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Impact of Phenolic Antioxidants on Structural Properties of Micellar Solutions

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Abstract

Antioxidants solubilized in micellar solutions can change micellar properties like the size and shape of micelles, critical micellar concentration (cmc) and viscosity. Interactions arising between antioxidants and the surfactant determine the locations of antioxidants and vice versa. The location and interaction are dependent on the type of both the antioxidant and surfactant. Influences of various antioxidants on the physical and structural properties were tested in micellar systems of cationic CTAB, non-ionic Brij 58 and anionic SDS. The antioxidants used to investigate the effects of gradually increasing lipophilicity were gallic acid (GA) and the gallate esters from methyl to octyl gallate (MG-OG). Hydroxy cinnamic acids (HCAs) like *p*-coumaric acid (pC), caffeic acid (CA), ferulic acid (FA) and sinapic acid (SA) were employed to observe effects of functional groups like hydroxyl and methoxy groups. Micellar size and shape determined by small angle neutron scattering (SANS), viscosity and cmc were chosen to characterize the antioxidant influence. In Brij 58 systems propyl gallate (PG) did not affect the cmc or aggregation number but decreased micellar size slightly due to an intercalation of PG into the region of the polyoxyethylene chain and the first adjacent alkyl chain methylene groups. In SDS systems the micellar size and cmc decreased in the presence of PG. This was attributed to PG residing in the Stern layer. However, in CTAB systems micelles swelled at low PG concentration and in the presence of GA, while higher PG concentrations and more lipophilic antioxidants led to a sphere-to-rod transition with a simultaneous increase in viscosity and decrease in cmc. This revealed the intercalation of antioxidants in the palisade layer of CTAB micelles entering into strong interactions of electrostatic and hydrophobic origin. It could be demonstrated that the interactions became stronger the more lipophil an antioxidant is and the more hydroxyl groups are attached to the aromatic ring. Differences in the location and interaction of antioxidant and micelles are proposed as being responsible for the effectiveness of antioxidants.

Keywords

Antioxidants; CTAB; SDS; Brij 58; emulsifier; localization

Introduction

Antioxidants are employed in emulsions to prevent lipid oxidation, but their effectiveness varies strongly among emulsions containing different emulsifiers/surfactants.[1,2] Several studies suggested that the reduced activity of an antioxidant is the result of interactions arising between the antioxidants and emulsifier/surfactants.[1–3] The type of interaction determines the specific locations of the antioxidant in emulsions and particularly in the emulsifier/surfactant rich oil-water interface. Thus, the location of an antioxidant in the emulsifier/surfactant environment can be of crucial importance for its activity. However, data demonstrating the exact location of antioxidants are lacking.

It is well known that ionic micellar systems are sensitive to interactions with counterions or solutes resulting in changes in micellar size and shape, viscosity or cmc. These changes can be correlated with the type of solutes and where these solutes are solubilized.[4–6]

Various studies using a variety of techniques such as rheological approaches,[7–12] birefringence,[8] NMR,[4,5,8,13,14] heat capacity,[15] acidity,[13] neutron reflectivity,[6] surface tension,[16] and SANS measurements[4,17,18] have been performed on the influence of organic counterions on cationic surfactants. It has been demonstrated that aromatic counterions are more effective at lower concentrations than salts or electrolytes in inducing the micellar growth,[6,13,19] which can be attributed to interactions with the aromatic π-electron system.[13] Particularly aromatic anions like hydroxy- and chlorobenzoates and phenols have been investigated for inducing the elongation of CTAB micelles.[4–6,9,11,12,18,20,21] Substituents and their position on the aromatic ring system play an important role in micellar growth. While hydroxyl groups in the *ortho*-position induce growth, those in the *para*- and *meta*-position do not.[15] For chlorobenzoates, the opposite was found. The growth inducing effect of *m*- and *p*-chlorobenzoates can be attributed to their intercalation among the surfactant headgroups, whereas *o*-chlorobenzoates prefer locations in the Stern layer tangential to the micellar interface.[4,5] There is an increase in viscosity the more hydroxyl groups are present,[13] but no effect on the viscosity could be observed in the presence of methoxy groups.[22] Vermathen et al.[5] summarized the main effects caused by aromatic counterions in terms of their substitution pattern and the nature of their substituents, size, hydrophobicity and degree of hydration that determine their preferred location and orientation within or at the cationic micellar interface. Additionally, a molecular modeling study of Rakitin and Pack[23] demonstrated the necessity of the aromatic counterion being planar. These properties may in turn influence the cmc, aggregation number, sphere-to-rod transition, Krafft point, degree of ionization, counterion affinity orders, catalytic activity of ionic aggregates[24] and in particular the solution properties.[4]

In this study the location of various antioxidants was investigated in micellar solutions using the surfactants CTAB, SDS and Brij 58. For the sake of simplicity, micellar solutions were considered to exhibit the important properties of surfactants located in the oil-water-interface of emulsions. To determine the importance of the lipophilicity of antioxidants on their solubilization, gallate esters with increasing chain length from methyl to octyl were employed (Figure 1). Furthermore, the effect of the number and position of hydroxyl groups on the aromatic ring was investigated using hydroxy cinnamic acids (Figure 2).

Experimental Section

Chemicals

Cationic CTAB (cetyltrimethylammonium bromide), anionic SDS (sodium dodecyl sulphate), non-ionic Brij 58 (polyoxyethylene-20-cetyl ether), gallates and hydroxy cinnamic acids were obtained from Sigma-Aldrich (Seelze, Germany) and used without further purification. D_2O (purity > 99%), acetic acid (analytical grade), and sodium acetate (anhydrous) were purchased from Roth (Karlsruhe, Germany).

Viscosity

Required amounts of antioxidants were weighed into 30 ml vials and added to 20 ml of 1% SDS, CTAB, or Brij 58 in H_2O acetic buffer solution

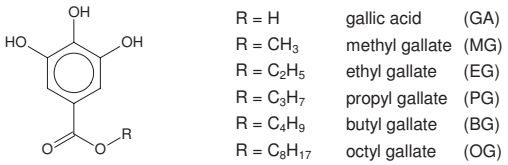

Figure 1: Structures of gallates

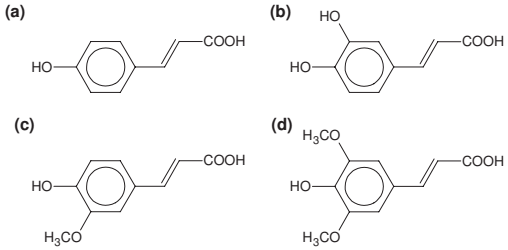

Figure 2: Structures of hydroxy cinnamic acids (HCA) (a) *p*-coumaric acid (pC), (b) caffeic acid (CA), (c) ferulic acid (FA), (d) sinapic acid (SA)

or to 5 ml of D_2O acetic buffer solution with equivalent surfactant concentrations. Samples needed to be heated to 60°C to ensure that they dissolved. Viscosity measurements of the samples in D_2O acetic buffer solution (0.2 mol/l, 5.0 pH) as well as the CTAB samples in H_2O containing increasing concentrations of PG were performed on a high resolution rheometer (NF Bohlin Instruments CVO 120) by the plate-plate method. During the measurements the sample volume of approx. 5 ml was placed on the plates, equilibrated at 25°C, and a constant shear stress of 1 Pa was applied. Every 10 s a data point was recorded so that 15 data points per sample were collected. The apparent viscosity was reported as the mean and standard deviation of the 15 data points. For low viscositly solutions, the sensitivity of the viscosity measurements was enhanced by using a capillary viscometer (Ubbelohnde viscometer with suspended ball-level for the determination of the viscosity according to DIN 51562, Schott, Mainz, Germany). The time required for the sample to run a certain distance was recorded and multiplied by the characteristic instrument constant ($K = 0.02954\,\mathrm{mm^2/s^2}$) in order to calculate the apparent viscosity. The viscosity measurement for each sample was carried out in triplicate and the result reported as the mean and standard deviation.

cmc

Surface tension measurements were performed on a Krüss K12 tensiometer (Krüss, Hamburg, Germany) using the platinum plate method (19.9 mm x 0.2 mm) according to Wilhelmy.[25] Prior to the measurements, the equipment was tested by determination of a surface tension of $\sigma = 72.7 \pm 0.2\,\mathrm{mNm^{-1}}$ of bidestilled water at 20°C. All stock solutions were prepared in acetic buffer solution (0.2 mol/l, 5.0 pH, $\sigma = 69.7 \pm 0.2\,\mathrm{mNm^{-1}}$) containing 0.1% SDS, 0.012% Brij 58, 0.032% CTAB or 0.0032% CTAB. Antioxidant stock solutions had the following concentrations: 0.4% PG, 0.2% PG, 0.1% PG, 0.01% PG as well as 0.01% GA, CA and SA. Each sample

had a total volume of 30 ml and contained either 15 ml of antioxidant stock solution or 15 ml of buffer solution (control), while the surfactant concentration was adjusted in the remaining volume of 15 ml. For each surface tension curve, 15 different concentrations were determined by recording 10 data points per concentration within a measuring interval of 2 s. The sensitivity was set to 0.010 g and the density of water was 0.998059 g/cm^3 for all measurements performed at 20°C.

Surface tension was plotted against the logarithm of the concentration. Origin 7.0 software was used to fit these curves and to determine the cmc, pre-cmc slope, and surface tension of the concentration at which micelles are formed according to the function:

$$\mathrm{f}(c_\mathrm{s}) = -\frac{\partial\sigma}{\partial \ln(c_\mathrm{s})} \left(\ln(\mathrm{cmc}) - \ln(c_\mathrm{s})\right)\ \mathrm{g}(c_\mathrm{s}) + \sigma_\mathrm{cmc}\ , \qquad (1)$$

$$\text{with} \qquad \mathrm{g}(c) = \begin{cases} 1 & \text{for} \quad c_\mathrm{s} \leq \mathrm{cmc} \\ 0 & \text{for} \quad c_\mathrm{s} > \mathrm{cmc}\ , \end{cases}$$

where σ_cmc is the surface tension at surfactant concentrations higher than the cmc and $\frac{\partial\sigma}{\partial \ln c_\mathrm{s}}$ describes the alteration in the surface tension as a function of the natural logarithm of the surfactant concentration c_s (slope). The cmc, σ_cmc and $\frac{\partial\sigma}{\partial \ln c_\mathrm{s}}$ were used as fitting parameters in a least-square fit. All values were reported as the mean and standard deviation according to the fitting curves. Since the antioxidants concentration ($\mathrm{c_{aox}}$) was kept constant and the surfactant concentration varied, the excess surface concentration can be calculated as:[26]

$$\Gamma = -\frac{1}{kRT}\left(\frac{\partial\sigma}{\partial \ln(c_\mathrm{s})}\right)_{c_\mathrm{aox}}\ , \qquad (2)$$

where σ is the surface tension [Nm^{-1}], R the gas constant, T the temperature in [K] and c_s the surfactant concentration [mmol/l]. In the case of ionic surfactants, counterions were considered by setting k to 2,[26] while for the non-ionic Brij k was 1. From the maximum excess surface concentration the specific excess surface concentration Γ^* was calculated, which is the ratio of surfactant headgroups (N_s) to micelle surface area (A_surf) in [Å^{-2}] given by:

$$\Gamma^* = (\Gamma\ N_\mathrm{A})^{-1} = \frac{N_\mathrm{s}}{A_\mathrm{surf}}\ , \qquad (3)$$

where N_A is Avogadro's number. The cmc is a measure of the free energy gain of micellization ($\Delta G^\circ_\mathrm{M}$) associated with micelle formation given by the relation:[27]

$$\Delta G^\circ_\mathrm{M} = RT\ \ln(\mathrm{cmc}) \qquad (4)$$

SANS

Micellar solutions containing 1% SDS, Brij 58 or CTAB were prepared in deuterium buffer solution (0.2 mol/l acetic buffer solution, pH 5.0). The required antioxidants were weighed (0.1-0.35%) and added to individual 3 ml micellar solutions. To dissolve the less hydrophilic antioxidants, samples needed to be heated to nearly 60°C. Small angle neutron scattering measurements were performed on the SANS-1 instrument at

the FRG1 research reactor at the GKSS research centre, Geesthacht, Germany.[28] To cover the range of scattering vectors q from 0.005 to 0.25 $Å^{-1}$, four sample-to-detector distances between $0.7\,\mathrm{m} < d < 9.7\,\mathrm{m}$ were used. The neutron wavelength λ applied for all experiments was 8.1 Å with a wavelength resolution of $\Delta\lambda/\lambda$ 10% (full-width-at-half-maximum value). Samples were kept in quartz cells (Hellma) with a path length of 2 mm and placed in a thermostated sample holder to ensure isothermal conditions of $T = 25.0 \pm 0.5\,^{\circ}\mathrm{C}$. Raw data were corrected for background from the deuterium buffer solution and sample cell and other sources according to conventional procedures described in detail by Cotton.[29] The two-dimensional isotropic scattering spectra were azimuthally averaged, converted to an absolute scale, and corrected for the detector efficiency by dividing by the incoherent scattering spectra of pure D_2O, which was measured with a 1 mm path length quartz cell.[30]

Analysis of SANS data

The SANS data were analyzed by the indirect Fourier transformation (IFT) method according to Glatter[31] in the version of Pedersen.[32] IFT is a model independent approach and requires only a minimum of prior information for analysis, i.e. the maximum size and dimensions of aggregates (spherical, rod or disk like). The IFT method makes it possible to calculate the pair distance distribution (correlation) function of scattering excess $p(r)$ for spherical aggregates or the cross-sectional pair distance distribution (correlation) function $p_{\mathrm{CS}}(r)$ for rod-like aggregates. From the $p(r)$ function the mass M can be obtained from which the aggregation number of micelles can be calculated. Furthermore, this equation gives the radius gyration of the scattering length density excess R_{g} for spherical aggregates as well as the corresponding mass per unit length (ML) and radius gyration of cross section ($R_{\mathrm{CS,g}}$) for rod-like aggregates.[33]

From the shape of the curve obtained by plotting $p(r)$ against the distance in the micelle r the shape of the micelle can be estimated. A symmetrical shape is related to spheres while an unsymmetrical shape indicates cylindrical micelles, with the axial length of the cylinder being responsible for the linear region of $p(r)$ for a large r.[18] The scattering curve $I(q)$ showed a q^{-1} dependence. This strongly supports the rod-like shape of aggregates. To obtain the total length of rod-like micelles, a second approach was additionally used where the lowest q range (< 0.01) was fitted using the Debye function[34] to obtain the apparent mass of aggregates. A similar analysis was performed for the polymer-like micelles formed by amine oxide surfactants.[35]

The aggregation number was calculated assuming that only surfactant molecules contribute to the micellar size and particularly to the micellar volume and scattering length density. The scattering length densities were calculated using table values from the studies by Chevalier and Zemb[36] (Table 1).

Table 1: Scattering length density per unit mass $\Delta\rho_m$ and molecular volumes V of CTAB, Brij 58, and SDS dissolved in deuterium buffer solution[36]

	$\Delta\rho_m$ [cm/g]	$V_{molecule}$ [cm^3]
CTAB	$-6.419 \cdot 10^{10}$	$606 \cdot 10^{-24}$
Brij 58	$-5.817 \cdot 10^{10}$	$1714 \cdot 10^{-24}$
SDS	$-5.980 \cdot 10^{10}$	$442 \cdot 10^{-24}$

Results

Viscosity

The apparent viscosity of the different micellar solutions was investigated in the presence and absence (control) of antioxidants (Figure 3). The dose dependency of the viscosity was determined for PG concentrations ranging from 0.1% to 0.35% in CTAB, Brij 58 and SDS micellar solution prepared with H_2O acetic buffer solution (shortened H_2O). The apparent viscosity of micellar solutions was about 1 mPas in the absence of antioxidants. The viscosity of Brij 58 and SDS micellar solutions was constant in the presence of PG regardless of its concentration. CTAB micellar solutions showed a constant viscosity up to a PG concentration of 0.2%. However, an increase in the viscosity to nearly 5000 mPas was found upon reaching a concentration of 0.35% PG (Figure 3a).

To determine the effect of the alkyl chain length of antioxidants, 0.2% of various gallates were added to the different micellar solutions (Figure 3b). No effect of the antioxidants could be observed in SDS or Brij 58 micellar solutions. However, in CTAB solution an increasing chain length of gallates had a marked effect. Addition of BG resulted in a threefold increase in the viscosity, but in the presence of OG the viscosity dropped to the same level as that measured for CTAB solution containing MG.

To correlate the viscosity results with SANS data obtained in D_2O acetic buffer solution (shortened D_2O) the impact of D_2O on the viscosity was also investigated (Figure 4). Comparison of the viscosities as a function of the antioxidant concentration in the different aqueous phases showed that in D_2O the viscosity increased with increasing antioxidant concentrations as well (Figure 4a). However, the sudden rise in the viscosity was seen in D_2O at a lower PG concentration of 0.2% than in H_2O (0.25%). At the same PG concentration the viscosity in D_2O was significantly higher than in the H_2O CTAB micellar solution and at a 0.35% PG concentration in D_2O the viscosity was more than twice as high as in the corresponding H_2O solution.

The increase in the viscosity caused by different gallates with increasing chain length could be observed in D_2O as well (Figure 4b). However, the viscosity was higher for EG, PG and BG in D_2O than in H_2O, but the same was true for GA, MG, and OG. Unlike in H_2O, in D_2O the viscosity maximum was already achieved by PG and decreased in the presence of BG.

In the presence of HCAs, only a slight rise in the viscosity was found (Figure 4c). While in H_2O no difference could be determined between the individual HCAs, in D_2O a slight increase in viscosity could be found in

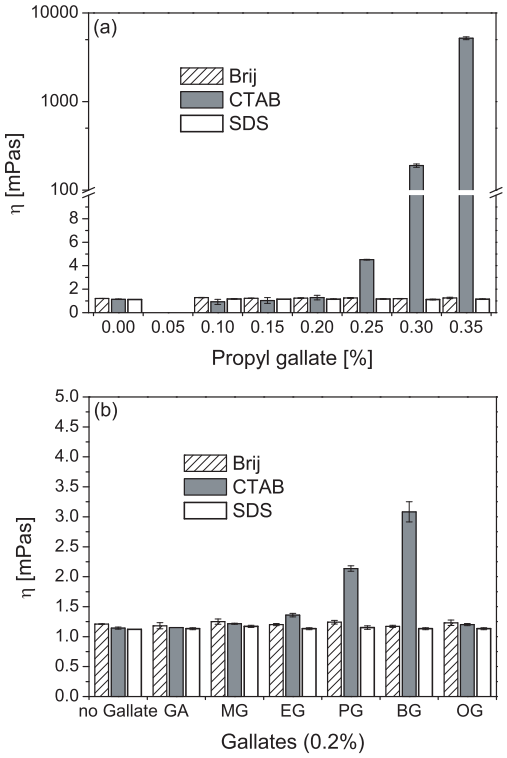

Figure 3: Apparent viscosity η of 1% CTAB, Brij 58, and SDS micellar solutions in the presence of increasing PG concentration (a) and in the presence of various gallates (0.2%, b). Micellar solutions were prepared in H_2O-acetic buffer solution, $n = 3$

the order SA = FA < pC < CA. The effect of HCAs containing methoxy groups on the viscosity was weaker in D_2O than in H_2O, whereas in the presence of pC and CA the viscosity was the same in both systems.

Surface tension

In Brij 58 micellar solutions, the surface tension above the cmc was found to be lower in the presence of 0.2% PG than in pure acetic buffer solution (control). However, the cmc was not changed by addition of PG (Figure 5a). In SDS micellar solution, the opposite effect could be observed. The surface tension at cmc was similar in the presence of 0.2% PG and in the control, but the cmc was lowered due to PG (Figure 5b). In CTAB systems, the cmc as well as the surface tension above the cmc were lowered in the presence of various antioxidants. Both the type of antioxidant (Figure 6b) and the concentration (Figure 6a) forced the CTAB monomers to start forming micelles that included fewer numbers of surfactant molecules.

Information obtained by fitting of the individual surface tension curves, including the cmc, surface tension above the cmc, the pre-cmc slope, the ratio of surfactant headgroups to micelle surface area and the free energy of micellization, are listed in Table 2. The cmc of the control systems showed great differences in the surfactant concentration required to form

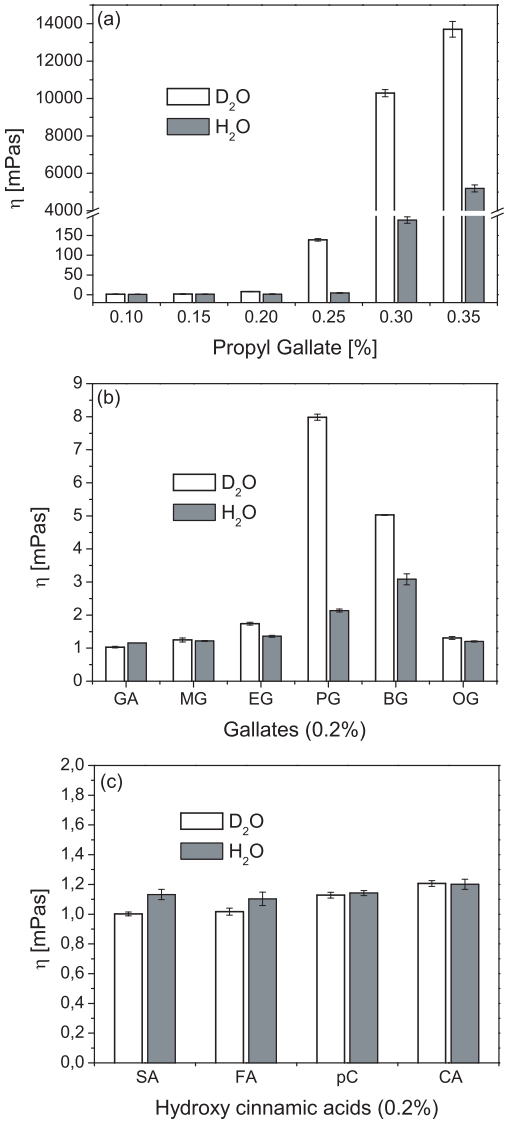

Figure 4: Comparison of the apparent viscosity η of 1% CTAB micellar solutions containing either H_2O or D_2O-acetic buffer solution (shortened H_2O or D_2O) in the presence of increasing PG concentrations (a) various gallates (0.2%, b) or various HCAs (0.2%, c), $n = 15$

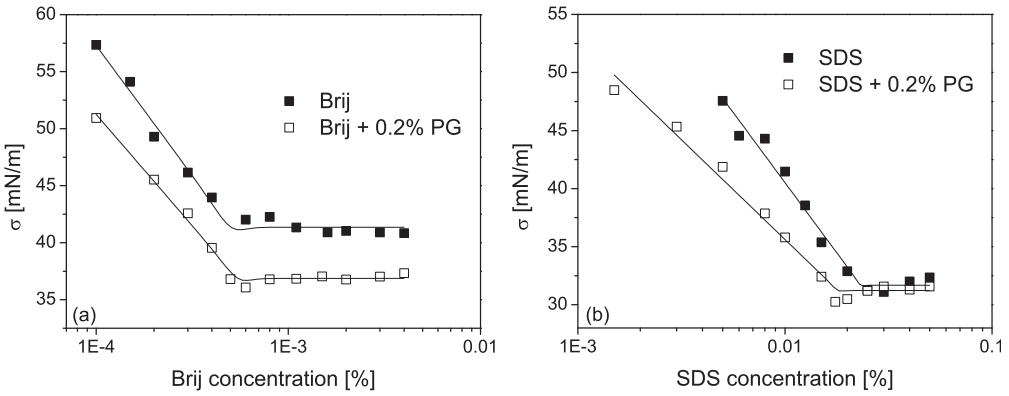

Figure 5: Surface tension σ as a function of Brij 58 (a) and SDS (b) concentration in the absence and presence of PG (0.2%), $n = 10$

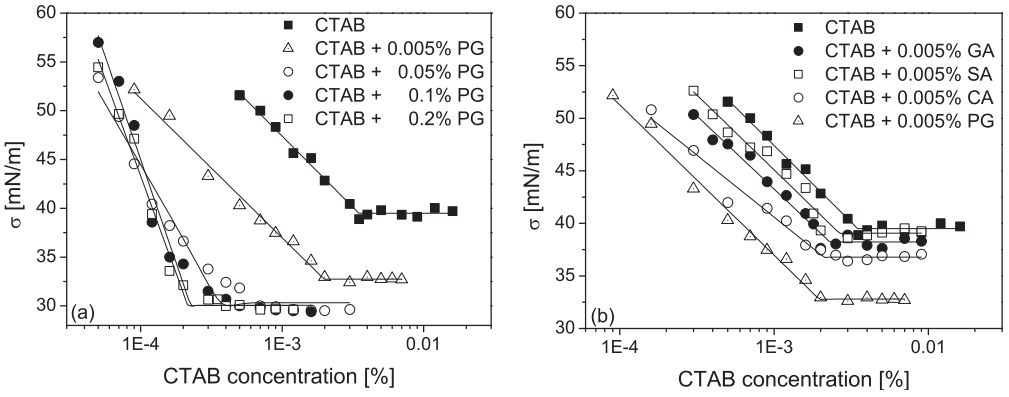

Figure 6: Surface tension σ measurements as a function of CTAB concentration in the absence and presence of different PG concentrations (a) and of various antioxidants (0.005%, b), $n = 10$

Table 2: Determination of the cmc, specific excess surface concentration, and free energy of micellization of CTAB, Brij 58, and SDS in the absence and presence of selected antioxidants

	cmc [mg/L][c]		σ_{cmc} [mN/m][cd]	$\frac{\partial \sigma}{\partial \ln c_s}$ [mN/m][ce]	$\Gamma^* \cdot 100$ [Å^{-2}][f]	ΔG°_M [kJ/mol][g]
	mean ± s.d.[a]		mean ± s.d.[a]	mean ± s.d.[a]	mean ± s.d.[b]	mean ± s.d.[b]
CTAB	34.8 ± 2.00	α	39.5 ± 0.17	6.3 ± 0.24	0.8 ± 0.03	-21.0 ± 0.13
+ 0.005% PG	19.8 ± 1.70	δ	32.8 ± 0.29	6.2 ± 0.25	0.8 ± 0.03	-22.3 ± 0.19
+ 0.05% PG	3.7 ± 0.40	η	30.3 ± 0.50	10.8 ± 0.84	1.4 ± 0.11	-26.1 ± 0.25
+ 0.1% PG	2.3 ± 0.20	ω	30.1 ± 0.59	18.3 ± 1.33	2.4 ± 0.18	-27.2 ± 0.20
+ 0.2% PG	2.2 ± 0.10	ω	30.1 ± 0.38	17.3 ± 0.83	2.3 ± 0.11	-27.3 ± 0.10
+ 0.005% GA	23.0 ± 1.90	β,γ	38.2 ± 0.28	6.0 ± 0.35	0.8 ± 0.09	-22.0 ± 0.19
+ 0.005% CA	21.1 ± 2.30	γ	36.8 ± 0.33	5.1 ± 0.34	0.7 ± 0.09	-22.1 ± 0.25
+ 0.005% SA	26.5 ± 2.70	β	39.1 ± 0.36	6.2 ± 0.41	0.8 ± 0.11	-21.6 ± 0.23
Brij	5.0 ± 0.30	α	41.4 ± 0.27	9.9 ± 0.61	2.6 ± 0.08	-28.0 ± 0.42
+ 0.2% PG	5.5 ± 0.20	α	36.9 ± 0.16	8.5 ± 0.33	2.2 ± 0.04	-27.8 ± 0.25
SDS	232.1 ± 19.1	α	31.7 ± 0.46	10.5 ± 0.76	1.4 ± 0.10	-16.2 ± 0.15
+ 0.2% PG	180.3 ± 14.4	β	31.2 ± 0.36	7.5 ± 0.37	1.0 ± 0.05	-16.7 ± 0.14

[a] Standard deviation obtained by fitting, n=10. [b] Standard deviation according to Gauss error propagation.
[c] Parameter obtained by fitting surface tension curve by equation 1. [d] Surface tension at which micelles form
[e] Slope of surface tension fit beneath cmc (pre-cmc slope). [f] Calculated from equations 2 and 3.
[g] Calculated from equation 4.
Greek letters that are not the same indicate significant differences in a micellar system (t-test, $p < 0.05$)

micelles, which was 232.1 mg/l for SDS, 34.8 mg/l for CTAB, and 5.0 mg/l for Brij 58. Addition of 0.2% PG to SDS decreased the cmc, and the free energy of micellization became more negative (from -16.2 to -16.7 kJ/mol), so that the SDS monomers started to form micelles already at a concentration of 180.3 mg/l. However, the calculations showed that in the presence of PG the ratio of SDS molecules to micelle surface area of 100 Å^2 decreased from 1.4 to 1.0.

The cmc of Brij 58 was not affected by the addition of PG. Since the surface tension above the cmc was lower, the pre-cmc slope was slightly lower as well. This led to a decrease in the ratio of Brij 58 molecules to micellar surface of 100 Å^2 from 2.6 to 2.2. However, the free energy of micellization remained constant in the presence and absence of PG.

The cmc of CTAB was decreased even in the presence of low antioxidant concentrations. Reduction of the cmc depended on the antioxidant used. The strongest effect could be observed for PG (0.005%) and the effect decreased in the order PG > CA > GA > SA (Figure 6b). In the presence of these antioxidants at the concentration of 0.005%, the ratio of CTAB headgroups to micellar surface area was constant, but the free energy of micellization became more negative. However, the higher the applied antioxidant concentration in CTAB solution the lower the cmc observed until saturation was achieved. This was found to be 16-fold lower than the cmc in the control system (Figure 6a). While in the presence of 0.05% PG the ratio of CTAB headgroups to surface area was doubled compared with this ratio in the control, addition of 0.1% or 0.2% PG enhanced this ratio threefold. The free energy of micellization became more negative changing from -21 kJ/mol in the absence of PG to -27.3 kJ/mol

in the presence of 0.2% PG. In addition, PG reduced the surface tension above the cmc to 30.1 mN/m relative to the control value of 39.5 mN/m. This was accompanied with a steeper rise in the pre-cmc slope.

Micellar size and shape

Comparison of the scattering curves and the corresponding $p(r)$ functions showed that all micellar solutions had spherical structures in the absence of antioxidants with the size increasing in the order SDS > CTAB > Brij 58 (Figure 7). In the presence of different antioxidants, changes in these micellar sizes and shapes were obtained by SANS data analysis. Values for the radius and length of the micelles as well as the calculated aggregation number of the surfactant molecules per micelle are compiled in Table 3. Whereas for spherical micelles the designated differences were indicated by the radii of micelles, the differences between rod-like micelles were indicated by their length. Additionally, the increases in aggregation numbers in the presence of antioxidants were compared.

The radius obtained for an SDS micelle in the absence of antioxidants was found to be 16.1 Å (Table 3). Upon addition of PG, the radius decreased significantly to an average value of 15.1 Å regardless of the antioxidant concentration. However, calculations of the aggregation number in the presence of PG indicated that the formation of micelles required significantly fewer surfactant molecules. This effect increased with increasing PG concentration.

In the case of Brij 58 micelles, which were twice as large as SDS micelles, there was likewise a decrease in the micellar radius from 30.9 Å to 29.2 Å in the presence of 0.1% to 0.3% PG, but the required aggregation number was not altered in any of the four measurements.

CTAB micelles adopted spherical structures in the control system as well as in the presence of 0.1% PG and 0.2% GA, SA, and FA (Table 3). The micelle radius was increased from 20.5 Å to 23.8 Å in the order control = SA < FA < GA < PG. In the presence of the other antioxidants, the CTAB micelle structure was transformed into a cylindrical structure possessing an average radius of about 21.8 Å and showed a one-dimensional growth depending on the antioxidant concentration or the type of antioxidant. Increasing PG concentrations led to a continuous increase in the length of the CTAB cylinders to approx. 700 Å at a concentration of 0.3% PG. Afterwards there was a slight decrease in length to 635 Å. At equal concentrations, the alkyl chain length of gallates also induced growth of the cylinder length. Although the length of the micelles in the presence of MG, EG and PG differed significantly, there was no increasing trend with increasing chain length. However, in the presence of OG a significantly longer one-dimensional growth was found. The HCAs without methoxy groups also induced structural changes in the CTAB spheres to give rod-like micelles. CA, which has two hydroxyl groups, caused the CTAB cylinders to grow more than pC, which has only one hydroxyl group on its aromatic ring. Comparson of the aggregation numbers showed that increasing the antioxidant concentration led to a strong increase in the number of surfactant molecules per micelle until addition of 0.2% of PG. At higher PG concentrations they seemed to approach a maximum, although the aggregation numbers differed significantly. The same effect could be observed for the different gallates. The aggregation number increased sharply until EG, followed by saturation at BG. With OG there

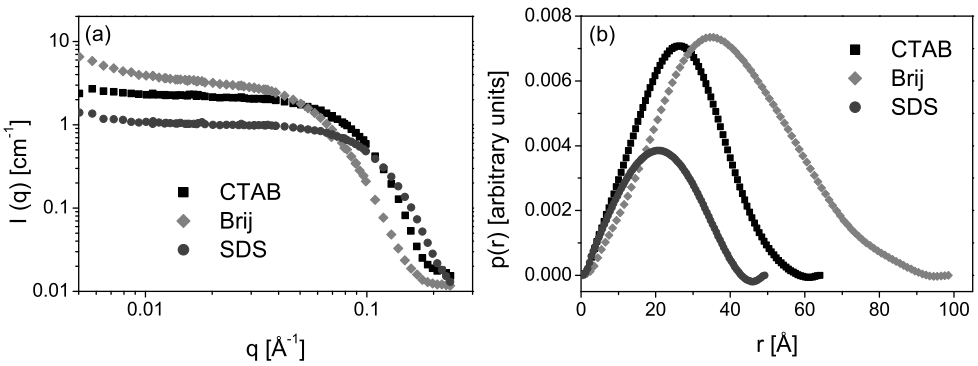

Figure 7: Scattering curves of 1% CTAB, Brij 58, and SDS micellar solution control systems obtained by plotting the intensity I versus the scattering vector q (a); Pair distance distribution function $p(r)$ obtained by IFT analysis for spherical structures (b)

was a renewed increase in the aggregation number but a simultaneous increase in the radius of the cross-section of the CTAB micelle.

Table 3: Calculations of the shape, size and aggregation number per micelle using to the fitted SANS data

	shape	R [Å][d] mean ± s.d.[a]	L_{app} [Å][e] mean ± s.d.[b]	R_{cs} [Å][f] mean ± s.d.[b]	N_{agg}[g] mean ± s.d.[c]
1% SDS	*sphere*	16.1 ± 0.1 $^{\alpha}$			**80** ± **1** $^{\alpha}$
+ 0.1% PG	*sphere*	15.1 ± 0.1 $^{\beta}$			**72** ± **1** $^{\beta}$
+ 0.2% PG	*sphere*	15.2 ± 0.1 $^{\beta}$			**69** ± **1** $^{\gamma}$
+ 0.3% PG	*sphere*	15.0 ± 0.1 $^{\beta}$			**68** ± **1** $^{\gamma}$
1% Brij	*sphere*	30.9 ± 0.3 $^{\alpha}$			**73** ± **1** $^{\alpha}$
+ 0.1% PG	*sphere*	30.5 ± 0.3 $^{\alpha}$			**74** ± **1** $^{\alpha}$
+ 0.2% PG	*sphere*	29.7 ± 0.3 $^{\beta}$			**75** ± **1** $^{\alpha}$
+ 0.3% PG	*sphere*	29.2 ± 0.3 $^{\beta}$			**75** ± **1** $^{\alpha}$
1% CTAB	*sphere*	20.5 ± 0.1 $^{\alpha}$			**103** ± **0** $^{\alpha}$
+ 0.1 % PG	*sphere*	23.8 ± 0.1 $^{\delta}$			**155** ± **0** $^{\eta}$
+ 0.15% PG	*rod-like*		421 ± 12 $^{\lambda}$	21.4 ± 0.1	**944** ± **17** ^{o}
+ 0.2 % PG	*rod-like*		609 ± 17 $^{\nu}$	21.6 ± 0.1	**1414** ± **10** $^{\theta}$
+ 0.25% PG	*rod-like*		659 ± 23 ^{o}	22.1 ± 0.1	**1610** ± **8** $^{\tau}$
+ 0.3 % PG	*rod-like*		712 ± 19 $^{\pi}$	22.1 ± 0.1	**1540** ± **6** $^{\sigma}$
+ 0.35% PG	*rod-like*		635 ± 21 $^{\nu,o}$	21.8 ± 0.1	**1525** ± **8** $^{\rho}$
+ 0.2% GA	*sphere*	22.0 ± 0.1 $^{\gamma}$			**118** ± **0** $^{\delta}$
+ 0.2% MG	*rod-like*		406 ± 9 $^{\lambda}$	21.4 ± 0.1	**780** ± **5** $^{\mu}$
+ 0.2% EG	*rod-like*		674 ± 25 $^{\pi,o}$	21.2 ± 0.2	**1269** ± **11** $^{\pi}$
+ 0.2% PG	*rod-like*		609 ± 17 $^{\nu}$	21.6 ± 0.1	**1414** ± **10** $^{\theta}$
+ 0.2% BG	*rod-like*		718 ± 23 $^{\pi}$	21.8 ± 0.2	**1438** ± **14** $^{\theta}$
+ 0.2% OG	*rod-like*		812 ± 32 $^{\rho}$	27.3 ± 0.2	**2340** ± **33** $^{\omega}$
+ 0.2% SA	*sphere*	20.5 ± 0.1 $^{\alpha}$			**100** ± **1** $^{\beta}$
+ 0.2% FA	*sphere*	20.9 ± 0.1 $^{\beta}$			**105** ± **0** $^{\gamma}$
+ 0.2% pC	*rod-like*		287 ± 8 $^{\eta}$	19.7 ± 0.2	**471** ± **6** $^{\lambda}$
+ 0.2% CA	*rod-like*		482 ± 15 $^{\mu}$	20.6 ± 0.1	**829** ± **6** $^{\nu}$

[a] Standard deviation obtained from fit data. [b] Standard deviation according to Gauss error propagation.
[c] Standard deviation obtained by relativ error of I_{cs}. [d] Radius of micelles approximating a homogenous sphere is related to R_g by $R = R_g\sqrt{5/3}$.
[e] Apparent length of micelles calculated by $L_{app} = M_{app}\Delta\rho_m^2 / I_{cs}(0)$.
[f] Radius of cross section in homogeneous circle approximation calculated by $R_{cs} = R_{cs,g}\sqrt{2}$.
[g] Aggregation number calculated for spherical micelles by $N_{agg} = I(0)N_A / (c_e - cmc)M_e\Delta\rho_m^2$ and for rod-like micelles by $N_{agg} = M_{app} / M_e$.
Greek letters that are not the same indicate significant differences in a micellar system (t-test, $p < 0.05$).

Discussion

SDS micelles

SDS micelles formed in the presence of PG showed a reduced cmc and lower aggregation number than in the absence of PG. Both parameters are due to the greater negative free energy of micellization (ΔG°_{M}) as discussed by Zana[27] for methanol, ethanol, and propanol in SDS solutions. These short alcohols were found to be adsorbed at the Stern layer of the SDS micelle and act as cosolvents.[27,37,38] Therefore, they act on the micellization process by modifying the properties of water and the chemical potential of the free surfactant[27] by altering the water structure.[37] This is also revealed by our SANS results showing a reduction in the radius of SDS micelles from 16.1 Å to 15.1 Å regardless of the antioxidant concentration. The smaller SDS micelles are due to more disordered aggregates resulting from a decreasing dielectric constant due to the cosolvent[27,37–39] and a simultaneously higher degree of orientation of the water dipoles.[40] As the dielectric constant and the Coulomb forces are inversely proportional, the Coulomb forces of the anionic headgroup increase. This results in the observed decrease to 70% of the ratio of SDS headgroups to micelle surface area promoting the spherical structure of SDS micelles. This promotion of spheres was found to be typical of cosolvents like highly hydrated and non-penetrating additives.[41] The solubilization of PG in the Stern layer of SDS micelles was in good agreement with the location and weak interactions of PG in the Stern layer shown by NMR data.[42] In this previous study the α-CH_2 group, which is the proton head group signal, was deshielded in the presence of PG indicating that the electron density of the headgroup protons was reduced by fewer available dipoles in the Stern layer due to fewer water molecules in the presence of PG. Using the "linear solvation free energy relationship" (LSER) theory Quinoa et al.[43] calculated the solubilization parameters of nonionic solute in different (pseudo)phases by multiple regression and could show differences in the intermolecular properties relative to those of water. The calculations showed that all three surfactants investigated were of equal strong hydrophobicity (v=3.3 - 3.6) but differed in their H-donor and acceptor properties. In the case of SDS micelles, the H-acceptor properties are weak towards the strong H-donors of OH groups or antioxidants, so that the amount of antioxidants partitioning into the SDS micellar phase, approx. 70% of the PG, is lower than into the other micelles.[2] The driving force for solubilization in the Stern layer may therefore be mainly due to hydrophobic effects that reduces the water content of this layer which is similar to cosolvent effects.[40]

Brij 58 micelles

The presence of PG affected the size of the Brij 58 micelle to a lesser extent than the size of SDS micelles with only a slight insignificant decrease in micellar radius being observed. In a recent study on Brij 35 micelles with different *n*-alcohols,[43]it was shown that short chain alcohols on butanol lead to a slight decrease in the Brij micelle radius. It was suggested that a cosolvent effect should be observed with methanol and ethanol, while propanol and butanol penetrate into the palisade layer and loosen the surfactant structure. This gives the hydrophilic polyoxyethylene chain more space to lay closer to the hydrophobic core resulting in a thinner hy-

drophilic micellar shell.[43] This is in agreement with the observed reduction to 85% of the ratio of Brij molecules to micellar surface in the presence of PG. In addition, NMR results indicated that PG is located in the region of the polyoxyethylene chain and the first adjacent alkyl chain methylene groups,[42] which is comparable with the location of propanol and butanol. However, the cmc, aggregation number, and free energy of micellization remain the same in the presence and absence of PG. Therefore, it may be assumed that there were no changes in micellization. This is in accordance with findings in the literature that changes in the physicochemical properties of micelles are mostly due to electrostatic interactions[44] or hydrophobic effects.[6,23] Since it is the properties of counterions such as distribution, hydration, planarity and hydrophobicity that mainly determine these influences on micellar structures, this explains why no significant alteration in the non-ionic Brij 58 micelles could be found.[45] According to the LSER theory, this means, that hydrophobic effects may be the driving force for solubilization of PG in Brij micelles. As PG penetrates into the the region of the polyoxyethylene chain and the first adjacent alkyl chain methylene groups, π and n-duplet (r=1,639) interactions[46] between the polyoxyethylene groups of Brij and the OH-groups of PG[2] may additionally arise. As the volume of the nonionic micelles is fourfold and eightfold higher than that of the SDS and CTAB micelles, respectively, these interactions may be too weak to induce any changes other than the slight decrease in micellar size that was found.

CTAB micelles

The increasing viscosity at higher PG concentration confirms that interactions between PG and CTAB resulting in a sphere-to-rod transition of CTAB micelles[7–12] influenced the physicochemical properties of the CTAB micellar system. It was found that the influence of antioxidants on CTAB micellar solution is comparable for H_2O and D_2O systems, but the viscosity increase in D_2O appeared at lower concentration and was twice as large at the highest PG concentration. This could be explained by the findings of Berr[47]showing that changes in micellar size and aggregation number with increasing D_2O content were caused by solvent isotope effects. Solvent isotope effects are due to small differences between the surfactant hydrocarbon-water interactions in H_2O and D_2O due to the stronger H-bonds formed by D_2O. The calculated free energy of transfer (ΔG°) from the aqueous to the micellar phase is more negative in D_2O than in H_2O. This means that as the D_2O content of the solvent increases monomers will be driven from the bulk to the micellar pseudophase resulting in larger and more micelles than in H_2O. Due to the promotion of micellization in D_2O, the viscosity increases by sphere-to-rod transition at a lower PG concentration than in H_2O. However, several studies showed that fractional charge and headgroup interactions of C_{14}TAB[48] and SDS[49] micelles do not change with solvent isotopic composition, indicating that these types of interactions are the same in H_2O and D_2O.[47]

The size of the spherical CTAB micelles was 20.5 Å in the absence of PG. In the presence of 0.1% PG the micelles swelled to a radius of 23.8 Å. With increasing PG concentration, the shape changed to a rod-like structure with an average radius of 21.8 Å and there was a one-dimensional increase in length up to approx. 700 Å as a function of the PG concentration. The aggregation number of the individual surfactant molecules per

micelle increased dramatically upon transition from 0.1% PG ($N_{agg} = 155$) to 0.2% PG ($N_{agg} = 1414$), which offers a lower energy state through higher surfactant accumulation.[50] This is consistent with the more negative free energy of micellization calculated in the presence of PG than when CTAB is dissolved in aqueous buffer solution in the absence of antioxidants. This phenomenon has been reported in numerous studies as clear evidence for interactions between solute and micelle that may be attributed either to electrostatic interactions[15,44,51] or hydrophobic effects.[6,52] Both effects ultimately lead to tighter packing. This tighter packing is revealed by the increase in the ratio of CTAB molecules to micellar surface, which is a factor of three when the PG saturation concentration was reached at 0.1%. On the one hand, a reduction mainly of the distance between the cationic headgroups by intercalation of the additive into the palisade layer[8,15,53] is consistent with specific interactions between headgroups and counterions, tighter packing and interfacial dehydration.[24] On the other hand, the effective volume of the alkyl chain could be increased by hydrophobic parts of the additive that insert themselves deep into the micelles.[8,9] The increased ratio of CTAB headgroups to micellar surface indicates that the Coulomb forces were weakened because PG is located predominately in the palisade layer. Our NMR experiments also provide clear evidence that the aromatic ring is located in the palisade layer of the micelle.[42] However, according to Rakitin and Pack[23] the positive charge of CTAB is uniformly distributed over the bulky α-CH_2-headgroup fragment. Therefore, electrostatic interaction cannot be excluded.

A drastic decrease in the cmc by a factor of 16 relative to the control system was observed in the presence of 0.2% PG, as the free energy of micellization became more negative in the presence of PG. Since this decrease was dependent on a PG concentration in the range from 0.005% to 0.2% PG with saturation reached at 0.1% PG, it can be concluded that antioxidants like PG act as cosurfactants.[39,41] This is also confirmed by the more negative free energy of micellization, the steeper rise in the pre-cmc slope, and a reduced surface tension at which micelles formed. Thus, PG intercalates into the palisade layer beneath the headgroup[5,6,41,54] as demonstrated for n-alcohols and n-amines with increasing alkyl chain length.[39]

Influence of antioxidant lipophilicity

Increasing the ester alkyl chain length up to $n \approx 3$ could lead to the intercalation of the gallate ester into the more hydrophobic palisade layer and contribute to the sphere-to-rod transition by screening electrostatic forces and dehydrating the palisade layer.[24] This difference between the longer ester alkyl chain and the unesterified gallate is already seen in the increase in viscosity and substantiated by comparison of the influence of GA and PG on the cmc. PG reduced the cmc and the surface tension at which micelles formed significantly more than GA did. However, at the cmc the free energy of micellization as well as the ratio of CTAB molecules to micellar surface area are the same in the presence and absence of both PG and GA. This may be due to both antioxidants intercalating into the palisade layer but GA was not located as deeply as PG in this layer as our NMR data showed (unplublished). In contrast, OG could penetrate deeper into the micelle,[5] causing a transition due to a larger alkyl chain volume but with a simultaneously increased cross-sectional radius. Therefore, the

aromatic ring and in particular the three hydroxyl groups are crucial for intercalation into the CTAB micelle. These results contrast with findings on the influence of increasing chain length of *n*-alcohols and *n*-amines on intercalation into CTAB micelles which suggest that these substances can be divided into cosurfactants and cosolvents depending on their alkyl chain length.[39]

Influence of antioxidant hydroxyl and methoxy groups

Comparison of the influence of substitution of two hydroxyl and two methoxy groups on the cmc showed a strong reduction of the cmc with CA, whereas the cmc reduction induced by SA was markedly smaller. However, at the cmc the ratio of CTAB headgroups to micellar surface area was the same in the presence of both antioxidants, and the free energy of micellization was slightly lower for CA than for SA. This could be explained by a deeper solubilization of CA in the palisade layer and stronger interactions due to its hydroxyl groups, as has been shown for cosurfactants.[39,41] The effect of CA was similar to the influences of the gallates. While HCAs with methoxy groups did not affect the spherical shape and size of micelles, the presence of HCAs with hydroxyl groups induced a sphere-to-rod transition. This finding was also consistent with a slight increase in viscosity in the presence of CA and pC, unlike in the presence of SA and FA. The two hydroxyl groups of CA induced formation of rod-like micelles that were twice as long as with pC. The aggregation number in the presence of FA and SA was the same as that in the control CTAB solution, while with pC it was four times higher and with CA eightfold higher. From these observations it could be deduced that an increase in CTAB molecules by one and a half the spherical structures are intact. A fourfold higher aggregation number than in the control led to structural changes. The length and aggregation number of the micelles were comparable to those obtained with the gallates for pC, which is below MG, and for CA, which is between MG and EG. Since this order is not consistent with the partitioning order, which is GA $(49.3\%)^2$ < MG $(86\%)^2$ < EG $(93.9\%)^2$ < CA (95.5%)[55] < pC (97.5%),[55] the solubilization of antioxidants is clearly determined by the substitution pattern of the aromatic ring. Our NMR data showed (unpublished) that the electron density in the palisade layer of CTAB is higher in the presence of CA than in the presence of MG or EG, which indicates the importance of the double bond and the carboxyl group that is deprotonated at the pH of 5.0. This may finally lead to a sphere-to-rod transition at lower concentrations due to a greater ability to screen electrostatic interactions.[44] Although the electron density increase in the palisade layer should be greater in the presence of pC than in the presence of CA according to their partition behavior, CA induced a one-dimensional growth that was twice as strong as that induced by pC. Thus, it can be concluded that hydroxyl groups are responsible for a stronger solubilization due to their ability to enter into interactions like H-bonds in an environment of highly ordered water structure.[56] This is in agreement with LSER theory, which showed that CTAB is a strong H-bond acceptor (a=1.023)[46] while the OH groups are strong H-bond donors.[2]

Summary

Changes in micellar properties investigated in the presence of antioxidants were attributed to where these antioxidants were solubilized in the micellar phase of the different surfactants. In SDS, antioxidants are located in the Stern layer and act as cosolvents, as shown by a smaller micellar size, decreased aggregation number and cmc due to more negative free energy of micellization (ΔG°_{M}). No changes in viscosity were found for SDS systems. Brij micelles were only slightly decreased in cross-sectional radius, but no alterations in cmc, aggregation number, or viscosity were observed. This was due to an intercalation of PG into the region of the polyoxyethylene chain and the first adjacent methylene groups of the alkyl chain. However, strong effects of swelling micelles or sphere-to-rod transition were found in the presence of antioxidants in CTAB systems. This was attributed to a solubilization of antioxidants in the palisade layer of CTAB, where they induced electrostatic screening and hydrophobic effects. Consequently, the antioxidants acted similar to cosurfactants, as the cmc was reduced with increasing antioxidant concentration and the ratio of CTAB headgroups to micellar surface area increased after formation of rod-like micelles, which means a tighter micellar packing. These effects were accompanied by an increase in viscosity as a function of antioxidant lipophilicity and antioxidant concentration. It could be demonstrated that an antioxidant that is more lipophilic or possesses more hydroxyl groups enters into stronger interactions. Although differences were found in the viscosity increase between the D_2O and H_2O solvent systems, several studies showed that fractional charge and headgroup interactions of ionic micelles do not change with solvent isotopic composition, which suggests that the interactions investigated are fully comparable in H_2O and D_2O.

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*** Response to Reviewer Comments**

Comments on reviews of "Impact of Phenolic Antioxidants on Structural Properties of Micellar Solutions" by Heins et al.

We would like to thank you for considering our paper for publication after revision.
We are grateful for intensive discussion of the reviewers, the helpful advices, questions and idiomatic corrections. We annotated questions and advices and reported the changes having done in the manuscript below. Comments are reported in blue.

Reviewer #1

Overall: This is an interesting article that reports on formation of mixed micellar structures using three model surfactants in combination with a number of phenolic antioxidants. The results are not entirely surprising though since stabilization of ionic surfactants by nonionic co-surfactants has shown to lead to very stable mixed micelles while having fairly minimal (or even detrimental) effects on nonionic surfactant micelles. Nevertheless, the results may have important implications for the functionality of these antioxidants in the presence of micellar surfactant solutions. However, I have an unusually large amount of comments and questions that I feel authors need to address prior to publication. Overall, I feel the article is a bit short on the discussion/interpretation side and should be expanded especially in light of the presented extensive surface tension data (I listed specific recommendations below). In this section, the proposed theoretical analytical models should also be revisited. The simple Gibbs model that authors used is not applicable for a mixed micellar (two surfactant) system and should not be used in its current form to calculate a headgroup area.
Regarding the surface excess concentration / headgroup area:
All published models for mixed micelles deal with two surfactants. This means calculating excess surface concentration for mixed micelles require cmc of the surfactant and the cmc of the co-surfactant (in our case propyl gallate). In contrast to long-chain alcohols which are able to build small and unstable aggregates, propyl gallate does not exhibit a cmc. Therefore we do not regard propyl gallate as a surfactant but as a solute which is incorporated into the micelle/palisade layer or as cosurfactant as defined by (Skoulios, Ann. Phys. 1978,3:421). In our case, as we kept the propyl gallate constant and varied the surfactant concentration, it is appropriate to calculate the excess surface concentration only for the surfactant (Janczuk et al, Colloids Surfac. A: Physiochem. Eng. Aspects 2003, 220:61). We corrected the Gibbs model to consider the counterions. With respect to the headgroup area we now calculate the number of surfactant headgroups per micellar surface area (A^{-2}) – or the ratio of surfactant molecules to micellar surface. We now use the expression "specific excess concentration" which is inversely related to the former expression of headgroup area.

On the other hand, the method section, especially in the SANS section could be substantially shortened. Currently, the SANS analysis section reads almost like a literature review. Authors should strip this section down to the essentials and refer to previously published papers on SANS with micellar systems instead.
We shortened this part. Please see specific comments for detail.

Finally, some of the statements that authors make should probably be reconsidered because they are speculative and lack additional experimental evidence. Most of these speculations could probably have been verified if authors had measured the surface charge of the mixed micelles using e.g. zeta potential, an experiment they may consider as they move their interesting studies further forward.

Lastly, there are quite a large number of language/translational issues that plague the paper and these need to be corrected as well prior to publication.
In conclusion, I recommend acceptance after these revisions have been made.
Please see specific comments for detail. In addition, a professional proof-reader went through the manuscript.

Specific comments:

P2,L2: Specify which changes in micellar properties authors refer to (size, charge, stability etc.)
We specified changes in micellar properties to size and shape of micelles, cmc and viscosity

P2,L3: shouldn't that be vice versa? i.e. the spatial arrangement of surfactanst within the micellar system is determined by the interactions (between the two surfactants and the surfactants and the solvent)
This question is difficult to answer, both ways may be true. Sentence has been revised to: Interactions arising between the solute such as phenolic antioxidants and the surfactant determine the location of the solute and vice versa.

P2,L14: the sentence reads as if viscosity, cmc , micellar size and shape were _all_ measured by SANS.
Sentence has been revised accordingly.

P2,L15: it did not change the size/shape, the structure may still have been changed (i.e. be composed of a two surfactants instead of one). NMR experiment would be needed to really elucidate on the structure..
The sentence was reworded "…did not affect the micellar structure" to" micellar parameters investigated"

P2,L17: this statement is speculative since no zeta potential measurements were done.
Extensive NMR data were evaluated to localize different antioxidants in micellar systems and emulsions with the emulsifiers CTAB, SDS and Brij which are as least as meaningful than data from zeta potential measurement. Manuscripts are submitted (one manuscript) or in preparation (2 manuscripts) to be published in LIPIDS. (The submitted manuscript is attached for further information - or please look on http://www.foodtech.uni-kiel.de/download/Thesis_Anja-Heins.pdf) In SDS systems an amount of 20-50% of antioxidants as a function of the antioxidant type is solubilized in the micellar phase (Stöckmann et al., JAOCS 2000) , however, no NOEs are found among antioxidant and emulsifier protons, and only minor changes were found in chemical shift or T1 relaxation. However, alteration in peak shape indicates faster rotation of SDS micelles due to decreasing size in the presence of antioxidants. So we concluded that antioxidants are solubilized in the Stern layer which leads to smaller and more disordered micelles. This is consistent with SANS data and decreasing cmc.

P2,L25: Authors state that driving forces are hydrophobic or electrostatic in nature. That seems to paint a somewhat incomplete picture and does also not highlight which of the interaction is more important. Clearly, the electrostatic repulsive interactions in anionic/cationic surfactant micelles is one of the key parameters that drives the integration into the micelle (antoxidants may act as "spaces" and reduce repulsive interactions). Authors could have somewhat distinguished these interactions by looking at salt or pH stability…

Application of the “Linear Solvation Free Energy Relationship” (LSER) theory makes it possible to consider solubilization parameters of the solute and of (pseudo)phases, which are calculated by multiple regression and revealed differences of properties relative to those of water. Parameters being considered are interactions with π and n-duplets (r), dipol character (s) H-bond acceptance (a), H-bonds donations (b) and hydrophobicity (v). Comparison of micellar pseudophases of SDS, CTAB, and Brij 35 (which interaction properties is comparable with Brij 58 (Schönfeld, 1976)) showed that the solubilization of solutes is governed by hydrophobicity (v) and h-bonds donations (b) of surfactants. The hydrophobicity is similar for all these surfactants (v=3,3 – 3,6) and contribute the most to the solubilization of the solutes. However, difference between these surfactants are found in π and n-duplets (r) and H-bond acceptance and donation (a, b) which leads in SDS micelles to a depletion (a=-0,084, b=-1,837, r=0,317), and in CTAB (a=1,023, b=-3,776, r=0,766) as well as in Brij micelles (a=1,621, b=-3,836, r=1,639) to an accumulation of solutes (calculated by Quina et al., 1995 J. Phys. Chem. 99:11708). For Brij the strong interactions by π and n-duplets (r) are governed by polyoxyethylene groups. Calculations after LSER for antioxidants showed, that the hydroxyl groups are strong H-bond donors which enhanced additionally the accumulation in Brij and CTAB and the depletion in SDS micelles. According to this theory, the ester alkyl chain of gallates leads only to an increase of hydrophobicity while all other parameters are equal. Thus, the higher solubilization of antioxidants in CTAB and Brij (at equal v) is extensively governed by H-bonds and in Brij systems by interactions with π and n-duplets (r). (Stöckmann et al., 2000 JAOCS 77:535) Therefore, relating the observed phenomenon to only one type of interaction is not appropriate.

P2,L29: I questions this last statement including the assertion of authors in the latter part of this study that these systems can be used to explain what is happening in emulsion based systems in terms of antioxidant activity. Micelles are completely different in nature and location of the co-surfactant in a micellar system may substantially differ from that in an emulsion interfacial layer. Curvature effects become dominant in micellar systems with sizes ranging from 5-100 nm.
Partitioning behaviour of antioxidants in micellar solution and emulsions are similar and are governed by the type of the emulsifier (Schwarz et al, 2000, J Agric Food Chem 48:4874; Stöckmann &Schwarz, 1999, Langmuir 15:6142; Pekkarinen et al.,1999, J Agric Food Chem, 47:3036) Therefore, the interactions between emulsifier and antioxidants are qualitatively equal and working with simplified micellar solution to evaluate these interactions is appropriate and a common method.

P2,L37: what is a “specific” interaction (or vice versa, what is a non-specific interaction?) also see previous, the location (spatial arrangement) is governed by the interactions, not the other way around
We cancelled specific. Please see previous comment.

P2,L41: “exact location” this raises an interesting question! – What are the important length scales that one should really care about when dealing with such antioxidant systems (i.e. how “exact” should one be - do we really need to care whether the antioxidant may be slightly buried in the interfacial layer say by a few Å?) Could diffusion of pro-oxidants simply take care of those differences?
The “exact location” of an antioxidant may give important hints on the type of interaction, which is responsible for the reduced or enhanced activity of an antioxidant. E.g. in the case of CTAB the low activity of propyl gallate is due to strong H-bonds (according to the LSER theory). This may result in low H-atom abstraction kinetic of the antioxidant. However, NMR data indicate

interaction between the phenolic moiety and the first adjectant methylen groups of the alkyl chain. I.e. in the latter case CTAB may function as barrier between the polar hydroxyperoxide and the antioxidant.
Further, extensive ESR data about the reaction kinetic and stoichiometry between different antioxidants and stable radicals with opposite polarities was evaluated in micellar systems and emulsions with above mentioned emulsifiers. Fast reaction and high stoichiometric factors were found for antioxidants (residing in the Stern layer) towards the hydrophilic radical (exclusively solubilized in the aqueous phase) but no reaction was found towards the lipophilic radical (exclusively solubilized in the micellar or lipid phase). Experiments with the homologues series of gallate esters showed deeper location in Brij micelles with increasing chain length (NMR) – and via ESR a faster reaction towards the lipophilic radical and vice versa slower reaction with the hydrophilic radical with increasing ester alkyl chain length. In conclusion, ESR data showed that the reaction between antioxidant and radical is governed by their proximity which is determined by the emulsifier (location and diffusion via interactions). Results are prepared for publication in two manuscripts and are available in a PhD thesis:
http://www.foodtech.uni-kiel.de/download/Thesis_Anja-Heins.pdf

P2,L 42: Don't understand this sentence - seems to relate two different things ..
We revised this sentence to: "It is well known that ionic micellar systems are sensitive to interactions with counterions or solutes, which result in changes in micellar size and shape, viscosity or cmc. These changes can be related to the type of solutes and to where these solutes are solubilized"

The following changes have been made accordingly:
P3,L4: strike "already much" – throughout the paper, pls. avoid emphasizing statements whenever possible
P3,L8: not sure "substituents" is actually a word..
P3,L9 role "in micellar growth"
P3,L11: the opposite "was found"
P3,L15: strike "an influence is given by"
P3,L17" the presence of" methoxy groups
P3,L19: strike "its" (3 times)
P3,L23: the aromat "being planar" or having a planar structure
P3,L23: may "in turn" influence
P3,L24: KraFFT point (double f)
P3,L27: The "location" (not localization) or maybe position or spatial arrangement may be better
P3,L43: Is Roth a city in germany or is it a company (where is it located if so)
P3,L45: Revise sentence to ...added to 20 ml of 1% aqueous micellar solutions or 5ml micellar solution in D2O...
P4,L1: "to ensure that they dissolved"?

P4,L4, what is NF? the Bohlin is a rotational rheometer.. Also, how can these very low viscous solutions be measured using a plate-plate geometry, they would never stay in the gap! This should have been measured with a coaxial cylinder geometry. Pls. also note in this section that without really measuring the full flow curve (and that was probably not done), authors cannot distinguish between newtonian and non-newtonian behavior. By the way, it would have been very interesting to see the full flow curves because in particular for the rod like micelles and

shear thinning effect might have been observed. Also, all viscosity data should be referred to as "apparent" viscosity because of that.
NF is the detailed specification of the rheometer form the manufacturer. The D2O solutions and the CTAB –H20 solutions with a higher concentration of 0.2% PG was measured with the plate-plate method and these samples stayed in gap. They (in particular the D2O solutions) could not be measured with a coaxial cylinder geometry, since the volume of D2O should be as little as possible (5 ml) due to commercial reasons. We agree, that we can not distinguish between Newtonian and non-Newtonian behaviour without measuring the flow curves. However, if the volume fraction in dispersed systems is <5%, we can assume that the viscosity of the solvent dominates. Nevertheless, we reworded “Newtonian liquids” to “low viscose solutions”
We refer now to apparent viscosity.

P4,L5, "equilibrated to 25C", P4,L14: "recorded" instead of stopped
P4,L16: "reported" instead of given
We revised these sentences

P4,L21: Pls. give a reference for method. What is the accuracy of the method (typically +-0.2 mN/m), surface tension of water is not between 72-73, its 72.8 mN/m at 20C!
P4,L24 pH 5.0 (double zero not needed), also was surface tension for buffer solutions measured??? should be listed!
We gave a reference. The accuracy of the method is ±0.2 so we changed surface tension to the accurate value. In addition we add the surface tension for the buffer solution of 69,7 mN/m in the experimental section.

P4,L29: While this is often done in this way for mixed micellar systems, it might have been better in this particular case to keep the ratio of surfactant/cosurfactant constant and then increase the concentration of the ratio-constant mixture to measure the surface tension. The way that authors did it, they are basically "diluting" the mixed surfactant structure by increasing concentrations of the base surfactant, thus the composition of the micelle changes as the surfactant concentration is increased
We found this method as the standard to measure the cmc. However, for future cmc experiments we will be glad to consider the given advice.

P5,L9: inconsitency in symbol, pls. either use sigma OR gamma throughout the paper, gamma is typically used for interfacial tensions, while sigma is used for surface tensions
We revised gamma to sigma.

P5,L15: Pls. see my previous comment, Gibbs equation in this form is not applicable to a mixed surfactant systems, there are appropriate models for binary surfactant mixtures (e.g. delta G = RT sum(Xi*ln gamma(i)), also I hope authors converted the mmol/l into the right units. Finally the calculation of A0 is therefore not valid. Note a single "headgroup area" for a mixed surfactant system makes actually little sense. Would that represent an average of surfactant - cosurfactant?? Finally, pls list a reference for this equation.

Table 2 has been shortened. The last three parameter which have been calculated based on the headgroup area have been cancelled. Instead the head group area we now list the specific excess concentration (s. our response to the general comment). The conversion from mg/L to mol/l was done when fitting the surface tension data to determine the slope of the pre-cmc curve

and calculating the excess surface concentration and the free energy of micellization.

P5,L23-27: see previous, also, are authors certain there were no detrimental effects when heating the systems to 60C?
We do not expect any detrimental effects as solute was after heating the micellar solution.

P5,L39: what are the "conventional" procedures?
Conventional procedures are multiple mathematical procedures of SANS analysis which could not be explained in an original article. These procedures are described in detail in the textbook we cited. We revised the sentence from “conventional procedure” to “procedures described in detail by Cotton (reference)..”

P6,L2: shouldn;t that be the scattering spectra of D2O?? and not water?
yes, we revised it.

P6,L5: Mostly??? what was used in the other cases? also I wouldn;t consider the listed informations as "minor"!
P6-7: pls. see my previous comment, I recommend to substantiall shorten this section.
We shorten this section and cancelled all equations and referred them to previous papers.

P8, top: This seems to be a table and if so should be listed as such with a proper heading..
A table has been created.

P8,L9-10: While 1 mPas is the viscosity of water, this does not mean that micelles had a newtonian behavior.
Please see previous comment about viscosity.

The following changes have been made accordingly:
P8,L13: pls. strike "extreme" and replace with the actual values (e.g. increased from x to y), I recommend to go through the entire manuscript and revise all such statements (see my previous comment as well)
P8,L23: "CORrelate".. which "were" obtained
P8,L27: the viscosity increased with ....
P8,L33: Pls replace I.e., its is typically not used at the beginning of the sentence, best would be to strike all such occurance
P8,L35" stronger??" how much, pls. avoid these vagues statements and be specific by giving percentage increases or other specific data.

P8,L44: While this may be "statistically significant", these are very minor increases in overall viscosity! I feel this is being overinterpreted..
The sentence has been changed: While in H_2O no differences could be determined between the individual HCAs, in D_2O a slight increase in viscosity could be found in the order SA=FA < PC < CA.

P9: What is "dynamic" viscosity?? (should be apparent). Also, Figure 3B needs to be revised, a bar chart would be much better, unless there is numerical value attached to the x-axis, no curve and especially no model that doesn't even seem to fit well should connect the points. These are

different chemical compounds. Also, It is nfortunate that authors did not go to igher concentrations than 0.35% it would have been interesting to see what happens at the higher concentrations. Finally, if authros wanted to point out differences between samples, it might have been better to use not the 0.2% gallate but the higher concentration, were values are really substantially different.
Dynamic viscosity is defined as the η = velocity gradient/shear stress with the units Pa*s in contrast to kinematic viscosity where the density is considered as well. As we annot finally clarify whether the investigated solutions show Newtonian behaviour, we will express the viscosity as apparent viscosity.
To show the trends in the viscosity increase, the figures 3a+b as well as 4a-c were changed to bar charts
All the measurements carried out in this paper were made to substantiate/compliment the abovementioned NMR data and to evaluate how and the viscosity was increased in CTAB solution, and what happens with micelles (see SANS) Therefore, the concentrations used in the NMR experiments were also used in the experiment of this paper.

P10: Pls. see previous comments rearding 4b. In terms of 4c, those differences are really quite small are should not be overinterpreted.
Please see previous comment.
what is n? (is that the number of repetitions?)
n is the common abbreviation for number of repetition.

P11: Figure 6, Table 1: Pls. see previous comments, I recommend to thermodynamically analyze in the discussion section the changes in the CMC as a fucntion of concentration, there are a number of excellent models/papers that describe similar data and that yield thermodynamic data that provides substantial insight. BTW: the data in Table 1 shoudl really be in the discussion section. In light of this, authors might also revisit their headgroup area discussion.
We briefly discussed some thermodynamic aspects. However, due to other aspects we have now included into the discussion along with the revision process, the paper has already lengthened quite a lot.

P12, L10-11: strongly, strongest, pls. reword...
P12,L29: should that be "curve" fits instead of "model" fits...
P13,L1-4: Can't understand this sentence (language) needs to be reworded.
P13,L14: Again, pls. be more specific and avoid such statements as ""resulted in different effects".
Since we decreased the number of figures (SANS scattering data), we revised and shorten this part as well.

P13,L33: These are very small changes, could authors give an estimation of the measurement accuracy /calculation from the SANS spectra?
Measurements with SDS and PG indicated an experimental error for SANS of +/- 0.3.

P14-16: Figures 8-10 are not needed since the analyzed data is actually presented in the table. Authors might as an example use maybe one figure to illustrate how a SANS spectra looks like, but the table (Table 2) is quite sufficient. This would also help to streamline the paper.
We agree and we have decreased number of figures.

P15: Table header is written with Caps, pls. revise to use small caps

Table header was revised accordingly.

P17,L10-15: This discussion is unfortunately not very detailed, authros might revisit and cite the specific results of more studies that have focused on interaction of nonionic surfactants with co-surfactants (neutral, anionic, cationic).
The discussion was complemented by a study on alcohols and non-ionic surfactants and LSER theory.

P17,L17-32: Again, thsi is not really a discussion as already almost a conclusion section and in light of my previous "headgroup area" comment questionable.
Please refer to the response to the general remarks at the beginning as well as the

P17,L41: What do authors mean by "solvent isotope effect"?
Please see review#2

P18,L38: increased significantly more??
No, decreased is correct based upon the cmc of CTAB in the absence of antioxidants.

P19,L25-36: This seems extremely speculative considering that no surface charge data is presented.
Please refer to the responses of the general remarks at the beginning and of P2,L17

Reviewer #2:

The present paper by Heins et al. describes the impact of certain antioxidants on the structure and organisation of the micellar systems CTAB, SDS and Brij58 applying SANS, surface tension measurements and viscosity measurements. Overall the data presented are sound and describe in great detail the structural arrangements and its changes due to the interaction of either gallates with various chain length and different hydroxy cinnamic acids. The results found demonstrate the effect of the antioxidants on the aggregation, inducing phase transition from sperical micelles to rod-like micelles for more hydrophobic gallates and cinnamic acids for CTAB. This was decribed as a balance between electrostatic and hydrophobic effects in the arrangement of the micellar structures. The present paper would profit if the author would add a short summary section at the end which briefly collects the main findings and effects described to the reader.
We shorten the SANS part and added a short summery instead.

Furthermore there is one point in the discussion not clear enough, i.e. the isotope effect of H2O and D2O with respect to the drastic change in viscosity given in Fig. 4b. A more appropriate discussion of the isotope effect should be added to the current text on p. 19. Oviously D2O weakens the electrostatic repulsion in the micelles giving rise to a higher viscosity.
We complemented the solvent isotop effect as follows:
Solvent isotope effects are due to small differences between the surfactant hydrocarbon-water interactions in H2O and D2O due to the stronger hydrogen bondings formed by D2O. The calculated free energy of transfer (Delta G°) from the aqueous to the micellar phase was found to be more negative in D2O than in H2O. This means that as the D2O content of the solvent increases, monomers will be driven from the bulk to the micellar pseudophase resulting in larger

and more micelles than in H2O. Due to this more promoted micellization in D2O, viscosity increase by sphere-to-rod transition proceeded at a lower PG concentration.
However, several studies showed that fractional charge and headgroup interactions of C14TAB \cite{602} and SDS \cite{601} micelles do not change with solvent isotopic composition, indicating that these types of interactions are the same in H2O and D2O \cite{439}.

By minor revisions referring to the above mentions aspects the paper can be accepted for publication.