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Interactions between sodium dodecyl sulphate and non-ionic cellulose derivatives studied by size exclusion chromatography with online multi-angle light scattering and refractometric detection

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Abstract

The novel approach described allows to characterise the surfactant–polymer interaction under several sodium dodecyl sulphate (SDS) concentrations (0–20 mM) using size exclusion chromatography (SEC) with online multi-angle light scattering (MALS) and refractometric (RI) detection. Three different cellulose derivatives, hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC) and hydroxyethyl cellulose (HEC), have been studied in solution containing 10 mM NaCl and various concentrations of sodium dodecyl sulphate. It is shown that this approach is well suited for successful application of both Hummel–Dreyer and multi-component light scattering principles and yields reliable molecular masses of both the polymer complex and the polymer itself within the complex, the amount of surfactant bound into the complex as well as appropriate values of the refractive index increment $(dn/dc)_{\mu}$, of both the complex and the polymer in question. The more hydrophobic derivatives HPC and HPMC adsorbed significantly more SDS than HEC. The inter-chain interactions close to critical aggregation concentration (cac) were clearly seen for HPC and HPMC as an almost two-fold average increase in polymer molecular mass contained in the complex.

Keywords: Size exclusion chromatography; Light scattering detection; Water-soluble cellulose derivatives; Hummel–Dreyer technique; Polymer–sodium dodecyl sulphate interaction

1. Introduction

Complex formation between water-soluble non-ionic polymers and negatively charged surfactants has been a subject of intense research for both fundamental and technological reasons [1]. Among synthetic water-soluble polymers, the interaction between poly(ethylene oxide) (PEO) and sodium dodecyl sulfate (SDS) was the most frequently studied [2]. For water-soluble cellulose derivatives, today used in a number of applications such as foods, building materials, cosmetics and pharmaceutical products [2], the interaction with anionic surfactants has attracted an increasing attention during the last decades. This attention has been mostly directed towards the ethyl hydroxyethyl cellulose (EHEC)/SDS system [3,4]. Cooperative hydrophobic interaction is assumed to be a driving force for this interaction. Contrary to the case of poly(ethylene oxide), where the adsorption takes place along the polymer backbone, the substituent groups on the cellulose backbone seem to be points of adsorption depending on their hydrophobicity. Other cellulose non-ionic derivatives should thus exhibit qualitatively similar complexation behavior to EHEC. A structure of micelle-like surfactant clusters bound to the polymer chains is the generally accepted picture of the complex [1]. A great number of experimental methods[1,3,4] has been used to investigate various aspects of these complexes.

Viscometry [1] has traditionally been employed to study the onset of polymer–surfactant interaction at some critical aggregation surfactant concentration (cac) which is lower

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than critical micelle concentration (cmc). At a fixed polymer concentration, an increase of the reduced viscosity as a function of increased surfactant concentration up to a maximum around cmc followed by its moderate decrease well above cmc is usually observed. The maximum reduced viscosity values usually increase with increased polymer concentration, reflecting an increased mass of the complex formed but the cac, indicating the onset of the process, remains more or less unchanged [1]. Occasionally, plot of reduced viscosity against surfactant concentration may pass through a minimum at low polymer concentration [3,4]. This behavior clearly indicates an intricate interplay among intra-chain, inter-chain and electrostatic interactions and an increased polymer concentration should strengthen inter-particle association. The critical polymer overlap concentration, c^*, is the upper limit of the low concentration interval where isolated polymer–surfactant clusters may exist. An important question whether polymer/surfactant complex formed at various surfactant concentrations contains just a single polymer molecule at least at polymer concentration well below c^*, is still not answered completely.

Dialysis equilibrium represents a traditional method for the study of protein/solute interactions and is widely used here to determine the mass amount of surfactant preferentially bound to the polymer investigated [1]. A serious drawback of this technique consists in extensively long times needed to attain true osmotic equilibrium. Equilibration times from 7 up to even 50 days have been reported [4–8] depending upon the macromolecule/surfactant system. The Hummel–Dreyer approach [9] already described in 1962 for studies of binding of low-molecular-weight solutes to proteins, frequently referred [10] to as dynamic dialysis equilibrium, can be applied here [1] to avoid necessary excessive duration and other difficulties in a classical dialysis experiment: when the preferential sorption of one component of binary solvent exists, the polymer solution establishes an osmotic equilibrium between the polymer coils and the bulk solution. When a solvent component having higher refractive index is preferentially adsorbed by a polymer, its refractive index increment (dn/dc) should increase relative to the original solvent composition. At the same time, a certain deficit of this preferentially adsorbed component exists in the solvent outside of the coils. A size exclusion chromatography (SEC) column can then be used to separate the polymer coils, in osmotic equilibrium with the mobile phase, from a deficit peak of that solvent component. The original Hummel–Dreyer technique uses that peak to quantify the amount of bonded solute to a protein molecule and this approach was also employed for the PEO/SDS system [11–14]. Assuming that the correct mass concentration of a polymer injected is known, its dialyzed dn/dc value can be calculated if the differential refractometer (RI) is properly calibrated [15,16].

Static and dynamic light scattering (DLS) techniques are nowadays widely used to investigate polymer–surfactant interactions. Dynamic light scattering allows to determine hydrodynamic size of the particles in question [17] and is able to separate contributions from polymer–surfactant complexes and free micelles if present in the solution [18]. Its use prevails to some degree because the handling and interpretation of data obtained in these multi-component systems is more straightforward compared to static light scattering. In static light scattering (LS), polymer dissolved in a strongly scattering micellar solvent can be viewed [19] as a ternary one (polymer and binary solvent) or as a binary one (polymer complex and surfactant solution in osmotic equilibrium with the complex). Cassasa and Eisenberg [20] and Strazielle [21] have shown that excess scattered intensity measurements must be performed under conditions of osmotic equilibrium to obtain true molecular masses in this case. Equations valid for evaluation of LS data for binary system can be used if also the dialyzed refractive index increment $(\mathrm{d}n/\mathrm{d}c)_{\mu,}$ of a polymer investigated is known. The true molecular mass of a polymer is then obtained even if the macromolecule preferentially adsorbs one component of the binary solvent [19,22,23]. If the solvent is strongly scattering, which is in general the case of surfactant solution above cmc, the excess LS intensity should be measured using dialyzed solutions [8] as well. When the amount of surfactant bound to the polymer is known, the concentration and $(\mathrm{d}n/\mathrm{d}c)_\mu$ of the polymer–surfactant complex can be calculated to obtain also true molecular mass of the complex. Rather complex evaluation of LS experiments on non-dialyzed polymer–surfactant systems [24] are frequently simplified using some approximations like the assumption that polymer adsorbs all SDS molecules [25] (mixed solvent becomes weakly scattering) or the use of some approximate equation [13] for calculation of $(\mathrm{d}n/\mathrm{d}c)_\mu$.

The aim of this study is to evaluate the ability of the on-line SEC-MALS/RI method to characterize the surfactant–polymer interaction for three cellulose derivatives under several concentrations of surfactant (0–20 mM). It will be shown that the dual multi-angle laser light scattering/refractometric detection is suitable for successful application of both Hummel–Dreyer and multi-component light scattering principles in the case of polymer–surfactant interactions. If the column is in thermodynamic equilibrium with SDS-containing mobile phases, this approach yields true molecular mass of both the polymer complex and the polymer itself within the complex, the amount of surfactant bound into the complex and appropriate values of $(\mathrm{d}n/\mathrm{d}c)_\mu$ of both complex and polymer in question.

2. Experimental

2.1. Materials

Three different cellulose derivatives were studied: hydroxypropylmethyl cellulose (HPMC), MS_{HPO} 0.25, DS_{Me} 1.9; hydroxypropyl cellulose (HPC), MS_{HPO} 3.7 and hydroxyethyl cellulose (HEC). The solid material (water content

3%) was dispersed in the actual mobile phase and stored in darkness under gentle stirring for 3–4 days. The final solute concentrations were for HPC 1.0 mg/ml, HPMC 0.75 mg/ml and for HEC 0.50 mg/ml. The mobile phase consisted of an aqueous 10 mM sodium chloride solution (p.a., Merck, Darmstadt, Germany) containing 0, 1.00, 1.75, 2.00, 2.50, 3.00, 5.00, 10.0 and 20.0 mM of sodium dodecyl sulphate (BDH Laboratory Supplies, Poole, England). The surfactant-free mobile phase containing only 10 mM sodium chloride was filtered using a 0.22 μm Millex-GS filter (Millipore Corp., Bedford, MA), the SDS-containing phases were filtered using 0.22 μm Millipore TCMF filter papers (Millipore Corp., Bedford, MA).

2.2. SEC-MALS/RI

The separation column was a TSK-GEL $GMPW_{XL}$ 7.8 mm × 300 mm, particle size 13 μm, linear mixed bed size exclusion column having a linear separation range of at least 1000–1,000,000 for poly(ethylene oxide). The column was rinsed for one week with the respective SDS containing mobile phases at flow rate 0.1 ml/min to get it equilibrated before the sample injections. The pump was a Shimadzu 10ADvp liquid chromatography pump (Shimadzu Corp, Kyoto, Japan). The degasser used was a ERC-3110 (Erma Optical Works Ltd, Tokyo, Japan). The flow rate of the mobile phase was held at 0.5 mL/min. The polymer sample was injected with a 717+ autosampler (Waters Corp. Milford, MA) equipped with a 100 μl sample loop. An on-line stainless steel High Pressure Filter Holder, 25 mm (Millipore Corp, Bedford, MA), with a 25 mm 0.025 μm VSWP filter (Millipore Corp, Bedford, MA) was positioned between the pump and the autosampler.

The light scattering photometer was a DAWN-DSP multi-angle light scattering instrument (Wyatt Technology, Santa Barbara, CA). Simultaneous concentration detection was performed using an Optilab DSP interferometric refractometer (Wyatt Technology, Santa Barbara, CA). Both detectors used a wavelength of 633 nm. The signals from the two detectors were analysed by ASTRA software (ASTRA for Windows 4.73) (Wyatt Technology, Santa Barbara, CA). The angular dependence of the scattered light was extrapolated to zero angle using the linear Berry fit method, which has been found to be advantageous in previous studies [26].

2.3. Determination of dn/dc

The non-dialyzed refractive index increment (dn/dc) in all mobile phases was determined by the injection of six different concentrations of each of the samples using 1 ml loop into the refractometer at 0.5 ml/min. The data were analyzed using the DNDC5 software (Wyatt Technology, Santa Barbara, CA). The recovery of the polymer samples in experiments without SDS was obtained from the ratio of the mass eluted from the column (determined by integration of the refractometer signal) to the mass injected using dn/dc values for HPMC = 0.133, HPC = 0.135 and HEC = 0.130.

Refractive index increment $(dn/dc)_{SDS}$ of SDS in 10 mM NaCl was determined in the same way. The value 0.126 found is in a good agreement with others [13]. The values of dialyzed refractive increment $(dn/dc)_{\mu}$ of the polymer were calculated from the SEC experiment using the data of differential refractometer (RI polymer peak area represents Δn after dialysis according to Hummel–Dreyer principle) and assuming that all polymer elutes from the column for all mobile phases containing SDS. This calculation is easily done using the option "100% recovery" and "known auxiliary detector constant" of Astra 4.73 software. Preferential adsorption parameter of SDS in terms of g of SDS bound to one g of polymer was then calculated from [22,23]

$$\gamma = \frac{[(dn/dc)_{\mu} - (dn/dc)]}{(dn/dc)_{SDS}}.$$

Having access to this value, corresponding concentration of the complex was calculated and dialyzed refractive index increment $(dn/dc)_{\mu,\,compl.}$ of the complex became directly accessible. This approach was preferred here because more common use of SDS vacant peak was hampered by a complex adsorption behavior of SDS to the column packing. When polymer and complex concentration and their $(dn/dc)_{\mu}$ and $(dn/dc)_{\mu,\,compl.}$ are available, LS data evaluation as a ternary preferential adsorption case as well as binary case complex/solvent is possible. The requirement of measuring excess light scattering intensity of dialyzed solutions is always fulfilled here.

2.4. Cloud point determination

A Mettler Toledo FP900 Thermo system (Mettler-Toledo, Zurich, Switzerland) was used in this study. Three photo sensors continuously measure the residual transmitted light (from the sample suspensions) from three samples (1.0% solutions). The temperature interval for analysis was 25–60 °C for HPC, 45–75 °C for HPMC and 25–100 °C for HEC. The details of this procedure are given elsewhere [27].

2.5. SDS/column packing interaction

A TSK guard PWL column 7.5 mm × 75 mm containing the same type of packing like the separation column was selected to speed up the saturation experiments. The equipment consisted of a VCR 40 HPLC pump (Academy Development Works, Prague, Czech Republic), a Model 7125 injection valve (Rheodyne, Cotati, CA) with a 50 or 630 μl loop and a R-401 differential refractometer (Waters Corp., Milford, MA) connected through a Black Star (Huntingdon, UK) 2308 A/D converter to an IBM compatible computer; a home-made software (©J. Horský, Institute of Macromolecular Chemistry) handled the data.

3. Results and discussion

3.1. Basic characterization of the cellulose derivatives

To initially characterize the molecular mass and size, all three cellulose derivatives were analyzed in surfactant-free 10 mM NaCl. This mobile phase is regarded to be a sufficiently good solvent for all of them and this expectation was confirmed by results showing good recoveries (>95%) and no signs of aggregation in MALS signals. The obtained weight-average molecular mass is close to 100,000 g/mol for HPMC and HPC samples whereas the HEC sample has a considerably higher M_w of about 350,000 g/mol. The molecular mass distributions (Fig. 1) are quite broad, the obtained polydispersity indices for HPC and HPMC are 2.1 and 2.2, respectively compared to 3.7 for HEC.

Aqueous solutions of cellulose ethers have the typical feature of a reversed solution behavior, i.e., reduced solubility and eventually phase separation at increased temperatures. This effect is strongly influenced by the chemical nature of the substituent groups. The type of substituent, the degree of substitution as well as the substituent pattern determines the hydrophobicity of a cellulose derivative and thus its phase behavior. One rather straight-forward way to achieve a crude estimation of differences in hydrophobicity is to study the onset of phase separation (clouding) at increased temperature. This has been done for the three different derivatives dissolved in 10 mM NaCl (Fig. 2). Clearly, there are major differences in the temperature where the onset of phase separation (or commonly called the cloud point) was observed. For HPC, the cloud point (defined here as the temperature where the transmission was reduced down to 96%) occurs already slightly above 40 °C. The HPMC sample starts to phase separate at 58 °C whereas no decrease in transmission at all is observed for the HEC sample in the selected temperature range (25–100 °C). It is well-known that HEC normally is quite hydrophilic which is confirmed also for this sample by the cloud point results. The almost 20 °C difference between HPC and HPMC reflects the high degree of hydroxypropyl-

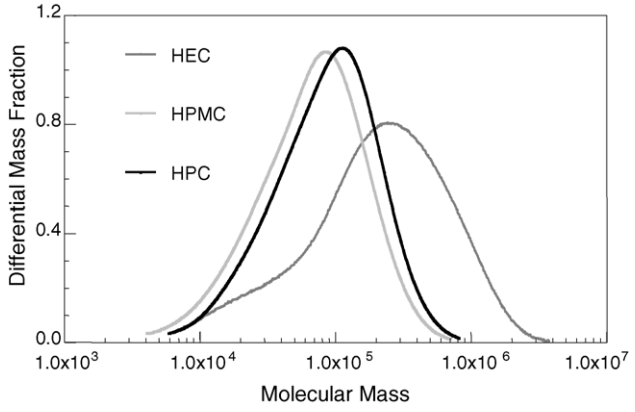

Fig. 1. Molecular mass distributions of the investigated HPMC, HPC and HEC samples obtained in 10 mM NaCl as a mobile phase.

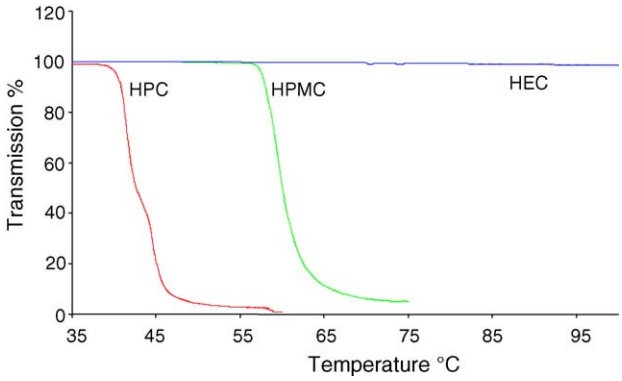

Fig. 2. Cloud point curves of the investigated HPMC, HPC and HEC samples obtained in 10 mM NaCl as solvent.

substituents (MS_{HPO} 3.7) along the HPC chain in this typical sample. The HPMC sample has a much lower amount of HP-groups (MS_{HPO} 0.25) which could explain the higher cloud point temperature; on the other hand, the randomly present methyl groups (DS_{Me} 1.9) have to be regarded as hydrophobic as well.

3.2. SDS/column packing interactions—effect of NaCl concentration

The separation column, which is expected to adsorb some SDS due to its ability to exhibit weak hydrophobic interaction, must be in thermodynamic equilibrium with any of the used SDS containing mobile phases. Break-through experiments in mobile phases containing 10 mM NaCl with 3 and 10 mM of SDS were performed to check the column behavior below and above the cmc of SDS in 10 mM NaCl (6.2 mM). 285 ml of 3 mM mobile phase was needed to obtain the break-through volume. The amount of SDS adsorbed to the column in this mobile phase was calculated to be 72.8 mg/g of packing. The injections of mobile phase without SDS as well as with an excess of 3, 5 and 10 mM SDS into the column after its equilibration gave completely no response of SDS as a result of its high retention. This result indicates that the expected Hummel–Dreyer vacant peak of SDS corresponding to the amount of SDS in the polymer-SDS complex will not be detected as well. The mobile phase containing 3 mM SDS/10 mM NaCl was then replaced by the next one containing 10 mM SDS/10 mM NaCl and an analogous saturation experiment was performed. The concentration of SDS eluted from the column remained the same as in 3 mM SDS mobile phase until the column attained its new equilibrium with the increased concentration of SDS in the mobile phase. A great similarity to the micelle-monomer equilibrium in solution is evident here. The concentration of SDS eluting from the column does not change until the amount of adsorbed “micelles” reaches a new equilibrium value corresponding to a situation where the mobile phase contains excess micelles, i.e., becomes strongly scattering. This also explains why no response of SDS was observed in

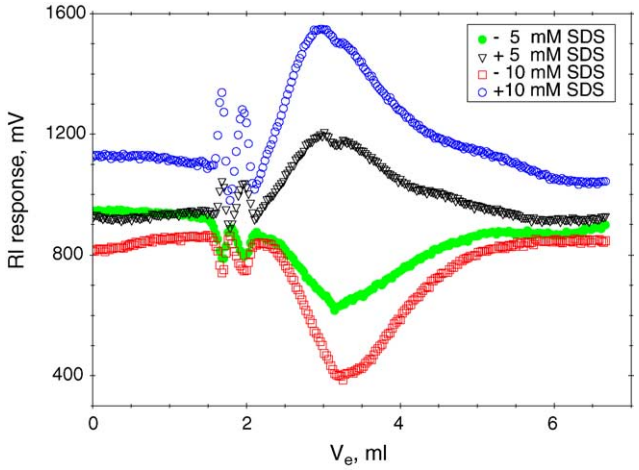

Fig. 3. The response of PWL 7.5 cm column equilibrated in mobile phase containing 10 mM SDS and 10 mM NaCl to variations of injected SDS concentrations.

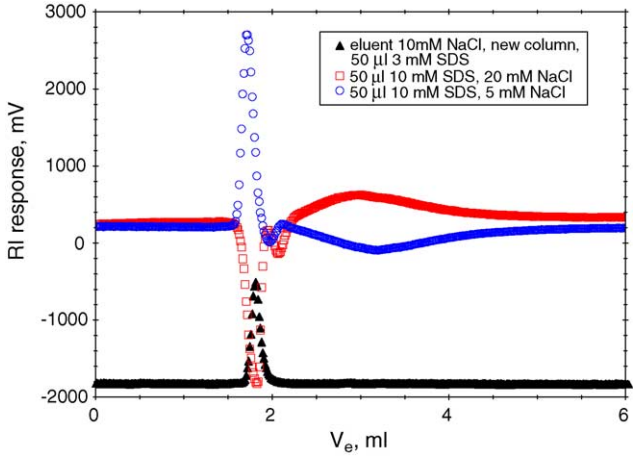

Fig. 4. The response of PWL 7.5 cm column equilibrated in mobile phase containing 10 mM SDS and 10 mM NaCl to variations of injected NaCl concentrations.

mobile phases containing concentrations of SDS below cmc. Additional adsorbed amount of SDS in this mobile phase (containing 10 mM of SDS) is obtained to be 28.6 mg/g and total adsorbed amount here increases up to 101.5 mg SDS/g of packing. Because the column is running under strongly non-linear chromatography conditions here, the difficulties concerning SDS retention and system peaks are anticipated [28]. The RI responses to the injections of SDS concentrations below and above its concentration in this mobile phase are displayed in Fig. 3. Positive/negative broad and asymmetrical peaks of SDS extending far behind the total permeation volume are obtained in this case when higher/lower SDS concentration is injected. These peaks may be used to estimate the amount of SDS in the case of injected polymer–SDS complex. The small peaks close to 2 ml (below 1% of injected mass) result from a small error in the salt content and from the dependence of cmc on ionic strength. This behavior appears to be a complex result of variations of SDS adsorption equilibrium under non-linear conditions and indicates a dominant role of electrostatic forces. Having the column in equilibrium in 10 mM SDS + 10 mM NaCl containing eluent, the effect of variations of NaCl concentration in the mobile phase at fixed 10 mM SDS content was investigated (Fig. 4). This result confirms the dominant role of salt ionic strength; the negative sharp peak in the case of increased NaCl concentration elutes close to the elution volume of NaCl peak from the non-equilibrated column and represents a difference between the positive signal of NaCl and a negative signal (being larger) of SDS micelles. Then, the equilibrium adsorption situation on the column surface is slowly restored, excess of adsorbed micelles is released and a broad positive SDS peak results. The opposite is true when the amount of NaCl in the injected solution is reduced in comparison to the mobile phase used. As expected, an increased SDS adsorption is observed when a salt concentration in the eluent goes up.

Fig. 5 shows the RI chromatograms of the three polymer samples dissolved in mobile phase containing 10 mM SDS and 10 mM NaCl and injected on $GMPW_{XL}$ column equilibrated in the same mobile phase. Two broad asymmetrical negative peaks at elution volumes about 8.5 and 10.5 ml are detected instead of only one (Fig. 3). No NaCl response confirms no difference of NaCl concentration between polymer solution and mobile phase, i.e., a correct preparation of polymer solution. The additional peak observed at 8.5 ml can be ascribed to the establishment of the osmotic equilibrium between the complex against the "infinite" large volume of solvent. When polymer is dissolved in the mobile phase, the mass balance due to osmotic equilibrium is $(c_{SDS})_{total} = (c_{SDS})_{\mu} + c_p \cdot \gamma$ [4]. Using maximum γ from Table 2 (see below), the value of $(c_{SDS})_{\mu} = 0.98$ mg/ml is obtained, which may be compared to 2.88 mg/ml SDS in mobile phase. Hence, the late eluting peak of SDS should correspond to the injected solution (restricted solution volume condition) and the early eluted peak should reflect further SDS uptake due to the osmotic equilibrium which establishes

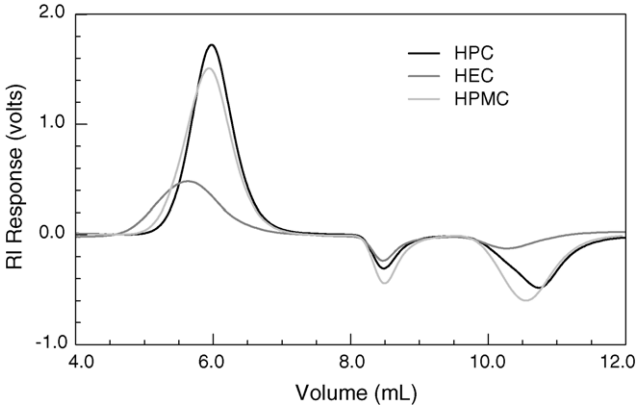

Fig. 5. RI responses of HPMC (light grey line), HPC (black line) and HEC (grey line) in mobile phase containing 10 mM SDS and 10 mM NaCl.

in the column after separation of the complex zone from the injected solvent zone. Let us note that most other interaction studies use only $(c_{SDS})_{total}$ to plot measured quantities. In our case, the relationship $(c_{SDS})_{total} = (c_{SDS})_{\mu}$ is always valid.

3.3. Interactions cellulose derivatives—SDS

The in-line use of a separation column predetermines some specific features of this technique when compared to non-separation techniques, like viscometry, DLS, etc., usually employed to investigate cellulose derivatives/SDS systems. First, it follows from Hummel–Dreyer principle, that the osmotic equilibrium here corresponds to a dialysis experiment against infinitely large solvent volume. In other words, our experiments are performed in a great excess of SDS. The results of Dubin and co-workers [8,29], although obtained for entirely different polyelectrolyte/surfactant systems, indicate in this case that the composition of the complex is independent of polymer concentration when the polymer concentration is low enough. The same result was found for methyl cellulose/SDS [30] and for the hydroxypropyl methyl cellulose/SDS system [31]. This is an important conclusion; the use of a SEC column implies variable polymer concentrations within the eluted peak due to intra- and extra-column band broadening processes. Let us note that approximate average dilution factor of our set-up is about ten. Second, the column adsorbs SDS, accumulates negative charge and a pronounced ion-exclusion effect for negatively charged complex is to be expected at too low ionic strength of the mobile phase. Therefore, all mobile phases contained 10 mM of NaCl to reduce ion-exclusion, and to some degree also polyelectrolyte expansion, in order to maintain the eluted complexes within the separation range of the column. Third, eluted peaks are monitored by the detectors immediately after the elution from the column where changes of polymer concentration take place. Fortunately, the system is a dynamic one; the kinetics of exchange of SDS in the complex has been shown to be fast enough [1] and inter-complex association, depending on polymer concentration [32], should be also fast being related to Brownian motion. Thus, it can be concluded that M_w values obtained reflect the equilibrium situation related to average eluted polymer concentration.

Obtained molecular masses of the complex of HPC, polymer in the complex, parameters of selective adsorption of SDS, *z*-averages of radius of gyration, and relevant refractive index increments as a function of SDS concentration in the mobile phase containing 10 mM NaCl are summarized in Table 1. As seen, nothing happens up to 1 mM SDS, the obtained molecular mass of the polymer in the complex, M_w, compares to that obtained in SDS-free medium within the experimental error. However, already at 1.75 mM SDS, there is a 50% increase of M_w, indicating that the interaction with SDS has been initiated. No significant change of $(dn/dc)_{\mu}$ is a clear sign that no massive incorporation of SDS into the polymer coil has started. The increase in M_w (polymer in the complex) can be thus conveniently explained only by the onset of intermolecular interactions between the hydrophobic parts of the HPC chains mediated by locally formed few small SDS clusters. It is likely that this level of SDS concentration is close to the cac for this system. Further additions of SDS caused first an increase in M_w up above 200,000 g/mol (2 mM SDS) and then its decrease back to the M_w obtained in the absence of SDS. At 2.0 mM, the first detectable increase of $(dn/dc)_{\mu}$ (caused by the corresponding γ) is observed indicating that the measurable redistribution of surfactant to the HPC coil has started. Note that small negative values of γ below 2 mM of SDS, corresponding to 1–2% of its maximum value, simply reflect the recovery values of the polymer injected which is not exactly 100% (see Section 2) and can be regarded as an acceptable experimental error of this determination. Maxima of $M_{w,\,compl.}$, $(dn/dc)_{\mu}$, and γ are obtained at 5 mM SDS, close to cmc, and are then seen to decrease somewhat for higher concentrations of SDS. The maximum M_w of the complex at 5 mM SDS is found to be 193% of polymer molecular mass. M_w of the polymer in the complex gradually gets back to the value obtained in the absence of SDS and indicates that the complex consists from only one polymer molecule (incorporating the relevant amount of SDS clusters) at 5 mM of SDS and above.

The indication of intermolecular association between cac and cmc in the case of SDS/EHEC system [33] as well as in other polymer/SDS systems below c^* has been reported by several authors [3,4,23,34]. Static light scattering measurements of M_w of PEO in the complex with hexadecyltrimethyl-

Table 1
Molecular masses of the complex of HPC, $M_{w,\,compl.}$, polymer in the complex, M_w, parameters of selective adsorption of SDS, γ, *z*-averages of radius of gyration $(r_G)_z$, and relevant refractive index increments as a function of SDS concentration in the mobile phase containing 10 mM NaCl

c_{SDS} (mM)	$(dn/dc)_{\mu}$	M_w	$(r_G)_z$	γ (g/g)	(dn/dc)	$(dn/dc)_{\mu,\,compl.}$	$M_{w,\,compl.}$
0.00	0.135	122000	31	−0.01	0.140	–	–
1.00	0.137	133000	32	−0.02	0.139	–	–
1.75	0.132	194000	28	−0.01	–	–	–
2.00	0.143	229000	31	0.04	0.138	–	–
2.50	0.147	175000	29	0.06	–	–	–
3.00	0.206	142000	32	0.53	–	0.135	217000
5.00	0.261	120000	28	0.97	–	0.133	236000
10.00	0.244	116000	22	0.83	–	0.133	213000
20.00	0.206	115000	22	0.53	0.139	0.134	177000

Average: 0.139.

ammonium chloride, although performed under low excess of SDS, have shown close to the maximum SDS binding conditions an increase of M_w of the polymer participating in the complex, on average, by a factor of two [23]. The similarity to our results is not so surprising when hydrophobic and electrostatic interactions are taken as main driving forces of complex formation. The only difference in the case of positively charged surfactant is found in weaker interaction [23] when compared to PEO/SDS system. In our case, at the very beginning of interaction very few and small SDS clusters are formed around polymer chains. The non-ionic polymer starts to accumulate charge but the localized charges are enough "diluted" to allow another polymer chain to participate in the complex because background electrolyte sufficiently reduces electrostatic interaction distance. Only when the amount of SDS in the complex becomes significant, repulsive electrostatic force prevails and the formation of multi chain complexes is no longer possible. The system should be in a dynamic equilibrium; a model of open association [32] seems to be appropriate here. Hence, the increased values of M_w in Table 1 should be average values reflecting dynamic "monomer/multimer" equilibrium. The open association process is known to be dependent on polymer concentration. Having variable polymer concentration during separation, a very complex separation mechanism must be expected under these conditions. The SEC size separation is expected to be blurred by the presence of multi-chain complexes having various sizes at various concentrations in all experiments where more than one polymer molecule may participate in the complex. This means, that no reliable conclusions can be drawn from the dependence of M_w and r_G against elution volume as well as from conformation plots in this range of SDS concentrations. Thus, the values of $(r_G)_z$ between 1.75 and 3.0 mM of SDS in Table 1 should be taken only as apparent values reflecting the presence of multi-chain complexes. Fig. 6, where the dependences of molecular mass of the polymer in the complex against the elution volume

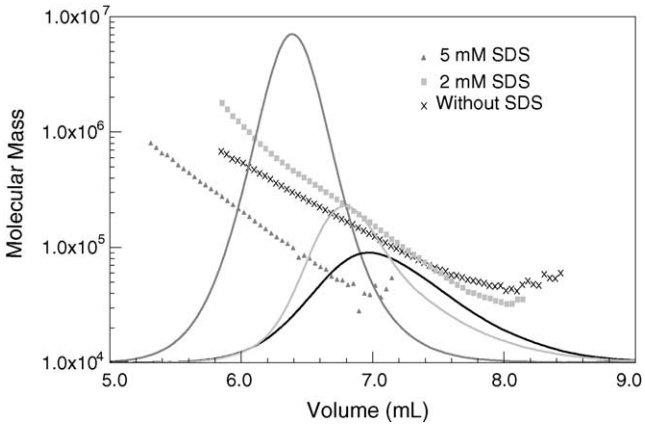

Fig. 6. The obtained molecular mass against the elution volume of HPC at various SDS concentrations in the mobile phase. Corresponding RI chromatograms: 5 mM (grey), 2 mM (light grey) and without SDS (black) are superimposed.

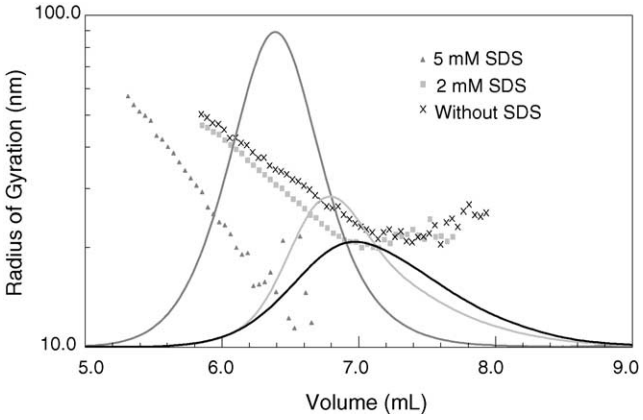

Fig. 7. The obtained radius of gyration against the elution volume of HPC at various SDS concentrations in the mobile phase. Corresponding RI chromatograms: 5 mM (grey), 2 mM (light grey) and without SDS (black) are superimposed.

are depicted for HPC at various SDS concentrations, illustrates this situation. The log M–V_e shape for 2 mM of SDS is entirely different from that obtained in 10 mM NaCl without SDS. The RI peaks reveal almost no shift in the case of 2 mM SDS but a significant shift toward lower elution volumes in the case of 5 mM of SDS. It was shown above that the column is strongly negatively charged due to the adsorption of the significant amount of SDS. The ion-exclusion behavior should thus explain the observed shift of RI peak at 5 mM of SDS where the polymer carries significant amount of SDS contrary to the low amount of SDS at 2 mM. The corresponding plot of log r_G–V_e is displayed in Fig. 7. An analogous situation to the previous figure is seen. An additional difficulty here consists in a broad molecular mass distribution extending to fairly low M values (see above) where r_G values are not accessible. Nevertheless, no significant change of r_G range (on Y axis) is seen between 0 and 5 mM of SDS, the corresponding r_G range is still say 20–60 nm in all three cases, indicating no pronounced coil expansion when a detectable amount of SDS is accumulated. Accordingly, the calculated values of the z-average r_G did not change dramatically within the range of SDS concentrations investigated. Again, this can be understood if the effect of the background electrolyte (reduction of the electrostatic interaction distance [35]) is taken into the account. The Debye length should be [35] around 5 nm in 10 mM NaCl and its variation due to changes in total ionic strength of the solutions should be thus comparable with the expected experimental error of measured r_G values. On the other hand, such changes of the repulsive interaction distance within the pores of column packing may create the observed ion-exclusion effect because of confined pore geometry.

There is a temptation to interpret the low conformation exponent obtainable from the conformation plot, its value decreases down to 0.3 for HPC between 1.75 and 3.0 mM SDS, as an indication of a strong contraction of the complex down to almost a solid sphere (its exponent should be 0.33). However, no significant changes of r_G observed (Fig. 7)

Table 2
Molecular masses of the complex of HPMC, $M_{w,compl.}$, polymer in the complex, M_w, parameters of selective adsorption of SDS, γ, z-averages of radius of gyration $(r_G)_z$, and relevant refractive index increments as a function of SDS concentration in the mobile phase containing 10 mM NaCl

c_{SDS} (mM)	$(dn/dc)_\mu$	M_w	$(r_G)_z$	γ (g/g)	(dn/dc)	$(dn/dc)_{\mu,compl.}$	$M_{w,compl.}$
0.00	0.133	82500	31	−0.01	0.134	–	–
1.00	0.132	102000	31	−0.02	0.135	–	–
1.75	0.130	97200	31	−0.03	–	–	–
2.00	0.131	155400	32	−0.02	0.134	–	–
3.00	0.188	188400	31	0.43	–	0.132	268000
5.00	0.373	95200	25	1.90	–	0.129	275000
10.00	0.307	88000	24	1.37	–	0.129	209000
20.00	0.275	98500	24	1.13	0.132	0.129	210000

Average: 0.134.

completely contradict this interpretation. The only relevant statement here should be that no conclusion about the complex density can be made from the conformation plot in the case of associating systems, in our case, between 1.75 and 3.0 mM SDS. To a large extent, the same applies to the interpretation of viscosity experiments in terms of coil size and expansion where a great caution is recommended as well. Some authors are aware of this situation [8,19,30,36–38]. It is possible to calculate mass/volume ratio for HPC without SDS and for 5 mM of SDS where maximum SDS binding occurs. This value should express segment density (segment + SDS density in the case of the complex) in the coil volume having radius $(r_G)_z$. Using $M_w = 122{,}000$ and $(r_G)_z = 31$ nm (Table 1), the value of segment density is obtained to be 0.0016 in the absence of SDS. This value will increase to 0.0031 using the M_w of the complex in the case of 5 mM SDS solution, taking into the account that almost no significant change of $(r_G)_z$ takes place. It follows from this simplified picture that any discussion in terms of dense spheres is irrelevant.

Obtained results for HPMC as a function of SDS concentration in the mobile phase containing 10 mM NaCl are summarized in Table 2. Qualitatively, a similar picture to the previous one is obtained. A substantial increase in M_w of the polymer in the complex is found at 2 mM of SDS. The maximum M_w is found at 3 mM of SDS and may be interpreted as indicating a maximized cluster association. Maximum values of $M_{w,compl.}$, $(dn/dc)_\mu$, and γ obtained at 5 mM SDS (close to cmc) are then seen to decrease somewhat for higher concentrations of SDS. The highest M_w of the complex at 5 mM SDS is found here to be 305% of polymer M_w. Again, M_w of the polymer in the complex gets back to the value obtained in the absence of SDS indicating that the complex consists of only one polymer molecule (incorporating the relevant amount of SDS clusters) at 5 mM of SDS and above. The plots of $\log M$ and $\log r_g$ against V_e were qualitatively very similar to the displayed ones for HPC (Figs. 6 and 7). For example, a difference in shape of $\log M$ against V_e was observed at SDS concentrations where inter-complex association takes place. No significant changes of r_G (when plotted against the elution volume) as well as of calculated values of $(r_G)_z$ could be observed up to 5 mM of SDS. The ion-exclusion behavior of HPMC was also very similar to the previous HPC sample. It follows from these experiments that the only significant difference between HPC and HPMC interactions with SDS is found in the higher amount of SDS adsorbed despite the higher cloud point value of HPMC. This apparent contradiction might be explained by fundamental differences behind these phenomena. Cloud point determination indicates the onset of macroscopic phase separation and should reflect rather the "average" hydrophobicity of polymer chains together with possible presence of non-derivatized sequences of the polymer chain which could also contribute to phase separation. On the other hand, clustering with SDS probably takes place rather on a microscopic scale, i.e. only parts of the coil (most probably side chains here) are involved in the cluster. Stronger interaction of HPMC with SDS may then result from a proper combination of hydroxypropyl and methyl groups along the cellulose backbone.

Obtained results for HEC as a function of SDS concentration in the mobile phase containing 10 mM NaCl are summarized in Table 3. HEC, having a cloud point above 100 °C

Table 3
Molecular masses of the complex of HEC, $M_{w,compl.}$, polymer in the complex, M_w, parameters of selective adsorption of SDS, γ, z-averages of radius of gyration $(r_G)_z$, and relevant refractive index increments as a function of SDS concentration in the mobile phase containing 10 mM NaCl

c_{SDS} (mM)	$(dn/dc)_\mu$	M_w	$(r_G)_z$	γ (g/g)	(dn/dc)	$(dn/dc)_{\mu,compl.}$	$M_{w,compl.}$
0.00	0.130	364000	65	−0.03	0.135	–	–
1.00	0.133	352000	64	−0.02	0.135	–	–
1.75	0.130	331000	62	−0.03	–	–	–
3.00	0.133	348000	64	−0.02	–	0.133	350000
5.00	0.163	416000	54	0.22	–	0.134	506000
10.00	0.212	360000	58	0.61	–	0.132	578000
20.00	0.187	338000	58	0.41	0.135	0.133	475000

Average: 0.134.

and thus being more hydrophilic, turns out to exhibit weaker interaction with SDS when compared to HPC and HPMC. An increase in M_w by only ~18% reflects the low tendency to intermolecular association at 5 mM of SDS. The maximum values of $M_{w,compl.}$, $(dn/dc)_\mu$, and γ obtained at 10 mM SDS are then seen to decrease somewhat for 20 mM of SDS. The highest M_w of the complex at 5 mM SDS is found here to be 164% of polymer M_w. The polymer molecular mass in the complex then returns to the value obtained in the absence of SDS at 10 mM of SDS. The behavior of $(r_G)_z$ follows the previous pattern. The SEC ion-exclusion effect described above persisted here as well above 5 mM of SDS.

The original Hummel–Dreyer technique uses negative peak to quantify the amount of bonded solute to a polymer molecule. The SDS/column packing adsorption experiments (see above) have shown that this approach cannot be used below cmc of SDS due to a peculiar SDS adsorption equilibrium. Nevertheless, the two negative peaks of SDS obtained on the RI trace from experiments in mobile phases containing 10 mM (Fig. 5) and 20 mM of SDS may be used to calculate γ. The calculations of γ were performed for all three polymers and the following values of γ were obtained for 10 and 20 mM of SDS, respectively, 0.97 and 0.65 for HPC, 1.58 and 1.17 for HPMC, 0.75 and 0.59 for HEC. A comparison with data of Tables 1–3 shows a reasonable agreement in most cases. However, the γ-values obtained using SDS peaks were found to be quite sensitive to baseline selection. This can be anticipated already from the inspection of Fig. 5 to be one main reason of observed differences. Hence, the use of dn/dc values was the preferred way to calculate γ also for mobile phases containing 10 an 20 mM of SDS.

4. Conclusions

The analysis of interactions between SDS and three different water-soluble cellulose derivatives in solutions of various SDS concentrations in presence of fixed NaCl concentration has been performed using SEC separation with online light scattering/refractometric detection. It was shown, using the full potential of the Hummel–Dreyer technique together with principles of static light scattering valid for multi-component systems, that this approach allows to measure true molecular mass of the polymer forming a complex, reliable molecular mass of the complex if composed from single polymer coils, the amount of preferentially adsorbed amount of SDS in the complex and refractive index increments of the polymer forming a complex as well as of the complex itself under the conditions of osmotic equilibrium which are necessary for a correct handling of light scattering data. Tedious dialysis experiments are thus conveniently avoided. It was found that there is a significant intermolecular association in the beginning of the complexation process when the amount bonded SDS is quite low. The increased amount of bonded SDS then prevents further intermolecular interactions and the complexes begin to be formed from single polymer coils carrying micellar clusters in agreement with the commonly accepted picture. The cluster formation roughly follows the differences in hydrophobicity and structure of side chains of the polymers used; the highest level of interaction was found in the case of HPMC, the lowest one in the case of HEC.

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