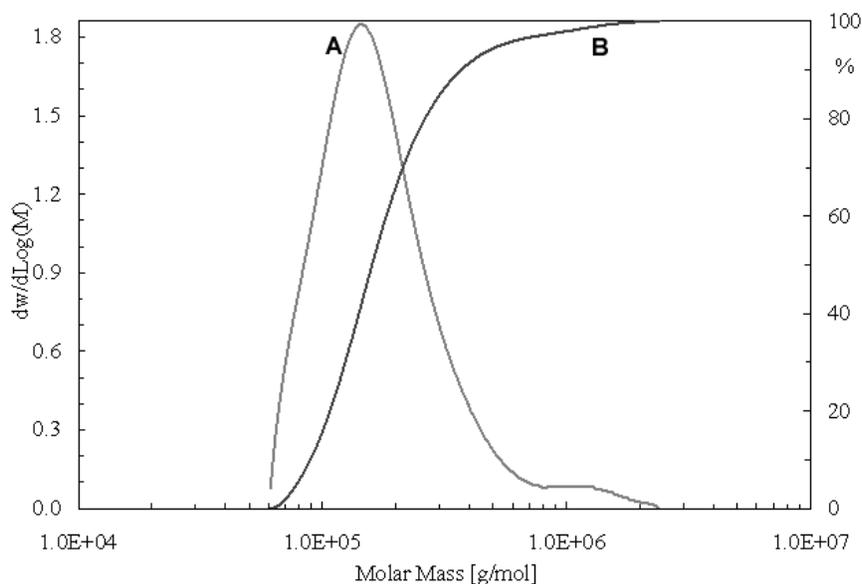


Fig. 7. Differential [$dw/d\text{Log}(M)$ for curve A] and cumulative (%) for curve B) molar mass distribution (MMD) from SEC-MALS measurements for the cHAL2 sample.

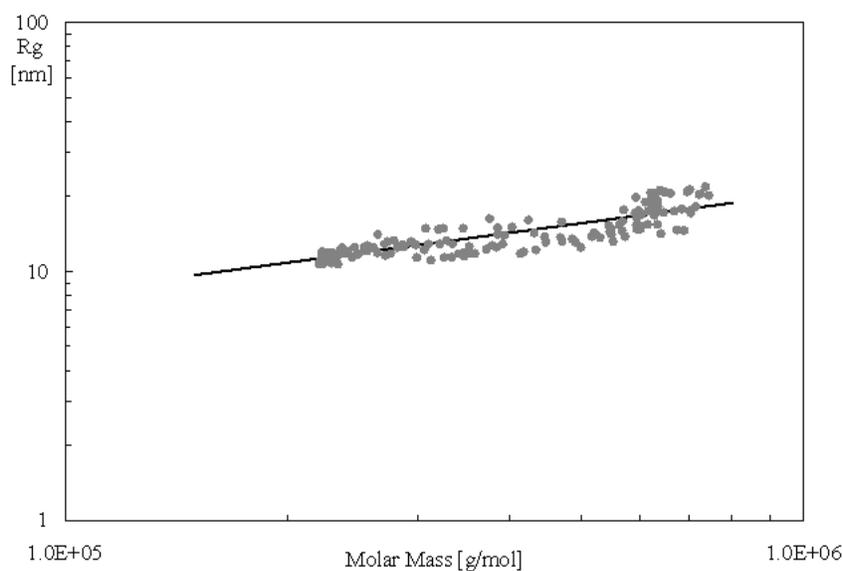


Fig. 8. Conformation plot from SEC-MALS of the cHAL2 sample; radius of gyration (Rg) as a function of molar mass.

Soft desorption ionization techniques, such as electrospray ionization (ESI), volatilize large ions for identification via mass spectrometry (MS), providing positive or negative ion mass/charge distributions that may represent the mass distribution of molecules within humic fractions (Sutton 2005). By this technique compounds may be analyzed from aqueous or aqueous/organic solutions at variable concentrations, similarly to the case of the above DSL measurements. As solvent evaporation occurs, the droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit). At this point a "Coulombic explosion" occurs and the droplet is ripped apart. This event produces smaller droplets that can repeat the process as well as naked singly or multiply charged analyte molecules. High resolution mass spectrometry makes it possible to assign the charge value to an ESI signal originated by an ionized molecule through the examination of the signal isotopic peaks intensity.

Our ESI measurements were performed on cHAL2 and SDBS solutions at the same pH and sample concentrations as for the DLS measurements. The results of the ESI measurements for cHAL2 showed that below the surfactant sample cmc value the signals intensity was too low to allow any useful analysis of the recorded mass spectra. Above the cmc value, several significant signals were observed over the investigated 200-1500 m/z range (Fig.8). For the main signals falling in the 400-450 m/z range, z was proven equal to 1 by the distinctive isotopic pattern for singly charged ions which was observed in the spectra acquired at 60000 resolving power. Also, above the cHAL2 cmc value, the relative intensity of these signals was shown to increase linearly with the sample concentration. The same could not be assessed for the signals at m/z > 500. Although several signals at these m/z values are present in Fig. 9, their relative intensity did not change significantly with the sample concentration. These signals therefore were assigned to the measurements background noise and were not further analyzed for our scope. By comparison for the SDBS solutions in the 0.02 and 2 g L⁻¹ concentration range, which included the 0.7-1.4 g L⁻¹ values reported for the cmc of this compound (Savarino 2007), the signal of the C₁₂H₂₅(C₆H₄)SO₃⁻ anion at 325 m/z was well observed, together with

other two signals at 311 and 339 m/z which were likely to arise from $C_{11}H_{23}(C_6H_4)SO_3^-$ and $C_{13}H_{27}(C_6H_4)SO_3^-$ impurities. For this compound, the intensity of these signals increased linearly with concentration, but the same did not occur for the higher m/z signals, which were therefore assigned to the background noise. The results obtained for SDBS demonstrate that ESI is not able to assess the presence of molecular aggregates, as the weak interactions holding these species are broken during spray ionization. However, for the present work, the ESI results are highly important, inasmuch as they prove the presence of small molecules with molecular weights around 450 in the cHAL2 humic-like material, nearly half the weight of the fragment represented in Fig. 2. The enlarged spectra in the lower m/z region revealed repetitious peaks at discrete 14 Da. These suggest the presence of molecules with methylene chain of different lengths and are consistent with the above discussed NMR and IR spectroscopy findings. The ESI results also do not exclude the presence of larger molecules, as these might not volatilize under the analytical experimental conditions. Although the results obtained by the above three techniques together suggest the presence of small molecules together with large macromolecules, the real molecular mass distribution in the cHAL2 sample cannot be assessed.

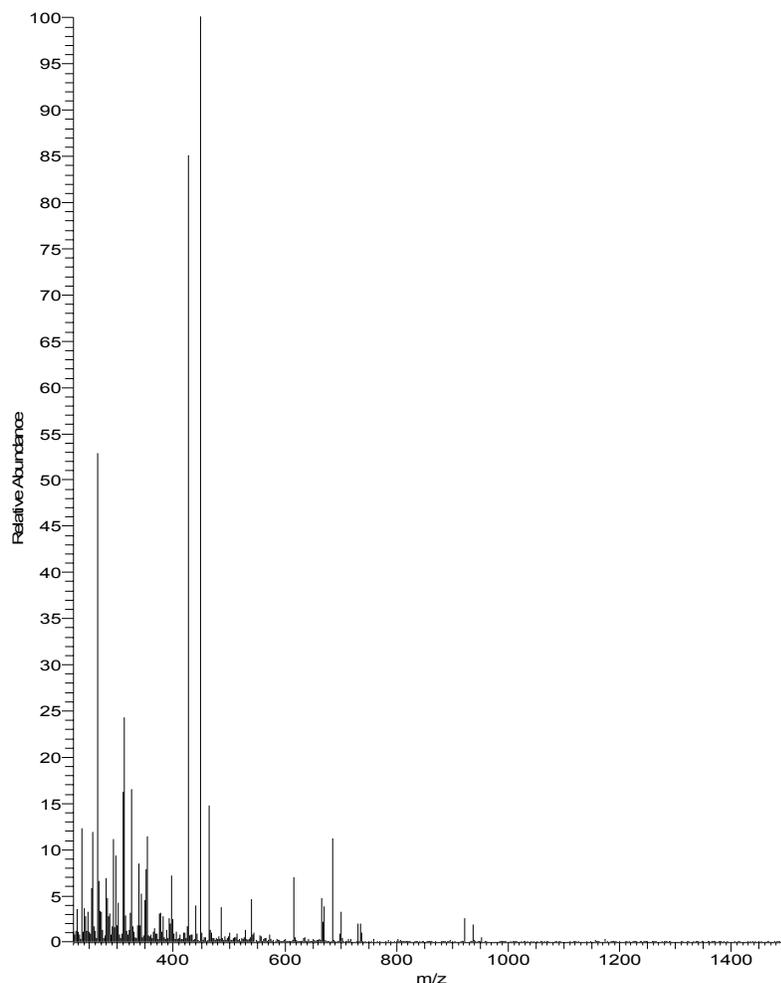


Fig. 9. Negative ion mode ESI spectrum acquired starting from 2.0g L^{-1} cHAL2 solution at pH7.

CONCLUSIONS

We have shown that two humic acid-like substances isolated from two different urban waste sources have different chemical structural features that significantly affect their surfactants properties. The results point out that biomass wastes may be an interesting source of biosurfactants with diversified properties that depend on the nature of waste and of its treatment process. Our structural investigation on cHAL2 suggests that, similarly to naturally occurring humic acids (HAs), it is likely to be a mixture of small molecules with molecular weight of the order of few thousands Da or less, and macromolecules with molecular weights of several hundred thousands. Whereas the size of the latter is not affected by their concentration in water, the small molecules probably can aggregate and be responsible for the surface tension versus concentration behaviour shown in Fig. 4. As to the potential performance of this humic acid-like substance in chemical technology, these materials could perform as classic surfactants due to their content of small molecules and capacity to yield micelles, and as dispersants due to their content of macromolecules (Showell 2006). In the next part of this work (Montoneri 2007, "Part 2") we will report the performance of cHAL2 as an auxiliary in a few important chemical technological applications and discuss the significance of the above structural features on performance rate.

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