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FAST TRACK FOR QUANTITATIVE ISOLATION OF LIGNOSULFONATES FROM SPENT SULFITE LIQUORS

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In this study, a novel approach for isolation and purification of lignosulfonates from spent sulfite liquor was established. This approach involves sorption onto macroreticular non-ionic poly(methyl methacrylate) beads (XAD-7 resin) and subsequent desorption with organic solvents to obtain lignosulfonates of high purity. The method was optimized, verified and tested on four industrial Lignosulfonate liquors from different processes and compared with an established ultrafiltration protocol. The method was optimized, verified, and tested on four industrial lignosulfonate liquors from different processes and compared with an established ultrafiltration protocol. The method proved to be reproducible, robust and significantly faster than ultrafiltration.

Introduction

Despite the relatively low annual production of lignosulfonates (LS) which accounts for approximately 10% of the total lignin output the development of many new approaches and their recent application in integrated forest biorefineries supports their high economic potential. Lignosulfonates have already found a variety of applications as surfactants, binders, and tanning agents, and they are also used in the production of fine chemicals.[1]

Lignosulfonates are the primary (but by far not the only) component of spent sulfite liquor which is generated in the sulfite pulping process. Sulfite pulping can operate at pH levels ranging from very acidic to alkaline depending on the process applied. Depending on the pH either HSO_3^- or SO_3^{2-} are the reactive species which cause sulfonation of lignin moieties that are this way rendered soluble and separable from the pulp. The presence of other components in the spent liquor and the difficulties with their neat separation cause certain limitations in the application of the entire sulfite pulping effluent as well as problems with conducting different types of chemical analysis of lignosulfonates and interpretation of the data obtained. Thus, new methods for lignin isolation and purification from spent liquors which could rapidly provide lignin with high purity and yield are currently of great interest for both analytical purposes and industrial recovery (i.e., to obtain lignins for value-added products). We were particularly interested in the development of fast analytical methods for the isolation and characterization of lignosulfonates which is a prerequisite to novel applications of the material.

Currently, several methods for the isolation of lignosulfonates are available, but they all fail to meet the demand for a fast yet sufficiently thorough separation from by-products and thus remain far too tedious and/or time-consuming for a general or even high-throughput analysis method. One of the first proposed protocols for lignosulfonate isolation and fractionation included cation exchange and precipitation of the lignosulfonate as a barium salt. Because barium lignosulfonates are weakly soluble in ethanol, the obtained precipitate can be further fractionated by ethanol-water mixtures along a column filled with cellulose.[2] In combination with ultrafiltration (UF) as a preliminary stage, this method provides reliable yields and relatively good fractionation of lignosulfonates with variation in the average molecular weight between 4,600 and 398,000 g mol^{-1} and dispersity Đ = *Mw*/*Mn* between 1.3 and 3.5.[3]

Another common approach for lignosulfonate isolation includes treatment with long-chain alkyl amines resulting in the formation of a water-insoluble lignosulfonic acid-amine complex which, in order to remove impurities, is then extracted with various solvents. The purified lignosulfonates are recovered by alkali extraction.[4-6] By varying the pH it was possible to fractionate lignosulfonates into portions with different contents of sulfonic, carboxylic, and methoxyl groups. This method involves many time-consuming steps and does not recover a substantial quantity of lignosulfonates due to foaming and emulsion formation.

In recent years, dialysis has been the most widely used method for lignosulfonate purification.[7] A wide range of membrane materials with various cut-offs is used. However, because dialysis relies on the diffusion of molecules with different hydrodynamic radii through a semi-permeable membrane, it

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cannot provide selective separation of lignosulfonates from their accompanying impurities (such as carbohydrates). Dialysis requires special selections of membranes for each liquor sample in order to reach an optimum yield and degree of purification. In addition, dialysis is highly time-consuming, and thus far has only been applicable for analytical purposes.

Isolation of lignosulfonates using liquid membranes was proposed recently.[8] Lignosulfonate separation is achieved by a two-way diffusion between a feed aqueous phase, which contains liquor components, and a stripe aqueous phase which contains alkali via a liquid membrane comprised of organic solvents and driven by a concentration gradient. The application of tri-*n*-octylamine as a carrier and dichloroethane as a solvent in the so-called bulk liquid membrane led to an almost quantitative separation of lignosulfonates. With the aim of making the process less error-prone, hydrophobic porous nylon membrane impregnated with an organic phase were used instead of the bulk organic layer.[9] These methods have certain drawbacks, among which, long separation times and low membrane stability were the most critical. The application of emulsion liquid membranes which has the same basic principle of isolation was also proposed.[10] This method appeared to be much faster and again allowed for almost quantitative Lignosulfonate isolation, albeit with unknown degree of purity. However, it required a long duration of ultrasonication for proper emulsification, which might impair the chemical integrity of the sample. Such effect of ultrasonication has recently been shown for celluloses, and similar radical processes are also expectable when lignosulfonates, or lignins in general, are sonicated.

Over the last few decades, the development of semi-permeable membranes led to the wide use of ultrafiltration (UF) in many fields, including, in particular, the isolation and fractionation of lignosulfonates. The possibility of relatively easy up-scaling and process control through the applied pressure set ultrafiltration apart from the methods described above. Previous studies have shown that UF allows for the quantitative isolation of lignosulfonates with high purity.[6, 11, 12] Moreover, the application of membranes with different molecular weight cut-offs can provide some rough, preliminary information on molecular weight distribution. Further fractionation of ultrafiltrated samples by means of gel-column chromatography produces lignosulfonates fractions with high uniformity.[11-13]

Currently, there is a high demand for selective adsorption methods in both industrial and laboratory analytical applications. Relative simplicity, the ability to scale up, possible high capacity, low cost, and easy-to-regenerate sorbents make ultrafiltration a highly attractive adsorption method. By involving many different sorbents, this method allows for the efficient isolation of organic low and high molecular weight compounds as well as inorganic molecules. The adsorbents are usually divided into further subgroups which include activated carbons, minerals, resins, industrial and agricultural wastes, fly ash, polysaccharide-based adsorbents, and biosorbents.[14-18]

Some attempts have been made to investigate lignosulfonate adsorption and desorption behaviour on minerals such as sandstone, limestone and dolomite.[16,19] It has been found that the adsorption capacity was rather low and that such processes take overly long time.

Of the previously mentioned adsorbents, synthetic polymeric resins are most attractive due to their durability, high adsorption capacity, selectivity, limited toxicity, and relatively low costs. Recently, the application of polymeric resins of different matrices including polyacryl-based or polyaromatic resins for adsorption of phenolic compounds in wastewater treatment, removal of inhibitors from fermentation media, and isolation of high value-added products was proposed.[15,19-22] Resins, such as Amberlite XAD-4, XAD-7, and XAD-16 are considered to have some of the highest adsorption rates. They possess a high capacity, are able to resist elevated temperatures (max. 150 ^{o}C) and are stable within the entire pH range. These resins can adsorb polymers with molar masses (MM) up to 60000 g mol^{-1} and can be easily regenerated and repeatedly applied. Several studies examine the possibility of modifying these resins. Acetylated or benzoylated XAD-4 could be used directly without a preliminary wetting step, and exhibited 20% higher capacity. In most cases, it was found that the adsorption process of phenolic compounds fits either the Freundlich or the Langmuir model, the thermodynamic parameters (free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), indicated a spontaneous and exothermic adsorption. Therefore, conducting the process at ambient temperatures results in a more favourable adsorption. In the case of phenolic compounds, the adsorption increases as the pH of the solution decreases. The ranges for time of contact and maximum adsorption capacity of different resins, applied for the adsorption of phenolic compounds of similar structure, vary greatly between studies (approximately 100-300 mg g^{-1}). Desorption of the adsorbate can be achieved quantitatively by flushing with aqueous alcohol or hot water.[19-22] Additional purification with diluted solutions of alkali and acid are beneficial before the resin is used once more.[19]

The aim of the present study was to evaluate lignosulfonate adsorption and desorption on a XAD-7 polymer resin at different pH levels, times, and adsorbent/adsorbate ratios and, thus, identify the optimal conditions of a single batch process in which the dominating and representative part of lignosulfonates can be isolated. Based on this optimized process, a novel general protocol for improved lignosulfonate isolation from spent liquors was developed according to which sampling with higher throughput and sufficient purification prior to instrumental lignin analysis is achieved.

Experimental

Materials and Chemicals

Four industrial ammonia and magnesium-based spent sulphite liquors were supplied by three different pulp mills (Table 1). The lignosulfonates are referred to as LS (1)-(4). The macroporous polyacrylate resin Amberlite XAD-7 (20–60 mesh), strongly acidic cation-exchange resin Dowex 50WX8 (50-100 mesh), ethanol, diethyl ether, chloroform, sodium

hydroxide, and anhydrous sodium tetraborate were obtained from Sigma-Aldrich GmbH, Schnelldorf, Germany.

Preparation of XAD-7 and DOWEX 50WX8 resins

The XAD-7 resin was washed and Soxhlet-extracted to remove micro particles and low molar mass contaminants.[23] XAD-7 resin was stored in ethanol (96%). Prior to the adsorption experiments, remaining ethanol was removed by exhaustive washing with deionized water. The resin was filtrated on a glass filter #3 at constant reduced pressure (900 mbar). For further adsorption experiments, the resin was used in the wet state; the complete removal of moisture negatively influences its adsorption capacity.[20] Moisture content was estimated according to a standard procedure from literature.[24] Two parallel runs yielded a 70% moisture content, which was used for further calculations of the LS adsorption.

The Dowex 50WX8 resin was thoroughly washed with distilled water. The resin was regenerated by stirring with diluted HCl solution according to following protocol: 0.5 M HCl (3 times for 10 min), then 1 M HCl (3 times for 10 min), and finally, 2 M HCl (3 times for 10 min). The regenerated resin was filtered on a glass filter and stored in a closed flask. Immediately before use, the resin was washed with distilled water until neutral.

Equipment

The analysis of the methoxyl group content was carried out according to the classical Zeisel-Vieböck-Schwappach method.[25] The sulfonic acid group content in isolated lignins was analysed with a titration protocol.[26] Elemental analysis was performed using a EURO EA 3000 CHNS-O instrument from HEKAtech (Wegberg, Germany). Oxygen was determined directly. UV absorption spectra of dissolved lignosulfonates were determined with a PerkinElmer Lambda 35 UV/VIS spectrometer using quartz cells.

IR Spectra were obtained with a Fourier transformation infrared spectrometer (FTIR) from Perkin Elmer (Frontier Optica, Waltham, Massachusetts, USA). The specimens were placed without any pre-treatment on an attenuated total reflection (ATR) Zn/Se crystal. Each sample was scanned from 4000 cm^{-1} to 600 cm^{-1} at 4 cm^{-1} resolution, taking the average spectrum of four scans. Processing of the spectra was performed with Spectra 10.3.2 software from Perkin Elmer for baseline correction and normalization.[27]

Residual carbohydrates were determined according to Sundberg et al. [33] GC-MS analysis was performed on an Agilent 6890N GC and an Agilent 5975B inert XL MSD quadrupole mass-selective detector (EI, 70 eV), using an Agilent HP-5MS capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) and helium as the carrier gas with a pressure of 0.94 bar, a flow rate of 1.1 ml·min^{-1}, a split flow rate of 7.5 $mlmin^{-1}$, and a split ratio of 7:1. The column oven temperature profile was as follows: initial temperature: 140 °C (1 min), increase to 210° C at 4 °C min^{-1}, increase to 300° C (final temperature) at 30 °C min^{-1}. The injector temperature was 260 °C, the temperature of the GC-MS transfer line was 290 °C, and that of the ion source was 230 °C.

Gel permeation chromatography (GPC) measurement were performed on an UltiMate® 3000 Standard LC system, equipped with a HPLC Kontron 420 pump and pulse damper. The detectors used were UV 280 nm and Shodex RI-101. Three PL GPC columns of 300 x 7.5 mm were calibrated by measuring the elution behaviour of polystyrene sulfonates as polymer standards of known molecular mass. The eluent used was DMSO/LiBr (0.5% w/v), filtered through a 0.45 mm filter prior to analysis. Flow rate: 0.50 ml min^{-1}, injection volume: 10 µl, column temperature: 40 °C. Data were evaluated using Chromeleon 6.8 software.

Thermogravimetric (TG) analysis in air atmosphere was carried out on the NETZSCH (Selb, Bavaria) TG 209 F1 Iris instrument over a temperature range between 25 and 1000 °C, with a heating rate of 10 °C min^{-1}. The purge gas velocity was 30 ml min^{-1} and the sample weight was 8-10 mg.

NMR spectra were recorded on a Bruker AVANCE II 400 (^{1}H resonance 400.13 MHz, ^{13}C at 100.61 MHz) with a 5 mm z-gradient, broadband (BBFO) probe head. Approximately 80 mg of the LS samples were dissolved in 600 µl of DMSO/Pyridine in a 4:1 ratio for the acquisition of NMR spectra. For the HSQC spectra, a spectral width of 9 ppm in ^{1}H- and 156 ppm in ^{13}C-dimension was chosen. Data were acquired in a 720 x 256 points data matrix with a scan number of 256 and a relaxation delay of 0.5 s. For processing, the acquired data were zero-filled to a final 2k x 1k data points, a Gaussian apodization in both dimensions and linear prediction with 32 coefficients in F1 was applied. The resulting experimental time was 11.5 hours. All samples were measured at 25° C. Data acquisition and processing were completed using Bruker Topspin 3.1. Post-processing of NMR-related illustrations was done in Adobe Illustrator CS5 and inspired by Ralph et al.[28]

Density of isolated lignosulfonates was determined according to ISO 1183-1:2012.

Extinction coefficient determination

A buffer solution with pH 12, containing 4.02 g of anhydrous borax $Na_2B_4O_7$ and 2.4 g of NaOH in 1 L of distilled water, was used for dissolution of LS that was isolated using the XAD-7 preparative method. An LS stock solution with a concentration of 0.25 mg ml-1 was prepared. Probes of stock solution ranging from 0.5 to 5 ml were quantitatively transferred to 25 ml volumetric flasks. The volume in the flasks was adjusted with the prepared buffer solution. The absorbance of solutions was measured at 280 nm against neat buffer solution. The extinction coefficient was calculated from the linear relation between LS concentration and UV absorbance.[26]

Lignosulfonate adsorption equilibrium and isotherm model estimation

Equilibrium experiments were carried out with a constant mass of wet XAD-7 resin (1 g base on dry weight) within lignosulfonate liquor solutions of increasing concentrations (3-80 mg ml^{-1}). The pH of the solutions was adjusted with 10% sulfuric acid to pH 2. The final volume of the lignosulfonate solutions was 50 ml. Closed bottles were gently shaken at 200

min^{-1} for 24 hours at room temperature. The content of lignosulfonate remaining in the solution was determined by measuring the UV absorbance at 280 nm. The extinction coefficient, as determined before for lignosulfonates isolated by the XAD-7 adsorption, was applied in calculations.

Adsorption kinetics

A stock solution of lignosulfonate liquor (4) at pH 2 and a solid concentration of 40 mg ml^{-1} was prepared. Prior to experiments, the content of lignin in the stock solution was analysed using UV-VIS. The stock solution (50 ml) was quantitatively transferred to 100 ml glass bottles containing wet XAD-7 resin. By taking into account the content of water in the resin, the following ratios between adsorbate (lignosulfonate) and adsorbent (XAD-7 resin) were set: 90, 110, 150, 220, 440, and 900 mg g^{-1}. The closed bottles were gently shaken at 200 min^{-1} for 40 hours at room temperature. The pH of the lignosulfonate solution remained unchanged after the addition of resin and during the adsorption process. Aliquots (100 µl) of the liquid phase were taken every 15 min during the first two hours, every 30 min during the next 9 hours, and once more after 12 hours. Each aliquot was transferred to a 25 ml volumetric flask and dissolved in borax buffer solution of pH 12. The content of lignosulfonates remaining in the solution was investigated by measuring the UV absorbance at 280 nm. The extinction coefficient determined for lignosulfonates isolated by the XAD-7 adsorption method was applied in the calculations. Adsorbed lignin was desorbed according to the procedure described below and was additionally analysed gravimetrically.

Preparative lignosulfonate isolation by adsorption on XAD-7 resin

Solutions of lignosulfonate liquors with a concentration of solids ~250 mg ml^{-1} were shaken with cation-exchange resin (Dowex 50WX8; approx. 4-5 g of wet resin per 1 g of total dissolved solids (TDS) present in liquor) for 3-4 hours, which decreased the pH to approximately 1.3. Treated lignosulfonate samples were filtered through a glass filter #3. Residues were washed with deionized water until a TDS content of ~60 mg·ml^{-1} was reached. The wet XAD-7 resin was added to the combined filtrates in the proportion 10 g of resin per ~1 g of TDS (adsorbate/adsorbent ratio ~100-150 mg g^{-1}) and shaken at 200 min^{-1} overnight. The liquid was thoroughly removed by vacuum filtration through a PTFE nozzle filter. Next, the resin was washed 3 times (10 ml per 10 g of wet XAD-7 resin) with acidified water (pH 2) and then 3 times with deionised water (10 ml per 10 g of wet XAD-7 resin). In between each washing step, the resin was gently shaken for 15 min and sucked dry.
The lignosulfonate was desorbed from the resin with alcohol (15 ml per 10 g of wet XAD-7 resin, methanol or ethanol can be used, ethanol (techn.) is preferred due to its lower toxicity) by gentle shaking for 30-40 min at 50 °C. The extract was separated by vacuum filtration as described above. In total, the resin was washed 4-5 times with alcohol and 1-2 times with deionised water. The ethanol from the obtained combined filtrate was removed by rotary evaporation at 40 °C. The remaining water solution of the isolated LS was quantitatively transferred to a plastic bottle and freeze-dried.

Lignosulfonate isolation on analytical scale by adsorption on XAD-7 resin

A polypropylene syringe of 5 ml was used as a convenient resin cartridge for analytical-scale isolation of lignosulfonates. The syringe spout was tightly filled with glass wool. Exactly 0.5 g of wet Dowex 50WX8 and 1 g of wet XAD-7 resins were placed into the syringe. The syringe was closed with the plunger. For extraction, the lignosulfonate liquor to be analysed and, if necessary, water were drawn into the syringe. The respective volume was controlled on an analytical balance such that the adsorbate/adsorbent proportion and the adsorbate concentration in the final mixture were in the range of 100-150 and 20-25 mg g^{-1}, respectively. Next, the filled syringe was closed and placed for 5-6 hours on a gyrator (e.g., a Heidolph REAX2 rotator). Finally, the liquid was pushed out of the syringe with the plunger. For regeneration, the resins were washed with acidified and distilled water and the lignosulfonate was desorbed in the same manner as described above for preparative-scale isolation. The obtained ethanol solution of lignosulfonate was evaporated in a nitrogen stream and the remaining aqueous lignosulfonate solution was freeze-dried.

Lignosulfonate isolation by ultrafiltration (UF)

The UF of lignosulfonate liquors was carried out on Millipore stirred UF cell model 8200 equipped with a membrane with a nominal molecular weight cut-off of 1000 kDa, diameter 63.5 mm, at 100 rpm stirring and ~3 bar pressure. Approximately 2-4 g (based on liquor TDS) of lignosulfonate liquor was used in each UF process. The volume of the lignosulfonate solution in the cell was kept at over 50 ml. The total volume of the collected permeate was ~600-1000 ml. The retentate, which contained purified lignosulfonate, was quantitatively transferred to a plastic bottle and freeze-dried. Three parallel experiments were performed for each lignosulfonate sample. After evaluating the yields of the prepared dry samples, the samples were dissolved in deionised water and additionally treated with cation-exchange Dowex 50WX8 resin (~4-5 g of wet resin per 1 g of isolated lignosulfonate). The resin was filtered off on a glass filter #3 and exhaustively washed with deionized water. The combined filtrates were freeze-dried.

Results and discussion

Four industrial lignosulfonate samples, obtained from the most common sulphite pulping processes in use today, were selected in order to establish the basic parameters of lignosulfonate adsorption on XAD-7 resin and to prove a general applicability, verify its reproducibility, and compare the results with the established UF method. Lignosulfonate liquor samples of different types (Table 1) were applied in this study in order to confirm the widest scope of the procedure, being applicable to various lignosulfonates originating from

the most common current sulphite pulping processes that result in lignosulfonates with rather diverse properties.

The XAD-7 resin has been selected because of its structure favouring the interaction with lignin mainly based on mixed modes of hydrophobic and hydrophilic contacts, due to both aliphatic chains and polar ester functionalities present in the resin. [29,30,31] XAD-7 resin has been widely used for adsorption of different kind of aromatic molecules and has been proven to be stable and reactive also after multiple recycling steps.[19, 29, 30, 31]

Lignosulfonate preparative-scale isolation

The moderate temperatures of the adsorption process onto XAD-7 resin and the speed used to mix lignosulfonates with the resin did not have a significant effect on the results. Mixing that was too vigorous caused mechanical destruction of the resin beads. The most influential and critical factors were found to be pH, resin capacity, and time of contact.

The lignin content in all experiments was monitored by UV-VIS measurements. It should be noted that this approach can only be used in a semi-quantitative way. It is limited through the presence of extractives and polysaccharide degradation products typical for industrial lignosulfonates liquors which cause an overestimation of the UV absorption. Still, the method allowed for a fast and sufficiently accurate estimation of the lignin content and provided reasonable results for judging the adsorption capacity.

The pH influencing the surface charge of the adsorbent and the degree of ionization of the adsorbate has been thoroughly investigated.[15,19-22] Highest adsorption of aromatic hydrophobic compounds was achieved at a pH below 4. Adsorption of liquor 4 which had a pH of 10.7 was very weak (Figure 1). At a pH of 1.3, which was achieved by treatment of lignosulfonates with cation-exchange Dowex 50WX8 resin, the maximum adsorption was significantly higher and was reached faster. Therefore, a pH of 1.3 was applied in all further experiments by treatment with a cation-exchange resin. In general, lignosulfonates - independent of their initial pH which can vary between acidic and alkaline depending on the process and handling at the pulp mill - are acidified prior to adsorption in order to convert acidic groups into their protonated forms, exchanging the counter ion from the sulphite pulping process, e.g. (NH_4^+, Mg^{2+}, Na^+, Ca^{2+}) for a proton, and hence levelling out any differences coming from the respective salt of the lignosulfonate. This can either be done by sulfuric acid treatment or by applying an acidic cation-exchange resin. The latter can be considered the milder protocol and is therefore

Table 1. Basic characteristics of lignosulfonate liquors and lignosulfonates isolated from them by ultrafiltration (1 kDa) and XAD-7 adsorption.

Basic characteristics								
Lignosulfonate #	1		2		3		4	
Pulping process	NH_4^+		Mg^{2+}		Mg^{2+}		Mg^{2+} + O_2 bleaching	
Density of dry liquor, g cm^{-3}	1.4		1.5		1.6		-	
pH	4.5		3.5		7.4		10.7	
Residual non-cellulosic polysaccharides, wt%	3.6		28.4		8.6		0.8	
Lignosulfonate content determined by UV-VIS								
Extinction coefficient, M^{-1} cm^{-1*}	15.3		17.4		14.0		12.0	
Lignin content, wt%	83.4		45.9		52.2		45.8	
Isolation								
Isolation method	UF	XAD-7	UF	XAD-7	UF	XAD-7	UF	XAD-7
Lignosulfonate yield, % of TDS	61.6	47.4	25.1	24.4	39.0	40.1	34.9	36.2
Characteristics of isolated lignosulfonates								
Density of isolated LS, g cm^{-3}	1.3	1.4	1.1	1.2	1.3	1.4	-	1.2
Residual non-cellulosic polysaccharides, wt%	0.7	0.7	0.4	0.5	1.0	0.6	0.6	0.3
-OCH_3, wt%	12.2	12.3	15.2	16.3	12.7	13.1	11.5	12.4
-SO_3H, wt%	11.4	11.3	13.3	9.2	16.6	14.5	9.9	7.8
-SO_3H, wt%**	14.9	14.2	13.4	11.9	18.0	14.4	9.6	8.6
C, mol%	52.0	52.3	48.1	53.3	49.0	49.8	53.7	54.5
H, mol%	5.2	5.6	5.2	5.4	5.2	5.4	5.6	5.6
O, mol%	34.4	33.3	35.7	33.9	35.5	35.8	34.1	33.6
S, mol%	5.9	5.6	5.3	4.7	7.1	5.7	3.8	3.4
N, mol%	1.1	1.7	0.4	0.4	0.2	0.2	0.4	0.3

*Determined for lignosulfonates isolated by XAD-7 adsorption.

** Calculated based on data obtained from elemental analysis.

usually applied to avoid possible unwanted changes in the lignosulfonate structure.

In order to obtain representative specimens, the lignosulfonates must be isolated quantitatively. Therefore, the optimal conditions for a single batch adsorption process were evaluated (Figure 2). It was evident that for quantitative adsorption of lignosulfonates, the adsorbate/adsorbent proportion must be approximately 100-150 mg of lignosulfonates per gram of dry XAD-7 resin. At that optimal ratio, the equilibrium was reached after approximately 3 hours. Further contact of lignosulfonate and resin did not increase the amount of adsorbed material. The lignosulfonate was desorbed from the XAD-7 resin and analysed gravimetrically in addition to the UV method. The average of the yield difference between both methods was 5%.

The optimized conditions for lignosulfonate adsorption were applied for preparative-scale lignosulfonate isolation from selected liquor samples (1-4). The yield determined by XAD-7 adsorption was compared to a widely applied ultrafiltration method (Table 1). As can be seen from Table 1, XAD-7 adsorption method and separation by UF gave very comparable data. The XAD-7 technique also proved reproducible results for Lignosulfonate liquors from different sulphite pulping processes. Several parallel experiments gave a relative standard deviation of less than 1% yield.

The application of XAD-7 in comparison to UF (1 kDa membrane) shortened the workup-time considerably. The time needed in UF experiments varied between 24 to 48 hours, depending on the amount of TDS; the time required for adsorption was 3 hours, independent of the amount of TDS.

All lignosulfonates isolated by XAD-7 adsorption had a different extinction coefficient (Table 1). In addition, when the amount of lignosulfonates was determined by UV spectroscopy based on the respective extinction coefficient, the lignin yield was slightly over-estimated compared to data obtained by UF and XAD-7 adsorption which are both based on gravimetric lignin determination (Table 1). The lack of a universal extinction coefficient prevents general application of the UV method to measure lignosulfonate contents very precisely.

Lignosulfonate adsorption equilibrium

The determination of adsorption equilibrium and further calculation of a proper isotherm model is a common technique for comparison and characterisation of adsorption processes. The Langmuir and Freundlich isotherms (which are the most frequently used isotherms) were used to characterize and compare the lignosulfonates adsorption process on XAD-7 resin. Equilibrium adsorption isotherms describe the equilibrium amount of solute adsorbed on XAD-7 resin (q_e) and the concentration of the solute in bulk solution (Ce). The range of liquor's concentration was selected based on the most probable applicable concentration for analytical and preparative purposes. The profiles of adsorption and isotherm fittings of three selected samples were located in a very narrow variation corridor, demonstrating the similarity of the adsorption process for various lignosulfonates (Figure 3). The Langmuir model provided better fitting results compared to the Freundlich model, having a maximum adsorption capacity q_e of ~600-900 mg g^{-1} and a specific Langmuir constant K_L of $3{\cdot}10^{-5}$ to $9{\cdot}10^{-5}$ L mg^{-1}. This high adsorption capacity can be explained by a multilayer adsorption, which is comprehensively described in the literature.[31, 32] Adsorption of lignosulfonates from concentrated liquor solutions causes problems at loadings larger than 900 mg g^{-1}. Overloading leads to difficulties in the subsequent desorption of LS with alcohol or alkali and must thus be avoided.

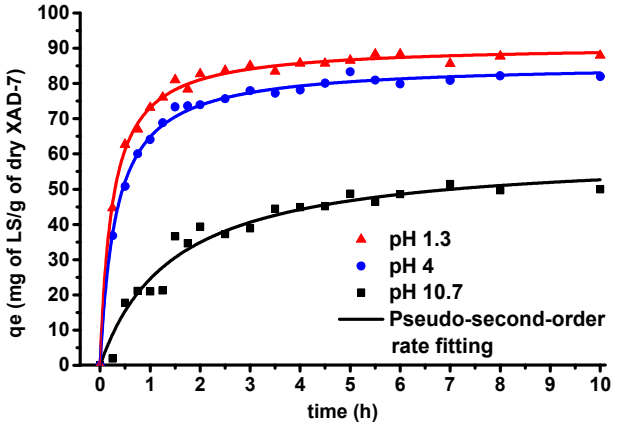

Fig. 1 The effect of pH on the adsorption of lignosulfonates on XAD-7 resin (adsorbate/adsorbent proportion: 130 mg g^{-1}) at 25 °C.

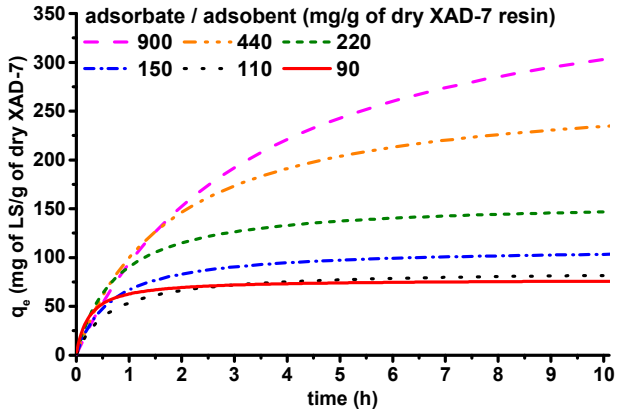

Fig. 2 The adsorption kinetic for the uptake of lignosulfonates onto XAD-7 resin at different adsorbate/adsorbent proportion at pH 2 and 25 °C.

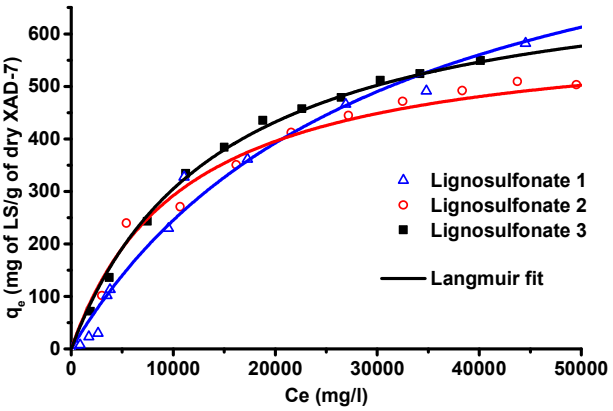

Fig. 3 Isotherm models of lignosulfonate (1-3) adsorption by XAD-7 resin at pH 2 and 25 °C.

Validation of preparative-scale XAD-7 adsorption

The amount of lignosulfonate which did not adsorb and was released in the washing stage from XAD-7 resin was estimated by UV spectroscopy. The amount released in all 6 washing steps is insignificant and the minute amount washed off is mainly lost in the first filtration stage. It is reasonable to assume that this small loss of lignosulfonate is not critical (as previously discussed), since the yields obtained with both purification methods, XAD-7 and UF, were either very similar or identical in most cases. Nevertheless, a series of validation experiments, aiming at confirming completeness of isolation in a single batch process, were performed. The lignosulfonates of three liquors samples (1-3) were isolated according to the proposed preparative-scale XAD-7 procedure. The filtrates and effluents obtained from the washing stage were combined, concentrated by evaporation, and used for a secondary preparative-scale adsorption. It was found that the total amounts which could be isolated at the second stage was less than 3 wt%. Such low yields confirmed the efficiency of the conditions determined for the single batch preparative-scale XAD-7 adsorption procedure.

The resin can easily be recycled by washing with alcohol and alkali. The only possible drawback would be the fact that only liquor solutions with concentrations of approx. 60 mg ml^{-1} can be applied, meaning that a typical industrial liquor stream has to be diluted down about four times.

Analytical-scale XAD-7 adsorption

The preparative scale isolation method can be used for a complete analysis of industrial lignosulfonates. The characteristics may vary considerably from one pulp cook to another, and thus quite often multiple analyses must be performed. Recent trends in lignin analysis focus on the application of fast methods (e.g., infrared spectroscopy) in combination with chemometric approaches to cope with the demand higher sample number and more complex structural data. In most cases, those techniques require isolated lignins, as they cannot easily correct for the complex matrix in the pulping liquor. To that end, we have developed an analytical-scale method (~5-50 mg) based on preparative-scale XAD-7.

In order to facilitate downscaling and easy handling, an approach similar to solid phase micro extraction was developed using a simple 5 ml syringe (Figure 4). To further accelerate the overall process and simplify handling, the treatment with cation-exchange resin (Dowex 50WX8) and the XAD-7 adsorption were combined.

It was proven that in relation to other methods, the analytical-scale approach quickly and easily provided purified lignosulfonates in quantities necessary for most routine analyses (~30 mg). Five parallel experiments were performed for each sample. The relative standard deviations for all experiments were below 5%. The relative content determined by both analytical and preparative-scale XAD-7 adsorption was similar. Several experiments on the resin capacity showed that overloading only occurred if the amount of liquor applied exceeded the recommended value by a factor of three.

The performance of the analytic-scale XAD-7 approach has been additionally compared with a small-scale centrifugal UF through a 1 kDa membrane (Pall Macrosep advance centrifugal devices) and a 3 kDa membrane (Amicon Ultra15 centrifugal filter units). The results show that XAD-7 is superior to UF due to its simplicity, a significantly lower isolation time, much higher throughput and a lower price.

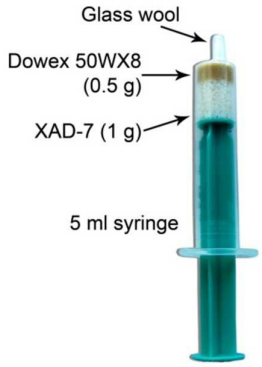

Fig. 4 Syringe filled with resins used for fast analytical-scale XAD-7 adsorption of lignosulfonates.

Characterization of lignosulfonates purified by UF and XAD7

Isolated Lignosulfonate purity. Lignosulfonates isolated by both the UF and XAD-7 methods contained less than 1% of residual, non-cellulosic polysaccharides, which were primarily composed of xylose, mannose, galactose, and glucose, as determined according to the methanolysis procedure.[33]

The content of the extractives, which was analysed using extraction with n-heptane, was below the limit of quantification.

Functional groups and elemental analysis. The comparison of the methoxyl group content after UF and XAD 7 purification produced slightly higher values for the XAD-7 purified lignin

(cf. Table 1). The methoxyl group content is accepted as one of the most important lignin parameters, as it displays lignin properties and species, and indirectly confirms lignin's purity. A higher relative content of the methoxyl group in lignin preparations, isolated from the same starting material, (for example, wood or black liquor), indicates a higher content of phenylpropane units, which are specific to lignin only. Thus it was concluded that lignosulfonates isolated by XAD-7 adsorption are of higher purity than those isolated by conventional UF. In addition, microanalysis was performed to complete the characterization. The data obtained are consistent, with minor deviations only in the sulphur content.

The sulfonic acid group content of the isolated lignins was analysed by titration (Table 1) and cross-checked with elemental analysis data. The calculations were based on the assumption that all sulphur contained originated from sulfonic acid groups. In most cases, the values were slightly higher when based on elemental analysis, but they did not significantly exceed those obtained by titration. Moreover, both methods showed that lignosulfonates isolated by XAD-7 had less sulfonic acid groups compared to lignosulfonate isolated by UF. This phenomenon can be speculatively explained by the specific equilibrium distribution of lignosulfonate molecules between the XAD-7 resin and the supernatant, causing minor loss of very small and at the same time highly sulfonated moieties. A higher content of sulfonic acid groups in lignosulfonates increases their hydrophilicity, thus lowering their affinity to the XAD-7 resin and facilitating desorption upon washing. This statement was verified experimentally by adsorption of potassium guaiacolsulfonate to XAD-7. The conditions of the experiment were exactly the same as in the adsorption kinetic experiments. Even at a high adsorbate/adsorbent proportion (850 mg g^{-1}) and relatively high solute concentration (15-20 mg ml^{-1}) the amount of adsorbed matter was very low and did not exceed 5%.

The same adsorption experiments were performed with pure phenol, and the phenol uptake by XAD-7 was almost as high as that of lignosulfonates. This observation suggested that the low adsorption of potassium guaiacolsulfonate was not related to its molecular weight alone, but its combination with the sulfonation. It is also reasonable to expect that the molecular weight does not have a significant effect on the overall adsorption.

Comparison of differently isolated lignosulfonates by FTIR spectroscopy. FTIR spectra of lignins of different origin are usually complex. Still, they are accepted as a fingerprint characteristic of lignin. Lignosulfonates have been thoroughly investigated by FTIR and bands have been assigned.[26,34-38] Purified lignins tend to produce the more useful results as interference from impurities can be kept to a minimum.

FTIR spectra of the isolated lignosulfonates in this study produced the expected standard pattern of bands. FTIR spectra of LS, isolated by XAD-7 and UF, were very similar (Figure 5, A). The only difference observed within each liquor sample was the intensity of the absorption bands at 1720 and 1680 cm^{-1}, which can be attributed to non-conjugated and conjugated carbonyl/carboxyl groups. A comparison of the lignosulfonates isolated by the analytical and preparative-scale XAD-7 adsorption approach by FTIR (Figure 5, B) revealed the identity of their characteristics.

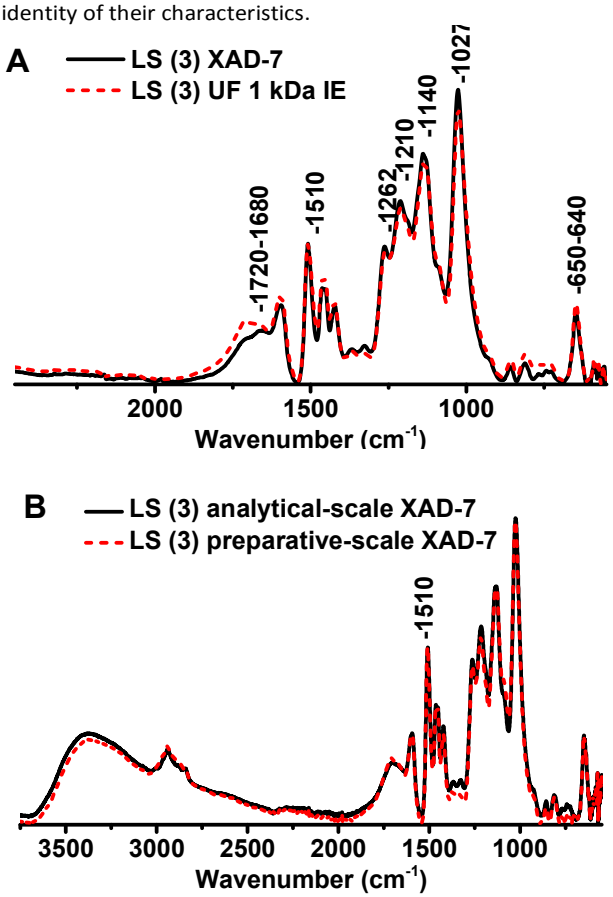

Fig. 5 A: FTIR spectra of Lignosulfonate (3) isolated by ultrafiltration (after cation exchange) and XAD-7 adsorption. B: FTIR spectra of the same Lignosulfonate (3) isolated by analytical and preparative XAD-7 adsorption

GPC characterisation of isolated lignosulfonates. Molar mass is one of the most important parameters of a polymer with regard to its physico-chemical properties and reactivity. Many attempts have been made to determine the molecular weight of lignosulfonates [38-40]; however, some fundamental obstacles, such as solubility, aggregation, dispersity, fluorescence, and absence of appropriate lignin standards for GPC column calibration, have prevented an accurate determination of their molar mass.[3,40-41] In this study, GPC was applied to determine whether different isolation protocols result in comparable molar mass distributions (MMD).

The isolation by XAD-7 yielded very well reproducible results, i.e. purified lignosulfonates with the same MMDs (Figure 6). Two independent isolations produced the same results, as demonstrated for three samples. Lignosulfonate (2) exhibited a significantly lower weight average molar mass compared to LS (1) and (3), probably due to more severe pulping conditions or the specific composition of the wood material applied at the mill.[40]

As expected, down-scaling of the XAD-7 protocol did not influence the MMD, and the results for preparative-scale and analytical scale isolation were the same. A comparison of the MMD of lignosulfonates isolated by both XAD-7 and UF did not show significant difference (Figure 7). Only small shoulders within the distribution, which could be caused by aggregation, were observed. With sample (3) a small shift to the lower molar mass region was observed when this Lignosulfonate is isolated by UF.

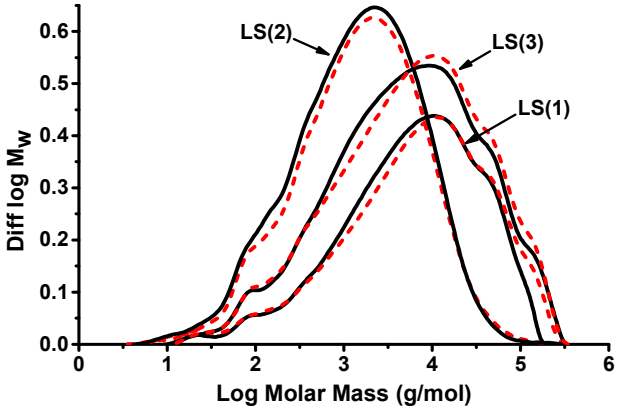

Figure 6. MMD of two isolations performed in parallel (dashed and solid curves) by preparative-scale XAD-7 for three lignosulfonates samples.

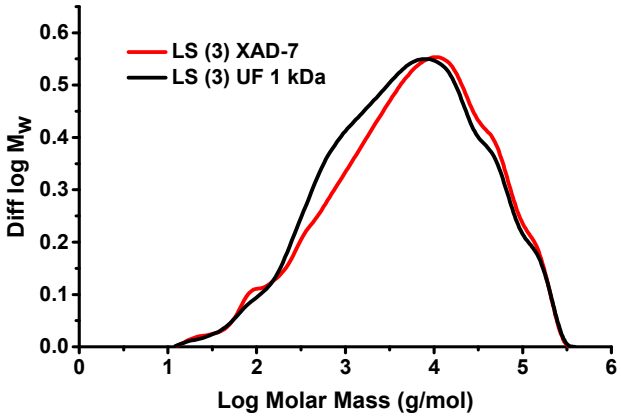

Fig. 7 Molecular weight distribution of lignosulfonates isolated by preparative-scale XAD-7 method and UF 1kDa.

Thermogravimetric (TG) analysis of isolated lignosulfonates. A thermogravimetric analysis generally produced similar degradation patterns for lignosulfonates which had been purified by both methods, XAD-7 resin adsorption and UF (Figure 8). As is typical for lignin preparations, rate maxima of thermo-oxidative degradation processes, estimated by differential thermogravimetry (DTG), were found at approximately 150 °C, 360 °C, and 450 °C.[42] A small shift in those maxima was observed for isolated samples. For Lignosulfonate (3) isolated by XAD-7, the degradation rate maxima shifted to the higher decomposition temperatures as compared to Lignosulfonate (3) isolated by UF. This can be attributed to small differences in molecular weight and functional group's composition in UF-purified resins lowering the degradation onsets. Importantly, no charred residue, which is inevitably found in the case of non-purified LS, was observed.

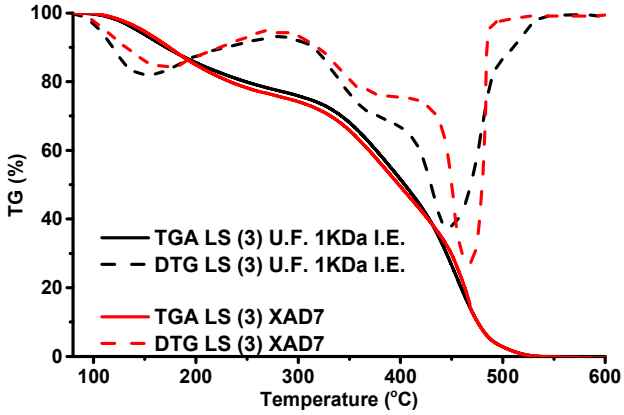

Fig.8 Thermogravimetry (TG) and differential thermogravimetry (DTG) curves of lignosulfonate purified by XAD-7 adsorption and ultrafiltration techniques.

Comparison of purified lignosulfonates by HSQC NMR. A qualitative comparison of the HSQC NMR spectra of lignosulfonates isolated by XAD-7 and UF provided greater insight into the quality of sample preparations. Overall, the samples were similar after both isolation techniques (XAD-7 vs. UF) and typical characteristics were obtained from the HSQC NMR spectra.[7,28, 43, 44]

Figure 9 shows the HSQC spectra of sample (3) after UF and XAD-7 adsorption. In addition to the general similarity mentioned above, minor differences and the presence of hitherto unidentified impurities were observed. The NMR evaluation of the purified samples is the topic of an ongoing study that will be communicated in due course. In the aliphatic-oxygenated region of the spectrum of LS isolated by XAD-7, two very prominent peaks (δ_H 5.3 / δ_C 79.5 ppm and δ_H 4.4 / δ_C 66 ppm) were absent compared to the Lignosulfonate isolated by UF. Although the definite nature of the peaks is yet to be determined, an impurity with a high degree of hydrophilicity and/or lower aromatic content appears likely as this would decrease affinity to the resin.

Another feature of the XAD-7 protocol worth mentioning was that the aliphatic region of the isolated Lignosulfonate showed a higher amount of aliphatic impurities (which are apparently not bound to the lignin). However, if spectra of UF-purified samples are scaled to a lower level, the spectra showed that a majority of these impurities are still present, but just in lower concentrations, compared to the relative amount of methoxyl groups in the respective samples. The high number of peaks in the aromatic region of the spectrum is owed to the fact that the shifts of the regular guaiacyl and syringyl units are different from those with a sulfonic acid group at Cα position.[7, 28, 43, 44] As the resolution in the shown spectra is too low in this area, further research is needed on the exact structure of these moieties. However, the relative composition of certain signals remains comparable for both purification methods.

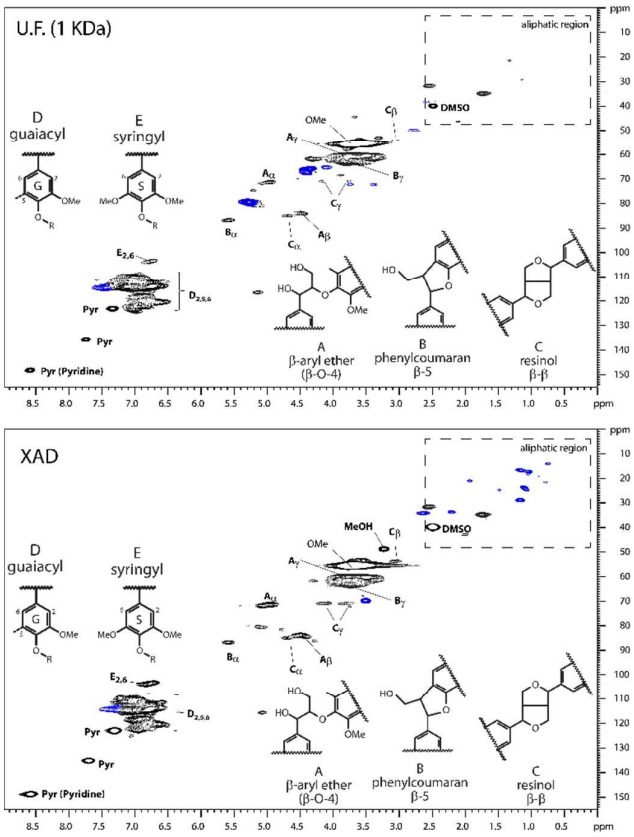

Fig. 9 Comparison of HSQC NMR spectra (acquired in DMSO/pyridine) of lignosulfonate samples purified by ultrafiltration through a 1 kDa membrane (top) and XAD-7 adsorption (bottom). Blue coloured peaks highlight major differences in the samples, the colour code having been introduced post processing.

Conclusions

A novel approach to isolation of LS from spent sulphite liquor by XAD-7 single batch adsorption was developed in two variants, for preparative scale and small analytical scale. The proposed method was tested on industrial Lignosulfonate liquors from different processes and compared with an established ultrafiltration protocol.

The XAD-7 method is superior to other methods mainly by its striking simplicity, reproducibility, and stability. The optimized protocol as given in the experimental includes all necessary steps by which the sample is cation-exchanged, adsorbed on a XAD-7 resin, purified by washing with deionized water, and eventually desorbed by alcohol extraction.

Another advantage of the XAD-7 method is its fast speed, at least in comparison to conventional and alternative purification approaches. Overall, it takes about 5-6 hours, which is much faster than UF purification through 1 kDa membrane which takes a few days. For XAD 7, the processing speed is independent of the liquor concentration. Both methods produce similar yields eventually.

An analysis of the equilibrium adsorption isotherms of Lignosulfonate samples showed a similar behaviour for various LS and gave a maximum adsorption capacity in the range of 600-900 mg g^{-1}. The preparative method can be easily scaled down and even more simplified for faster processing of a high number liquor samples. This is then done simply and cleanly in filled syringes. It can provide analytical amounts of purified lignosulfonates with purity characteristics equal to those obtained with a preparative method, but for much higher sample numbers.

It was shown that – as does UF – XAD-7 provides lignosulfonates of high purity. Isolated samples contained less than 1% of hemicelluloses, negligible amounts of extractives, and no inorganic impurities.

HSQC NMR, FTIR, GPC, and TGA analysis confirmed the similarity between both the XAD-7 and the UF method. The only noticeable differences in lignosulfonate characteristics were found in the content of methoxyl and sulfonic acid functional groups. It was shown that XAD-7 is more selective to lignosulfonate molecules and reports them to have a slightly higher content of methoxyl groups than UF. At the same time, the XAD-7-isolated lignosulfonates contained a somewhat lower amount of sulfonic acid groups, which could be explained by the high hydrophilicity of highly sulfonated moieties that are preferentially desorbed during the washing stage. Overall, XAD-7 single batch adsorption proved to be a fast, robust, and efficient way to isolate lignosulfonates with high purity. The provided protocols will allow a faster analysis and handling of larger sample amounts. We are thus confident that the method has the potential to advance lignin research and lignin application in general, and that it will be favourably accepted among lignin chemists in the worldwide community.

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