

EFFECT OF MOLECULAR WEIGHT DISTRIBUTION ON CHEMICAL, STRUCTURAL AND PHYSICOCHEMICAL PROPERTIES OF SODIUM LIGNOSULFONATES

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ABSTRACT

Chemical, structural and physicochemical properties of six sodium lignosulfonates (SLS) fractions with high purity and narrow molecular weight distribution (M_w) ranked between 2307 and 19583 g.mol^{-1} were studied. Structural characterization of these fractions, using ^{31}P Phosphor Nuclear Magnetic Resonance (^{31}P NMR) and Fourier Transformed InfraRed (FTIR) analysis, was reported. ^{31}P NMR and FTIR analyses show that these fractions present an important variability of hydroxyl, carboxyl and sulfonic group content. The adsorption isotherms of SLS fractions; fitted by Guggenheim–Andersen–de-Boer model; present different isotherms profile and different values of binding energy and adsorption capacities. SLS fractions were found to be highly charged and present the behaviour of soft particle. Intermediate fractions with M_w of 4297 and 2471 g.mol^{-1} give the highest surface activity and antioxidant capacity. Moreover, fractions with the highest molecular weight, M_w more than 6953 g.mol^{-1} , present the greatest charge density and apparent viscosity. This data can help to develop new niche applications for the SLS.

Keywords: Sodium lignosulfonates, diafiltration, physicochemical properties, antioxidant capacity.

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INTRODUCTION

Lignosulfonates (LS) are commercially available lignins obtained from sulphite pulping of wood. More than 1,000,000 tons are annually marketed¹. The presence of the sulfonate groups confers to them a high solubility in water. Taking into account to their structure, LS exhibit dispersive, stabilizing, binding, complexing, antioxidant and antifungal properties²⁻⁶. These properties open to LS several applications. So, they can be used as concrete admixtures, gelling additive in resins preparation, stabilizing agent of emulsions and foams, raw material in the production of fine chemicals (vanillin, pyrocatechol ...), as well as binders in feed due to their antioxidant and sequestering metal ions capacity⁷⁻¹¹. However, their effective industrial use takes place mainly in dispersing and binding applications characterized by very low added values. This limitation is due to the pulping method, the salt used and the origin of wood^{12, 13}. Thus the obtained LS are characterized by a relatively complex chemical composition, a wide range of molecular weight distribution and therefore a high heterogeneity of their physicochemical properties¹². Depending on the pulping method, unless four LS (Calcium LS, Sodium LS, Ammonium LS and magnesium LS) are available. The main studied ones are calcium LS (CLS). The obtained results with these biopolymers showed that the structure, the molecular weight distribution and as well as additives affect strongly the physicochemical properties and application performances of LS^{14, 15}. Due to the difficulties of fractionation of LS few papers investigated the effect of molecular weight distribution on the reactivity and physicochemical properties of these biomolecules. For CLS, Ouyang et al.¹⁶ studied the dispersive and adsorption capacity of different fractions obtained by ultrafiltration, and observed that, in aqueous suspension, these two properties increase when the molecular weight increases. Moreover, Yang et al.¹⁷ reported that for fractions obtained with higher molecular weight the hydrophobic interactions is the main driving forces for adsorption, while for fraction with lower molecular weight the hydrogen-bond and the attractive power of anionic groups are the main driving forces for adsorption. These latter showed also, that the potential zeta is depending on mass concentration and lowest values are reached with

fractions characterized by intermediate molecular weight. For Sodium lignosulfonates (SLS), Li et al.¹⁸ showed that the dispersion ability increased with molecular weight and increasing sulfonic groups. Yang et al.¹⁹ studied the effects of the molecular weight of SLS on their capacity to reduce the viscosity of the coal water slurry, the adsorption behaviour and the zeta potential. They reported that fractions with molecular weight between (10 kDa and 30 kDa) lead to a better effect on reducing viscosity while fraction greater than 10 kDa is more adsorbed on the coal surface. The potential zeta is more linked to the presence or the absence of the sulfonic and carboxyl groups.

As it was mentioned previously the main study was focused on the CLS properties. Few studies were dedicated to SLS where these biopolymers exhibit interesting properties of adsorption and viscosity reducer²⁰. The aim of this work is to study the effect of molecular weight distribution on the structural and physicochemical properties of different SLS fractions, obtained by diafiltration process using five membranes with a cut off in the range of 5 to 300 kDa. To remove salts and to obtain fractions with a narrow molecular weight distribution, the diafiltration was fed by a five volume of demineralised water/volume of SLS solution. For each fraction chemical and structural characteristics (molecular weight distribution and functional groups) and physicochemical properties (hydration and charge properties, surface activity, antioxidant capacity and rheological property) were investigated and discussed.

EXPERIMENTAL

Materials

Sodium lignosulfonates (SLS) composed by 90 wt. % of LS, 4 wt. % of reducing sugars and 6 wt% of total impurities and AAPH 2,2'-Azino[2-methyl-propionomidin] dihydrochlorid 97% purity, were furnished by (Aldrich, Deutschland).

ABTS 2,2-Azino-bis(3-ethylbenzo-thiozoline-6-sulfonic acid) 98% purity was provided by (Sigma, Deutschland).

FL Fluoroscetin (free acid) was furnished by (Flucka, Deutschland).

Diafiltration

Diafiltration (DF) in feed and bleed mode was carried out to remove impurities, like sulphur, ash and salts and to obtain different fractions with a narrow molecular weight distribution. The washing step was realized at a constant volume by adding continuously demineralised water until 5 volume of SLS solution. Five ceramic tubular membranes (TAMI, France) with successively molecular weight cut-off (MWCO) (300000, 150000, 50000, 15000 and 5000 Da) were used. The retentat was concentrated using a rotary evaporator, freeze-dried and weighed. After each step the obtained permeate was then fractionated using the next MWCO membrane. SLS was separated into six fractions with molecular weight ranges: more than 300000 (F₁), 300000~150000 (F₂), 150000~50000 (F₃), 50000~15000 (F₄), 15000~5000 (F₅) and less than 5000 (F₆). DF was performed under pressure and temperature of 5 bars and 50°C, respectively.

SLS determination

The SLS content of the commercial sample and the different fractions was determined as reported by Ringena et al.²¹, at 280 nm, using UV detector 6000LP (Thermo, France). Commercial SLS were used as a calibration standard due that their content of LS is known (90%).

Reducing sugar content

Reducing sugar was determined using method described by Miller²².

Size exclusion chromatography analysis

Commercial SLS and their fractions were analysed by Size Exclusion Chromatography (SEC) (HPLC LaChrom Merck, Deutschland). The system consists of a pump L-2130, an autosampler L-2200, and a Superdex 200HR 10/30 column (24ml, 13 µm, dextran/cross linked agarose matrix). Detection was performed using UV detector diode L-2455 at 280 nm. Before analysis, the samples were filtered using regenerated cellulose membrane (0.22 µm) and aliquots of 50 µl were injected to the SEC system. Commercial SLS and their fractions were dissolved in 0.1% solution of Buffer Phosphate pH=7, 0.15 M

NaCl. The same solution was used as an eluent. The flow rate was 0.4 ml at 25°C and 11 bars. The calibration was performed using polystyrenes sulfonate (PSS) with weight average molecular weight between 73900 Da and 1100 Da as standard.

Fourier transformed infrared analysis

Transmission infrared spectra of the SLS fractions were performed using Fourier Transformed InfraRed (FTIR) spectrometer tensor 27 (Bruker, France) equipped with a Platinum ATR optical cell and a deuterated triglycine sulphate (DTGS) detector. Samples (powder) were placed directly on the crystal. The diaphragm was set to 6 mm and the scanning rate to 10 kHz. Each spectrum was recorded 156 scans. The wave number range used is 4000 and 800 cm⁻¹ with resolution of 2 cm⁻¹. The spectra were baseline corrected for further analysis.

³¹P NMR

NMR experiments were performed on a Bruker Avance-400 spectrometer (Bruker, France), using an inverse-gated decoupling (Waltz-16) pulse sequence with a 30° pulse angle and 25 s pulse delay. The analyses were done by derivatising 30 mg of each fraction with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP)²³. ³¹P NMR data were processed offline using NUTS NMR data processing software (Acorn NMR Inc.).

Commercial SLS and their fractions were phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane in presence of cyclohexanol as an internal standard and analyzed with quantitative ³¹P NMR according to a method described by Granata and Argyropoulos²³.

The concentration of each hydroxyl functional group (mmol/g) was calculated using the internal standard (cyclohexanol) with known hydroxyl functional number. Besides, the C9 unit was calculated from the elementary analysis performing for commercial SLS and their fractions. From the concentration of hydroxyl functional group (mmol/g) and the C9 unit formula, the number of functional group per C9 unit (group/C9) was also calculated.

Dynamic vapour sorption

Water sorption isotherms of commercial SLS and their fractions were determined using dynamic vapour sorption (DVS) technique (Surface Measurement Systems, United Kingdom). The principle of this method is an evaluation of sample weight changes over time at 25°C and at relative humidity (RH) between 0% and 95%. About 40 mg of sample was loaded into the quartz sample pan. A first step (drying phase) consists to control the humidity at 0% for 10 h to obtain internally equilibrates. The sample was then subjected to successive ten steps of 10% RH increase, up to 95%. For each step the mass changes (m) were plotted against time. The equilibrium was considered to be reached when changes in mass with time (dm/dt) was lower than 0.002 %/min. The accuracy of the system was ±1.0% and ±0.2 °C for RH and the temperature respectively. Error for each sample is 2%.

The water vapour adsorption isotherms were described by using GAB (Guggenheim–Andersen–de Boer) model, which is the most commonly used isotherm model for moisture sorption isotherms of foods²⁴:

$$X = X_m \frac{C_{GAB} K_{GAB} a_w}{(1 - K_{GAB} a_w)(1 - K_{GAB} a_w + C_{GAB} K_{GAB} a_w)} \quad (1)$$

Where X_m is the monolayer moisture content, X is the equilibrium water content, C_{GAB} is the characteristic energy constant, K_{GAB} is the characteristic constant correcting the properties of the multilayer molecules with respect to the bulk liquid. The parameters were calculated by Origin software²⁵.

The electrophoretic mobility investigation

The velocity of a particle in a unit electric field is referred to its electrophoretic mobility (μ_E). Determination of μ_E values permit to assess the electrical charge of macromolecules. Soft particles are characterized by the presence of an ion penetrable layer at the outer surface exposed to the continuous medium. Ohshima²⁶ proposed a model (equation 2) for μ_E that allowing determining $1/\lambda$, which is related to the length of accessible layer and ZN when Z corresponding to the valences of charge density in the

polyelectrolytic region and N represents the electrical charge density in the polyelectrolytic region (charges/m³). The μ_E of soft particles is described in the equation 2:

$$\mu_E = \frac{\epsilon_0 \epsilon_r}{\eta} \frac{\psi / Km + \psi_{DON} / \lambda}{1 / Km + 1 / \lambda} + \frac{eZN}{\eta \lambda^2} \quad (2)$$

η is the viscosity of the medium (Pa/s)

ϵ_0 is the absolute permittivity of vacuum

ϵ_r is the relative permittivity of the electrolyte solution

e is the elementary charge (1.6×10^{-19} C)

ψ_{DON} is the Donnan potential define like :
$$\psi_{DON} = \frac{KT}{ze} \ln \left\{ \frac{ZN}{2zn} + \left(\left(\frac{ZN}{2zn} \right)^2 + 1 \right)^{1/2} \right\} \quad (3)$$

ψ_0 is the potential at the boundary of the surface region define like :

$$\psi_0 = \frac{KT}{ze} \left\{ \ln \left\{ \frac{ZN}{2zn} + \left(\left(\frac{ZN}{2zn} \right)^2 + 1 \right)^{1/2} \right\} + \frac{2zn}{ZN} \left(1 - \left(\left(\frac{ZN}{2zn} \right)^2 + 1 \right)^{1/2} \right) \right\} \quad (4)$$

Km is the Debye Hückel parameter in the surface layer that involved the contribution of the fixed-charges

$$ZeN \quad Km = k \left[1 + \left(\frac{ZN}{2zn} \right)^2 \right]^{1/4} \quad (5)$$

k is the Debye Hückel parameter define like :
$$k = \left(\frac{2z^2 e^2 n}{\epsilon_0 \epsilon_r kT} \right)^{1/2} \quad (6)$$

z is the valence of the electrolyte solution.

T is the thermodynamic temperature.

K the Boltzmann constant and n is the bulk concentration of the electrolyte solution.

NaCl was the electrolyte used so $z=1$, e the elementary electric charge.

μ_E of commercial SLS and their fractions was performed using Zetasizer ZS equipment HPPS 5001 (Malvern Instrument, United Kingdom) by means of laser Doppler electrophoresis.

Determination of surface tension

Measurements of surface tension of SLS solutions prepared in demineralised water, at concentration ranging from 0 to 10 g.L⁻¹, at 25°C, were made using a tensiometer model K12 (Krüss, deutschland). The results have been accomplished by the method of Wilhelmy plate. This method based on placement of a sheet over the surface of the solution. The plate is immersed in the solution and the force necessary to return it to its original position equals to the surface tension.

Rheological measurements

The rheological measurements were carried out in a Stress Tech Rheometer (Reologica AB, Sweden) using a cone and plate geometry (volume sample 1.2 mL, cone angle 4°, diameter 40 mm). A lid was added into the sample to prevent evaporation at high temperatures.

The rheometer was connected to a thermostatically controlled bath. Samples at range of concentration from 5% to 20%, dissolved in demineralised water, were allowed to equilibrate at 25 °C and shear rate increasing from 0.03 to 0.2 s⁻¹ were imposed. The GNF (generalized Newtonian fluid) model was used to determine the apparent viscosity of commercial SLS and their fractions; where the shear stress τ is

proportional to the strain-rate $\dot{\gamma}$.

$$\tau = \eta_a \gamma \quad (7)$$

Antioxidant activity determination

The antioxidant activity was performed using Xenius multidetection microplate reader (Safas, Monaco). 96-well with black (Trolox equivalent antioxidant capacity assay) and clear (Oxygen radical absorbance capacity assay) polystyrene microplates were used.

Trolox equivalent antioxidant capacity

The antiradical activity of commercial SLS and their fractions was evaluated by scavenging the radical ABTS^{•+}. ABTS radical cation (ABTS^{•+}) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16h before being used. Next ABTS^{•+} solution was diluted with PBS (Phosphate Buffer Saline) (pH 7.4) to an absorbance of 0.5 at 734 nm and equilibrated at 30°C. 200 µl of ABTS^{•+} and (10, 20, 35, 50, 70, 80 µl) of SLS solutions (10 mg.L⁻¹) was mixed and adjusted to 300 µl with buffer solution. After 15 min incubation, the optical density was measured.

The percentage inhibition of ABTS^{•+} is calculated by using equation 8.

$$\% \text{ inhibition} = \frac{DO_{\text{initial}} - DO_{\text{final}}}{DO_{\text{initial}}} \times 100 \quad (8)$$

The Trolox equivalent antioxidant capacity TEAC represents the molar concentration of trolox (µM) with the same antioxidant activity of 1 mg.L⁻¹ of SLS solutions.

Oxygen radical absorbance capacity

Oxygen radical absorbance capacity (ORAC) measures the ability of a molecule to prevent the oxidation of FL by free radicals from the decomposition of AAPH at 37°C. Fluorescence was read with an excitation wavelength of 485 nm and an emission filter of 528 nm. AAPH solution and FL were prepared in a phosphate buffer solution at 75 mM at pH 7.4. The control of this reaction is the trolox which is also prepared in the same buffer.

Six different quantities (10, 20, 35, 50, 70, 80 µl) of commercial SLS and their fractions at a concentration of 10 mg.L⁻¹ were placed in the wells of the microplate with FL and adjusted at the same volume with buffer solution (80µl). The mixture was preincubated for 30 min at 37 °C, before rapidly adding the AAPH solution (220µl) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 6 min for 240 min. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution was used. The inhibition capacity was expressed as Trolox equivalents (µM), and is quantified by integrating of the area under the curve (AUC). All reaction mixtures were prepared in twenty times. The area under the fluorescence decay curve (AUC) was calculated using the equation 9.

$$AUC = 1 + \sum_{i=6}^{i=240} f_i / f_0 \quad (9)$$

where f_0 and f_i are the initial fluorescence read at 0 min and at i min, respectively. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank.

RESULTS AND DISCUSSION

Chemical and structural characterization

For all fractions of SLS, obtained after each step of diafiltration, the main structural and chemical characteristics were monitored.

Composition and SLS recovery of different fractions

Recoveries rates, percentage of LS and percentage of reducing sugar of SLS fractions are given in Table 1. The obtained results showed that fraction F₁ is the most important one in commercial SLS. It represents more than 48% w/w of the total mass. While for F₃ (50 ~ 150 kDa) and F₄ (15 ~ 50 kDa) the recoveries rates were only about to 6% and 5%, respectively. Similar results for recovery of intermediaries fractions obtained by ultrafiltration were reported by Ringena et al.²¹. The percentage of SLS of each fraction was also determined. It is equal to 99%, 97%, 95%, 93% and 75% respectively for F₁, F₂, F₃, F₄ and F₅. For F₆ this percentage is relatively low (19 %) and percentage of reducing sugar is high (19%) compared to the proportion in the other fractions. Therefore, except for F₆ and F₅, diafiltration allows obtaining high pure fractions. These fractions will be deeply characterized to enhance the development of new niche applications.

Determination of molecular weight distribution

Figure 1 shows the chromatogram profiles of commercial SLS and their fractions analyzed by SEC-UV. Except for F₆, SLS fraction profiles, compared to that of commercial SLS, showed a narrow and symmetrical distribution. The behaviour of F₆ can be explained by the high impurities content in this fraction. Moreover, the weight average molecular weight (M_w), the number average molecular weight (M_n) and the polydispersity D (M_w/M_n) were calculated and summarized in Table 2. It appears that the polydispersity decreases progressively with the decrease of the cut off of membrane. It is equal to 6.17, 3.46, 2.15, 1.78, 1.44 and 1.67 for commercial SLS, F₁, F₂, F₃, F₄ and F₅ respectively. The M_w of the fractions varied from (2307 to 19543 g mol⁻¹) and M_n from (1385 to 5659 g mol⁻¹). Due the high impurities of F₆, molecular weight and polydispersity of this fraction were not determined. Similar behaviour was observed by Ringena and al.²¹. This result confirms, as it was indicated by these authors, that no correlation can be established between the cut off of used membranes and the actual molecular weight of SLS fractions. This is due to the complexity of LS structures compared to the PSS standards used as reference for weight determination. In fact, PSS are rather linear²⁷ while the structure of SLS are highly heterogeneous and complex²⁸. This structural difference can leads to the underestimation of the molecular weight values of SLS. Due to the high heterogeneity of F₆ (low LS content and high impurities content), the next analyses were not realized to this fraction.

Determination of functional groups

FTIR investigation

The functional properties of SLS depend strongly on the nature of the different groups forming the skeleton of these polymers. FTIR analysis allows the identification of these groups. The assignments and the intensity (ATR units) of these spectres are given in table 3. These results indicate that several variation of the profile and the intensity of adsorption were occurred in different regions of the spectrum. The band at 3392 cm⁻¹ was assigned to hydroxyl groups. F₃ presents the highest adsorption of hydroxyl groups. The bands between 1690 cm⁻¹ and 1650 cm⁻¹ are due to carbonyl/carboxyl groups, F₃ and F₄ have higher adsorption of carbonyl/ carboxyl groups than the other fractions. Similar results were found for the band at 1040 cm⁻¹ which mean that these fractions (F₃ and F₄) contain more sulfonic groups than F₁, F₂, F₅ and commercial SLS. The FTIR analyses show that fractions characterized by a low M_w (F₃, F₄ and F₅) absorb at 1125 cm⁻¹ which mean that this fractions contain syringyl units specifically. This observation was confirmed by the highest absorption of these fractions at 1420 cm⁻¹ related to the C-H deformation of OCH₃ groups. The variation in the functional groups can be in the origin of new and specific surface properties which can be exploited for the development of new applications to these polymers.

³¹P NMR analysis

The concentration of hydroxyl functional groups of commercial SLS and their fractions are presented on Table 4. These results show that all fractions contain aliphatic OH, guaiacyl OH and carboxylic acid. For Commercial SLS and their fractions, OH-guaiacyl and OH-syringyl groups were detected. F₃ and F₄ contain the highest OH-guaiacyl (0.125/C9) and carboxylic acid (0.087/C9), respectively. OH-Syringyl groups (0.013/C9), (0.005/C9) and (0.025/C9) were identified only for F₃, F₄ and F₅, respectively. Observations of ³¹P NMR spectra confirm FTIR results. Both of them, show that F₃ has the highest OH

hydroxyl groups content and F₄ a highest carboxyl groups content and also confirm the presence of syringyl groups in the lowest fractions. It appears from these results that the fractionation of SLS by diafiltration allows the production of lignosulfonate with a wide range of M_w and structural properties which could in the origin of the development of new applications.

Physicochemical properties

Chemical and structural investigations revealed that DF can furnish SLS fractions with different molecular weight distribution and functional groups. The variation of molecular weight and the composition can affect the colloidal and physicochemical properties. So, deep investigations of the main colloidal properties of the obtained fractions were performed and compared.

Sorption isotherms study

The quality of most raw materials depends to a great extent upon their physical and chemical stability. This stability is mainly a consequence of the relationship between the equilibrium moisture content and the corresponding water activity (a_w), at a given temperature. These water sorption isotherms are unique for a given composition of the raw materials. Many empirical and semi-empirical equations describing the sorption characteristics of different raw materials have been proposed in the literature. The kinetic models based on a multi-layer and condensed film (GAB model) is considered to be the most versatile sorption model available in the literature. As for several applications the capability of the water retention of LS is an important criterion, thus the sorption isotherm of commercial SLS and their fractions were investigated at 25 °C. The results are summarized in Figure 2 and according to Brunauer²⁹ classification they belong to type II curve shape. The GAB model was used to fit these data. The three parameters of this model X_m (monolayer moisture content), k_{GAB} , and C_{GAB} values are reported in Table 5 together with the mean relative percentage deviation module (E) and R^2 . Examination of these results indicates that the GAB model fits well the experimental adsorption kinetic for SLS and the different fractions throughout the entire range of water activity. The GAB model, gives E values ranging from 2.64% to 7.26 %, with average value of 4%. The GAB model parameters X_m (monolayer moisture content dry basis), k_{GAB} , and C_{GAB} values provide an indication of the monolayer water adsorption capacity, the binding energy of the water and the monolayer heat of sorption respectively. For several material particularly foodstuff and biopolymers, X_m varies from 0.5 to 15, k_{GAB} between 0.7 and 1 and C_{GAB} is in the range of 1 to 20. It appears that for the three GAB parameters the obtained values for SLS are comparable to those reported for foodstuff. The lowest value of X_m (8.8) was obtained with F₁ and the highest value (28.5) with F₅. The variation of GAB parameters can be attributed to a combination of factors, which include the conformation and topology of molecule and the hydrophilic/hydrophobic sites adsorbed at the interface. The observed variation of X_m and C_{GAB} confirms the structural and chemical differences occurred between SLS fractions. Despite the importance of knowledge on the mechanism of water-binding of LS the data on isotherm sorption are scarce and no comparison can be made. The results obtained in this study can help to understand the behaviour of the biopolymer when used in different formulation.

Electrophoretic measurements

The variation of the morphology and the functional groups of the different fractions of SLS can affect their interactions and complex formation with other macromolecules. To quantify these possible effects, the electrophoretic mobility (μ_E) and conformation of commercial SLS and their fractions were investigated. The obtained μ_E profiles for different fractions were reported in Figure 3. Negative μ_E were observed due to the negative charge of sulfonic groups. Moreover, the absolute value of μ_E across non-zero values and decreases when the NaCl concentration increases. These profiles are characteristic of soft particles. In fact, SLS was previously described like ellipsoid particles would have a dense core surrounded by a less dense surface layer of polymeric chains containing hydroxyl and sulfonic groups²⁰. Table 6 reports the values of charge density (ZN) and the particle softness parameters ($1/\lambda$). ZN seems to be molecular weight dependent and present a high value for high molecular weight. As an example, ZN for F₁ and F₅, is equal -40,5 mM and -27.5 mM respectively. Also, $1/\lambda$ values, about 2 nm, doesn't

appeared depending on molecular weight, it was assumed that this parameters depend on the polymer conformation³⁰.

Surface tension

The surface tension of aqueous solutions of commercial SLS and their fractions is shown in figure 4. It appears that the increase of the concentration in the solution lead to a decrease of the surface tension. However, the commercial SLS and their fractions are not able to form micelle. This behaviour could be attributed to the spherical shape of their hydrophobic skeleton, which hinders the formation of a regular arrangement at the interfacial phase and thus affects their surface activity¹⁷. In spite of the no formation of micelle, some of these fractions; particularly F₃ and F₄ lead to an interesting decrease of surface tension with an increase of the concentration. For 10 g/L these two fractions reduce the surface tension to a value around of 52 mN/m. This behaviour could be attributed to the presence of the hydroxyl and sulfonic groups as it was shown previously by Infrared spectra analysis and ³¹P NMR. The effect of a high density of sulfonic and hydroxyl groups of a modified LS on the surface properties was reported Pang and al.³¹. These authors studied the effect of hydroxylation and sulfonation of CLS and they demonstrated that the content of the hydroxyl groups and sulfonic groups are important to enhance the surface activity.

Rheological investigation

In order to evaluate the effect of the fractionation of LS on their colloidal properties, the apparent viscosity of the different fractions was determined and compared to commercial SLS. The obtained data, for the imposed shear rate, were modelled as a Newtonians fluid. The calculated apparent viscosity (η_a) of SLS solutions at three different concentrations (20%, 10% and 5%) are given in table 7. The highest apparent viscosity $2.19 \cdot 10^{-3}$ and $2.16 \cdot 10^{-3} \text{ N.m}^{-2}.\text{s}$ were obtained respectively with fraction F₁ and F₂ at 20%. For others fractions η_a was about of $1.5 \cdot 10^{-3} \text{ N.m}^{-2}.\text{s}$. These apparent viscosity decreases as the concentration and the molecular weight of the fraction is decreasing. The lowest value ($1.28 \cdot 10^{-3} \text{ N.m}^{-2}.\text{s}$) is obtained with F₅ and F₄ at 5 %. This difference, observed between η_a values of SLS solutions can be attributed to the presence of more sodium in the fractions characterized by low molecular weight. In fact, as reported by Browning et al.²⁰, the presence of sodium can promote the repulsive forces and therefore reduce the viscosity. The effect of sodium on the viscosity was stated by comparing the viscosity of SLS and CLS. SLS viscosity is lower compared to that of CLS due to the stronger electrokinetic repulsive force of the sodium²⁰.

Antioxidant capacity

The antioxidant activity of commercial SLS and their fractions, evaluated by TEAC and ORAC assays, are reported in table 8. It indicates that the TEAC values of commercial SLS and their fractions ranked from 1.67 to 2.86 μM and from 1.76 to 2.53 μM for ORAC values. F₃ (2.83 μM) and F₄ (2.86 μM) showed the greatest TEAC values. ORAC method are also showed that F₃ (2.47 μM) and F₄ (2.53 μM) are the fraction which have the greatest antioxidant capacity. Compared to the most known antioxidant molecules, such as vitamin C (TEAC, 5.68 μM), vitamin E (TEAC, 2.32 μM) and rutin (TEAC, 3.97 μM)³², SLS fractions exhibit a relatively interesting antioxidant power. The fractionation allows obtaining fractions (F₃, F₄) with a relatively highest antioxidant activity compared to SLS. Both the used methods (TEAC and ORAC) lead to a similar conclusion. The variation of the antioxidant power between fractions could be attributed to the structural difference observed previously. In fact, Zhou et al.³³ studied the antioxidant capacity, using TEAC method, of different phenolic acid (4-OH benzoic, vanillic, and syringyl acids) and they observed that the presence of methoxyl groups (OCH₃) in the ortho position to the hydroxyl position on the phenyl ring (syringyl acid) enhances the antioxidant activity. The presence of syringyl units in fractions F₃, F₄ and F₅ may be in the origin of the more important antioxidant capacity. However, values obtained by TEAC are slightly lower compared to ORAC. This difference is attributed to the fact that TEAC assay uses exogenous ABTS^{•+} radicals, whereas the ORAC assay uses more physiologically relevant peroxy radicals, and can react with non-lignosulfonates components³⁴. F₅ evaluated by TEAC present an antioxidant activity less then commercial SLS due to the presence of impurities.

CONCLUSION

Diafiltration achieved five SLS fractions with different molecular weight and polydispersity ranking from 1385 g.mol⁻¹ to 19543 g.mol⁻¹ and 1.44 to 3.46, respectively. Structural investigation by FTIR analyses and ³¹P NMR show a high content on hydroxyl and sulfonic groups for fractions with molecular weight ranking from 2471 g.mol⁻¹ to 4297 g.mol⁻¹ (F₃ and F₄). The physicochemical properties of SLS fractions were also investigated and compared to the commercial SLS. The adsorption isotherms of commercial SLS and their fractions present different isotherms profiles and well fitted by GAB model. The parameters of this model indicated that the energy binding and adsorption capacities differ from one fraction to another. Thereafter, surface activity were evaluated, results emphasize that fractions with molecular weight with M_w of 4297 and 2471 g.mol⁻¹ (F₃ and F₄) have a higher surface activity than commercial SLS. These two fractions display also the highest antioxidant activity. In this work, we also demonstrated that LS are soft particle and we determined the charge density. F₁ (with M_w of 19543 g.mol⁻¹) and F₂ (with M_w of 6953 g.mol⁻¹) present the highest charge density (-40.5 mM and 41 mM) and the highest apparent viscosity (2.19 10⁻³ and 2.16 10⁻³ N.m².s). The analysis of the whole results indicated that diafiltration permits the obtaining for at least one fraction that shows greater activity in a given property compared to commercial SLS.

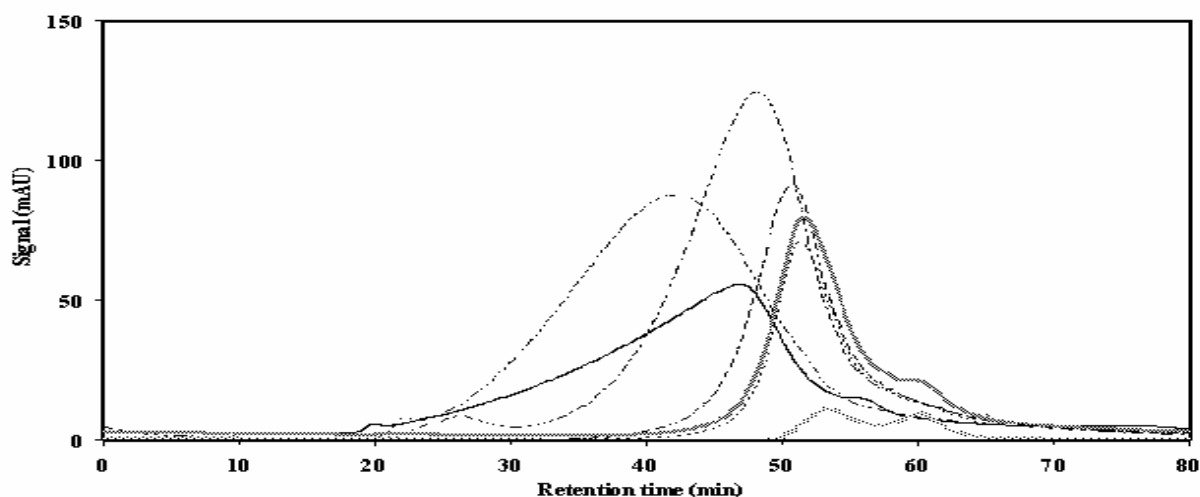


Fig.-1: UV detected SEC elution profiles of commercial SLS and their fractions obtained after diafiltration.

(—— Commercial SLS - - - - F₁ - - - - F₂ - - - - F₃ - - - - F₄ - - - - F₅ - - - - F₆)

Table-1: Percentage of recovery rate, percentage of SLS content and percentage of reducing sugar of commercial SLS and their fractions.

Fraction	Cut-off range (Da)	%Recovery rate	% SLS content	%Reducing sugar
Commercial SLS	----	-----	91.45 ± 1.47	3.68 ± 0.06
F ₁	More than 300000	48.38±2.66	99.59 ± 0.82	1.33 ± 0.04
F ₂	150000~300000	14.88 ± 4.96	97.08± 0.27	1.65 ± 0.14
F ₃	50000~150000	6.55 ± 1.49	95.34 ± 1.91	2.55 ± 0.01
F ₄	15000~50000	5.31 ± 0.20	93.98 ± 2.36	4.23 ± 0.10
F ₅	5000~15000	10.48 ± 2.72	75.59 ± 0.52	4.52 ± 0.11
F ₆	Less than 5000	4.19 ± 0.92	19.81 ± 1.81	19.22 ± 0.28

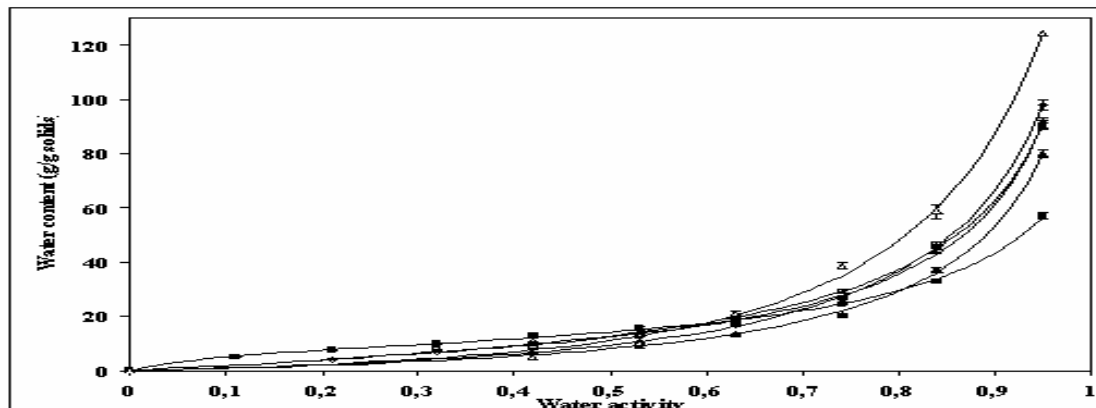


Fig.-2: Sorption isotherm profile obtained for commercial SLS and their fractions estimated at 25 °C from 0% to 98% RH and modelled up with the GAB model.

(♦ Commercial SLS ■ F₁ ▲ F₂ ◇ F₃ □ F₄ △ F₅ — GAB FIT)

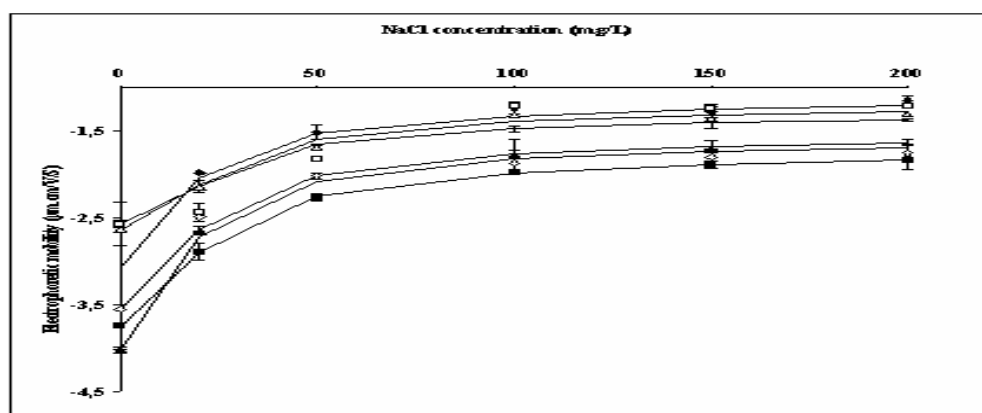


Fig.-3: Profile of electrophoretic mobility measurements of commercial SLS and their fractions. Solid lines represent the best-fitted theoretical mobility curves. (♦ Commercial SLS ▲ F₁ ■ F₂ ◇ F₃ △ F₄ □ F₅ — FIT)

Table-2: Molecular weight distribution and polydispersity obtained by SEC-UV of commercial SLS and their fractions.

Fraction	Cut-off range (Da)	Mn (g.mol ⁻¹)	Mw (g.mol ⁻¹)	Polydispersity
Commercial SLS	----	2896 ± 364	17783 ± 1482	6.17 ± 0.33
F ₁	More than 300000	5659 ± 388	19543 ± 707	3.46 ± 0.11
F ₂	150000~300000	3236 ± 89	6953 ± 63	2.15 ± 0.03
F ₃	50000~150000	2408 ± 153	4297 ± 430	1.78 ± 0.09
F ₄	15000~50000	1722 ± 85	2471 ± 48	1.44 ± 0.05
F ₅	5000~15000	1385 ± 33	2307 ± 87	1.67 ± 0.03

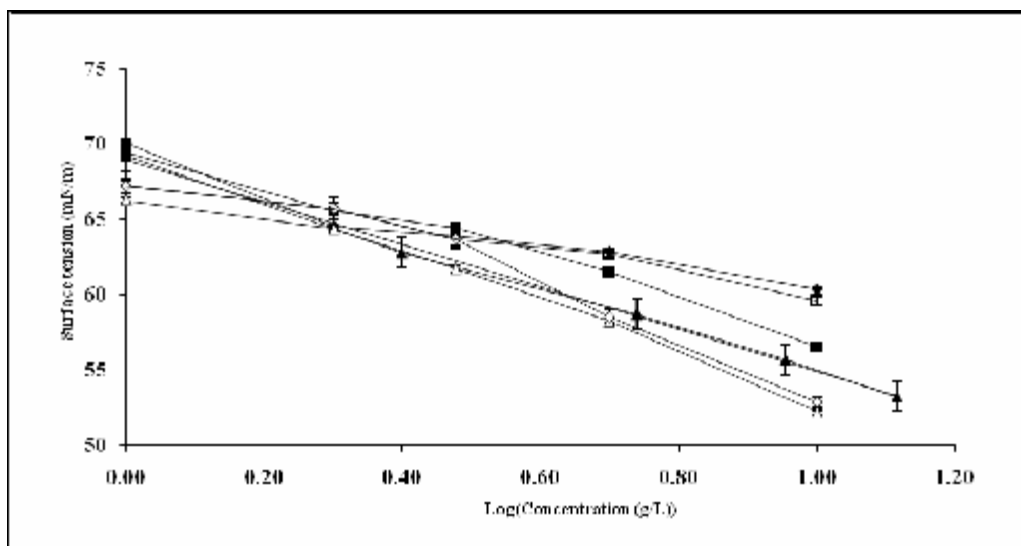


Fig.-4: Surface tension of commercial SLS and their fractions according to concentration (g/L).
(▲ Commercial SLS ◆ F₁ ■ F₂ △ F₃ ◇ F₄ □ F₅ — FIT)

Table -3: Assignment of FTIR spectra and their intensity (ATR Units) of commercial SLS and their fractions.

Wave number (cm ⁻¹)	Functional Groups	Signal Intensity (ATR Units)					
		Commercial SLS	F ₁	F ₂	F ₃	F ₄	F ₅
3392	OH Stretching	0,151	0,070	0,101	0,230	0,142	0,082
1690	C=O stretch unconj	0,074	0,069	0,072	0,086	0,102	0,081
1650	C=O stretch conj	0,097	0,079	0,082	0,122	0,117	0,097
1565	Aromatic squeel vibration	0,145	0,153	0,156	0,163	0,17	0,111
1420	CH deformations of OCH ₃ groups	0,074	0,068	0,070	0,84	0,114	0,078
1180	C-H deformation of Guaiacyl units	0,112	0,091	0,132	0,154	0,152	0,131
1125	C-H deformation of Syringyl units	-	-	-	0,101	0,134	0,097
1040	C-S elongation	0,118	0,103	0,135	0,154	0,156	0,120

Table-4: Commercial SLS and their fraction characteristics calculated from ³¹P NMR data.

- (a) Determined by integration with cyclohexanol as an internal standard.
(b) Calculated on the basis of C9 units from elemental analysis performed on commercial SLS and their fractions.

Chemical shift range δ ³¹ P-NMR	Assignment	Commercial SLS		F ₁		F ₂		F ₃		F ₄		F ₅	
		mmol/g ^a	Group/C9 ^b	mmol/g ^a	Group/C9 ^b	mmol/g ^a	Group/C9 ^b	mmol/g ^a	Group/C9 ^b	mmol/g ^a	Group/C9 ^b	mmol/g ^a	Group/C9 ^b
145.5–150.0	1-Aliphatic OH	2,240	0,601	1,832	0,419	2,432	0,624	1,581	0,426	2,235	0,729	1,619	0,504
141.8–	2a-Syringyl	-	-	-	-	-	-	0,049	0,013	0,015	0,005	0,080	0,025

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143.5	OH												
138.7– 140.1	2b- Guaiacyl OH	0,314	0,084	0,478	0,109	0,434	0,111	0,465	0,125	0,266	0,087	0,300	0,093
134– 135.7	3- Carboxylic acid	0,070	0,019	0,033	0,008	0,056	0,014	0,057	0,015	0,266	0,087	0,232	0,072

Table-5: Parameters X_m , C_{GAB} and k_{GAB} obtained from the fitted curves with GAB for commercial SLS and their fractions.

Fraction	X_m	C_{GAB}	k_{GAB}	E	R^2
Commercial SLS	17.79	0.48	0.90	4.39	0.99
F ₁	8.79	11.89	0.89	3.62	0.99
F ₂	11.14	0.72	0.92	4.50	0.99
F ₃	12.30	1.34	0.92	2.64	0.99
F ₄	13.31	1.15	0.91	4.87	0.99
F ₅	28.53	0.36	0.89	7.26	0.99

Table-6: Determination of the surface charge properties (ZN, the spatial charge density in the polyelectrolyte region, and $1/\lambda$, the softness parameter) of commercial SLS and their fractions using the Ohshima's method.

Fraction	ZN(mM)	$1/\lambda$ (nm)
Commercial SLS	-31.0 ± 1.41	1.91 ± 0.01
F ₁	-40.5 ± 0.28	1.98 ± 0.01
F ₂	-41.0 ± 0.07	2.10 ± 0.01
F ₃	-39.5 ± 0.42	2.00 ± 0.01
F ₄	-31.5 ± 0.28	1.95 ± 0.01
F ₅	-27.5 ± 4.41	2.20 ± 0.01

Table-7: Apparent viscosities at different concentration (5%, 10% and 20%) of commercial SLS and their fractions.

Fraction	$\eta_a (10^{-3} \text{ N.m}^{-2}.\text{s})$		
	200g/L	100 g/L	50g/L
Commercial SLS	1.99 ± 0.04	1.57 ± 0.09	1.43 ± 0.06
F ₁	2.19 ± 0.07	1.76 ± 0.22	1.36 ± 0.11
F ₂	2.16 ± 0.01	1.74 ± 0.23	1.30 ± 0.07
F ₃	1.55 ± 0.02	1.32 ± 0.01	1.29 ± 0.03
F ₄	1.53 ± 0.05	1.33 ± 0.04	1.28 ± 0.05
F ₅	1.54 ± 0.05	1.37 ± 0.03	1.28 ± 0.02

Table-8: Antioxidant capacity evaluated by TEAC and ORAC methods of commercial SLS and their fractions.

Fraction	TEAC (μM)	ORAC (μM)
Commercial SLS	2.52 ± 0.08	1.92 ± 0.13
F1	2.51 ± 0.21	1.91 ± 0.23
F2	2.45 ± 0.10	1.76 ± 0.13
F3	2.83 ± 0.12	2.47 ± 0.17
F4	2.86 ± 0.18	2.53 ± 0.10
F5	1.67 ± 0.19	2.18 ± 0.06

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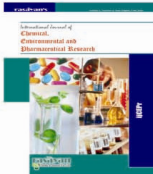
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