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A fluorescence study of sodium hyaluronate/surfactant interactions in aqueous media

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Abstract—The interactions between sodium hyaluronate, an anionic polysaccharide, with surfactants (anionic and nonionic) were investigated using pyrene fluorescence measurement methods. The change of micropolarity produced by the interaction was monitored by the measurement of emission intensity ratio between the first and third bands (I_1/I_3), and the intensity ratio of the excimer and the third vibration monomer band (I_E/I_M). Because the hydrophilic heads on the SDS were attracted by the domains formed by the hydroxyl groups of hyaluronate, the I_1/I_3 ratio was reduced by the addition of hyaluronate at lower than 0.06% of sodium dodecyl sulfate (SDS) concentration. No aggregation was observed between hyaluronate and nonionic surfactants (Tween-80 and Cremophor EL) in the whole concentration range studied. At a higher concentration of surfactant, the I_1/I_3 ratio of hyaluronate/surfactant was influenced by the addition of saccharide (glucose, lactose, or mannitol). However, the effect of saccharide could be reduced by the addition of salt.

Keywords: Hyaluronic acid; Surfactants; Saccharide; Tumor drug delivery system

1. Introduction

Natural and synthetic polymers and surfactants are often used in combination in formulating products in the food, cosmetic, pharmaceutical, chemical, and other industries.[1] In combination, unexpected interactions may occur between selected polymers and surfactants. These interactions may have great influence on the formulation and its applications. Understanding and predicting possible interactions that may occur between certain polymers and surfactants would be useful in both academic and commercial applications. Using various numbers of tools and techniques, interactions between polymers and surfactants in formulations have been extensively studied and published in recent years.[2–6] It was evident from published studies that polymer–surfactant association can be influenced by many factors such as their ionic character, the hydrophilic/hydrophobic nature of their molecular structures, the conformation and flexibility of the polymers[7] and the presence of additives.[8]

Fluorescence probe techniques have proven to be one of the powerful tools in investigating the association of polymer–surfactant systems.[9–12]

A pyrene fluorescence probe has been used to examine the interactions between polymers and surfactants. The polarity-induced changes in photophysical properties of pyrene can be quantified by measuring the ratio of emission intensities between the first and third bands (I_1/I_3). These variations can be correlated with the polarity of the immediate environment in which the pyrene probe was placed.[13,14] This makes it a useful tool to study the formation and properties of molecular association and aggregation.

Hyaluronic acid is a linear polysaccharide and a naturally produced highly viscous glycosaminoglycan. It is made from alternating units of a disaccharide of D-glucuronic acid and 2-acetamido-D-glucose (*N*-acetyl-D-glucosamine) as a repeating unit linked at the β-(1→3)- and β-(1→4)-positions. It plays an important role in

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providing mechanical and transport function in body tissue.[15] Its specific binding properties with certain proteins enables hyaluronic acid to provide interesting biological functions.[16] Hyaluronic acid has been widely used as a medical material in surgery, radiotherapy, cosmetics, and in pharmaceutical preparations. In particular, hyaluronic acid has been used as a drug targeting delivery system for anti-tumor cytotoxic agents.[17–22] It has been demonstrated and documented that hyaluronic acid has specific binding with CD44 and RHAMM receptor on the cancer cell surface.[23] Because hyaluronic acid is highly hydrophilic and some cytotoxic agents are hydrophobic, surfactants are often employed in the formulations to improve the active ingredient's solubility and bioavailability. Understanding possible interactions between hyaluronic acid with surfactants and effects of other additives on the hyaluronic acid/surfactant association will be important in the final design of a hyaluronic acid drug delivery system.

The interaction between the anionic polysaccharide sodium hyaluronate with cationic surfactants has been studied by NMR spectroscopy,[24,25] viscosity,[26] surface tension,[27] dye solubilization, and conductivity methods.[28] In these previous papers, interaction between an anionic charged polymer and a positively charged surfactant was found to be mainly electrostatic. It has also been found that association was influenced by many factors, including surfactant chain length, polymer molecular weight, electrolyte concentration, etc.

In our studies, the hyaluronic acid is used as a sodium salt (at pH 7). The glucuronic acid residues are completely dissociated at neutral pH, giving a weakly charged polyelectrolyte containing one negative charge at every repeating unit. Although little or no interaction has been indicated between a nonionic surfactant and a polymer or between a surfactant and a polyelectrolyte of the same charge,[28] a better understanding of the influence of sodium hyaluronate on the solubilization character of surfactant may help in the formulation of the polymer–surfactant systems used in pharmaceutical preparations.

In the present work, the association between sodium hyaluronate and nonionic surfactant and anionic surfactant is studied using a pyrene fluorescence method. The effect of saccharide (glucose, lactose, or mannitol) added to sodium hyaluronate/surfactant system has also been investigated.

2. Results and discussion

Pyrene is a very hydrophobic molecule with a low aqueous solubility. It is expected to line up preferentially in the hydrophobic domains of polymers. The fluorescence spectrum of pyrene is related to its vibronic fine structure, and the relative peak intensity is strongly dependent on the microenvironment polarity. With increasing polarity, intensity of the first band (I_1) was enhanced, whereas no effect is seen on the intensity of the third band (I_3). This feature is often used to study the change in environmental polarity of surfactant/polymer upon association in aqueous solution. Therefore, the ratio of I_1/I_3 was used to determine the critical micelle concentration (CMC) of surfactants and to examine the interaction between surfactants and polymers.[1,9]

One of the most important properties of surfactants is to form micelles in aqueous solution. In the presence of micelles and other macromolecular systems, the pyrene molecule is preferentially solubilized in interior hydrophobic regions of these aggregates. When two pyrene molecules enter into the same hydrophobic micelle core, the fluorescence spectrum of the excimer can be detected and quantified. With surfactant concentration increasing, the number of micelles is increased. The probability of two pyrene molecules entering into one micelle is gradually diminished, resulting in proportional disappearance in the spectrum band.[12,29] Therefore, it is also possible to determine the critical micelle concentration (CMC) of surfactants by measuring the intensity ratio of the excimer and the third vibrational monomer band (I_E/I_M) as a function of surfactant concentration.

Figure 1 shows the variation of I_1/I_3 ratio of pyrene emission as a function of hyaluronate concentration at pH 7. It can be seen that there is a rapid drop of the I_1/I_3 ratio, followed by a gradual decrease when hyaluronate concentration reached 0.1% (w/w). At 0.25% (w/w) the ratio value reaches about 1.77, which is lower than that for pyrene in aqueous solution (1.85–1.90). The behavior suggests that with the increase in hyaluronate concentration, the pyrene molecules partition between the hydrophobic microdomain of hyaluronate and the aqueous solution. This results in a decrease of the ratio I_1/I_3. As the concentration of hyaluronate is above 0.1% (w/w), hyaluronate molecular chains start to overlap with each other and form a transient network structure, and the change of I_1/I_3 ratio is reduced.

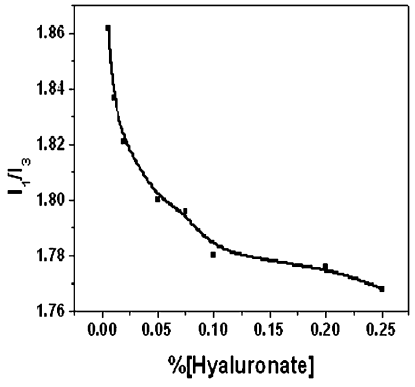

Figure 1. I_1/I_3 ratio of pyrene as a function of hyaluronate concentration. λ_{exc} = 334 nm; detection wavelengths: I_1, 373 nm; I_3, 384 nm.

Although the ratio was decreased with increasing hyaluronate concentration at the studied concentration range, the ratio I_1/I_3 in aqueous solutions of hyaluronate is slightly lower than in pure water.

To examine the interaction behavior of hyaluronate with various concentrations of nonionic surfactants (Cremophor EL, Tween-80), the ratio of I_1/I_3 and I_E/I_M of pyrene as a function of surfactant concentration was measured in the presence or absence of hyaluronate, respectively (Figs. 2 and 3).

A typical plot of I_1/I_3 as a function of surfactant concentration is as shown. With increasing surfactant concentration, the I_1/I_3 ratio experiences an initial flat plateau, then undergoes a sharp decrease and maintains less change afterwards. It was observed that for Tween-80 concentration higher than 0.0008% (w/w) the I_1/I_3 ratio began to decrease until the surfactant concentration was reached for 0.02% (w/w). Tween-80 CMC in pure water was around 0.02–0.03%, which is in accordance with the previously reported value.[30] The addition of Tween-80 to a pyrene-containing solution of hyaluronate was monitored under the same conditions. The curve of I_1/I_3 as a function of Tween-80 concentration showed a transition at 0.02% (w/w), a concentration nearly equal to the CMC of Tween-80. The data indicated that the interaction between hyaluronate and a nonionic surfactant was extremely weak. The plot of I_1/I_3 ratio versus Cremophor EL concentration showed that for surfactant concentration lower than 0.0006% (w/w), the I_1/I_3 ratio is little affected. With a further increase in Cremophor EL concentration, the I_1/I_3 ratio decreased until a surfactant concentration was reached for 0.01% (w/w). Cremophor EL CMC in pure water was about 0.01% (w/w), which coincides with the CMC of Cremophor EL alone.[31] Compared to the plot of I_1/I_3 ratio as a function of Cremophor EL in the presence and absence of hyaluronate, the result showed an almost identical CMC value, approximately 0.008–0.01% (w/w), which indicate that the Cremophor EL does not interact with hyaluronate.

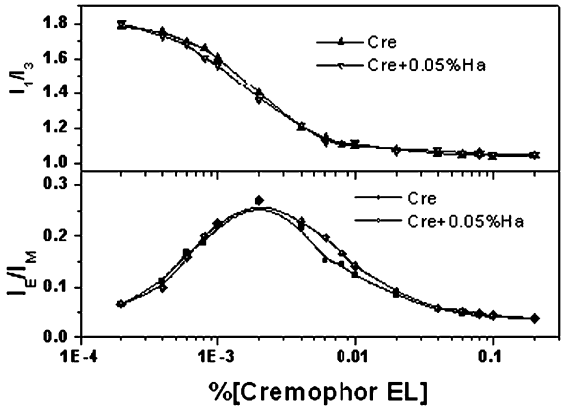

Figure 2. Change of I_1/I_3 and I_E/I_M ratios of pyrene with Cremophor concentration in the absence and presence of 0.05% hyaluronate. λ_{exc} = 334 nm; detection wavelengths: $I_1 = I_M$: 373 nm; I_3: 384 nm; I_E: 475 nm.

The final I_1/I_3 value showed a slight difference between the Tween-80 and Cremophor EL. This result might be ascribed to the difference between the hydrophile–lipophile balance of Tween-80 (HLB = 15) and Cremophor EL (HLB = 12.5).[31] On the other hand, the two surfactants showed similar I_E/I_M ratios comparable to the I_1/I_3 ratios.

The addition of saccharide to a solution of hyaluronate and surfactant was monitored (Fig. 4). It can be seen that the effect of three saccharides on the I_1/I_3 ratio is almost the same. Below the CMC the addition of a saccharide to the hyaluronate/Tween-80 system caused a decrease in the I_1/I_3 ratio; in contrast to this, the I_1/I_3 ratio increased at higher surfactant concentration. The observation suggests that the addition of a saccharide lowered the value of I_1/I_3 in aqueous solutions of

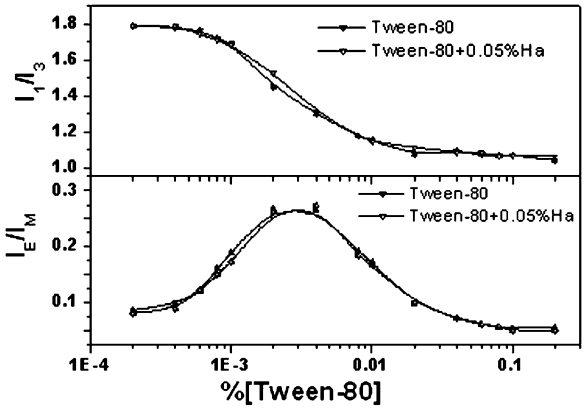

Figure 3. Change of I_1/I_3 and I_E/I_M ratios of pyrene with Tween-80 concentration in the absence and presence of 0.05% hyaluronate. λ_{exc} = 334 nm; detection wavelengths: $I_1 = I_M$: 373 nm; I_3: 384 nm; I_E: 475 nm.

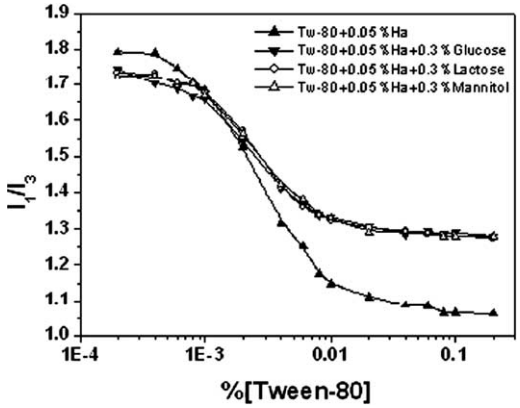

Figure 4. Change of I_1/I_3 ratio of pyrene with Tween-80 concentration in the presence of 0.05% hyaluronate and 0.3% saccharide including glucose, lactose, and mannitol. λ_{exc} = 334 nm; detection wavelengths: I_1: 373 nm; I_3: 384 nm.

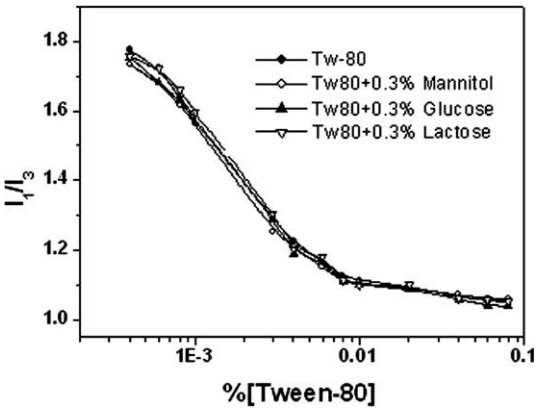

Figure 5. Change of I_1/I_3 ratio of pyrene with Tween-80 concentration in the presence of 0.3% saccharide including glucose and lactose. λ_{exc} = 334 nm; detection wavelengths: I_1: 373 nm; I_3: 384 nm.

the polymer–surfactant system at lower surfactant concentration. This result is similar to that of the control experiment (Fig. 5). In an aqueous solution of fixed saccharide concentration in the absence of hyaluronate, the I_1/I_3 ratio is slightly lower than that for the solution of the surfactant alone. Similarly, in aqueous solutions of the saccharide in the absence or presence of hyaluronate, the I_1/I_3 ratio is lower than in pure water (Table 1). That is to say, the I_1/I_3 ratio of a pyrene-containing solution can be decreased by the saccharides. Above the CMC, the final I_1/I_3 ratio was 1.2–1.3 (Fig. 4), which is slightly higher than that in the absence of saccharide. But the effect of the saccharide on the I_1/I_3 ratio can be reduced by the addition of salt solution, whereby the I_1/I_3 ratio does not change until 0.2 M of salt concentration is reached (Table 2). This observation suggests that the increase of the I_1/I_3 ratio above CMC may be caused by the change of micelle polarity due to the addition of a saccharide.

The behavior of the system containing hyaluronate and SDS with the same charge as the polymer is shown in Figure 6. The variation of the I_1/I_3 ratio of the control experiment with the surfactant in dilute salt solution in the absence of polymer (at 1.25 mM), which is similar in ionic strength to sodium hyaluronate solution, was observed as in Figure 7. In both systems, it was observed that the CMC of SDS is approx 0.2% (w/w). The experimental result was in accordance with previously reported values.[9,32] The CMC of SDS was lowered by addition of electrolyte due to the screen of electrostatic repulsion. This indicated that sodium hyaluronate affects the micellar properties of the surfactant in the same manner as a low-molecular-weight electrolyte, which is consistent with previous reports.[1] However, there was a difference in I_1/I_3 ratio between them as shown in Figure 6. For SDS at concentrations less than 0.1% (w/w), a small, slow decrease of the I_1/I_3 ratio was observed in the

Table 1. I_1/I_3 ratios of pyrene in different solutions with sugar in the absence or presence of HA without surfactant

Groups	Ratio value of I_1/I_3	
	Water	0.05% HA
Water	1.8636	1.850
Glucose (0.25 M)	1.8485	1.8310
Lactose (0.25 M)	1.8410	1.8377
Mannitol (0.25 M)	1.8259	1.8143

λ_{exc} = 334 nm; detection wavelengths: I_1: 373 nm; I_3: 384 nm.

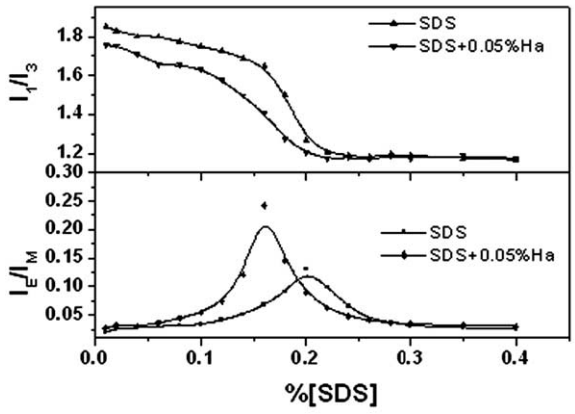

Figure 6. Change of I_1/I_3 and I_E/I_M ratios of pyrene with SDS concentration in the absence and presence of 0.05% hyaluronate. λ_{exc} = 334 nm; detection wavelengths: $I_1 = I_M$: 373 nm; I_3: 384 nm; I_E: 475 nm.

Table 2. Change of I_1/I_3 ratios of pyrene with Tween-80 concentration with sugar or NaCl or their mixture in the presence of 0.05% hyaluronate[a]

Groups	Ratio value of I_1/I_3				
	0.0004%	0.0008%	0.002%	0.006%	0.02%
Tw80–0.01 M salt	1.7248	1.6366	1.4468	1.2156	1.1180
Tw80–0.05 M salt	1.7046	1.6563	1.4382	1.2074	1.1154
Tw80–0.1 M salt	1.6910	1.6518	1.4210	1.2193	1.1150
Tw80–0.2 M salt	1.6917	1.6234	1.4070	1.1916	1.1173
Tw80–0.01 M glucose–0.2 M salt	1.7118	1.6394	1.4224	1.2017	1.1103
Tw80–0.1 M glucose–0.2 M salt	1.7163	1.6299	1.4286	1.1992	1.1154
Tw80–0.28 M glucose–0.2 M salt	1.7123	1.6212	1.4330	1.2108	1.1202

[a] λ_{exc} = 334 nm; detection wavelengths: I_1: 373 nm; I_3: 384 nm.

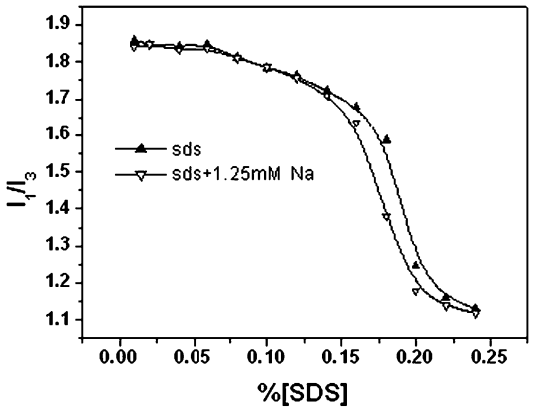

Figure 7. Change of I_1/I_3 ratios of pyrene with SDS concentration in the absence and presence of 1.25 mM NaCl. λ_{exc} = 334 nm; detection wavelengths: I_1: 373 nm; I_3: 384 nm.

presence of hyaluronate (Fig. 6). This might imply that the hydrophilic sulfonate groups of SDS were attracted toward the microdomains formed by the hydroxyl groups in the hyaluronate chain. At higher concentration of SDS, the free micelles are formed and the value of I_1/I_3 approaches that of SDS micelles in the absence of hyaluronate.

Parallel to the behaviors of I_1/I_3 it is also seen from Figure 6 that in the presence or absence of HA, the addition of SDS was accompanied by a gradual increase in the I_E/I_M ratio until the CMC was reached. Above the CMC of SDS, the ratio of I_E/I_M decreased due to the formation of normal micelles. However, in the presence of hyaluronate, the peak of the I_E/I_M ratio showed an obvious shift from 0.2% (SDS alone) toward lower SDS concentration (0.16%), which follows a similar trend in the I_1/I_3 ratio.

3. Conclusions

It has been proved, using pyrene fluorescence methods, that the interaction between hyaluronate and a nonionic surfactant is weak. This indicates that the addition of hyaluronate to a surfactant solution has little effect on the solubility properties of nonionic surfactant. Although the I_1/I_3 ratio of hyaluronate/surfactant system was increased by the addition of saccharide, this effect was reversed by addition of salt. These observations indicate that the micelle polarity is affected by saccharides. The addition of hyaluronate to solution of SDS resulted in a lowering of the CMC of the SDS due to the reduced electrostatic repulsion. Below 0.1% (w/w) SDS concentration, in the presence of hyaluronate, the I_1/I_3 ratio was lower than that of a solution without hyaluronate, which might be assigned to the solubilization of the hydrophilic heads on the surfactant in the domains formed by the hydroxyl groups of hyaluronate.

4. Experimental

4.1. Materials

Hyaluronic acid (MW 1.2×10^6 Da) was purchased from Fred Biochemical Ltd (China) in the form of its sodium salt. Polyoxyethylene 20 sorbitan monoleate (Tween-80™) was purchased from Fluka. The polyether of castor oil and ethylene oxide (Cremophor EL™) was purchased from BASF. Pyrene (99%), purchased from Sigma Chemical Co., was purified by recrystallization from ethanol. Ultrapure water was obtained by reverse osmosis (Milli Q, Millipore, Spain). All other chemicals were of analytical grade. Hyaluronate concentration was expressed in weight percent (w/w %) or in millimolar of the repeating monovalently charged disaccharide unit (at pH 7, the carboxylate groups are almost fully dissociated); 0.05 wt % of sodium hyaluronate corresponds to 1.25 mM of repeating units.

4.2. Preparation of sodium hyaluronate/surfactant dispersions

Concentrated stock solution of hyaluronate and surfactants were prepared by dissolving each ingredient separately in ultrapure water with a low rate of stirring. To prepare various sample mixtures for testing, various proportions of concentrated aqueous hyaluronate and surfactant solutions were mixed and diluted with ultrapure water to obtain a constant hyaluronate concentration of 0.05% (w/w) with a wide range of surfactant concentrations. The solutions containing the probe were prepared by adding methanol stock solution of pyrene to the hyaluronate/surfactant or surfactant alone solution. The final pyrene concentration was formulated to contain 1.0×10^{-6} M and stored at 25 °C for 24 h.

4.3. Steady-state fluorescence measurement

Pyrene emission spectra (λ = 350–500 nm) were recorded in a Cary Eclipse fluorescence spectrophotometer (Varian, USA), with an excitation wavelength of 334 nm and detection wavelengths of I_1 = I_M = 373 nm, I_3 = 384 nm, and I_E = 475 nm. Excitation and emission slits were set to 5 and 2.5 μm, respectively. All experiments were carried out in triplicate at 298.0 K. The concentration of sodium hyaluronate was 0.05% (w/w).

Acknowledgements

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