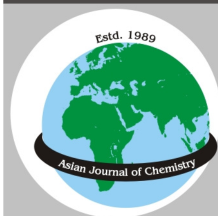
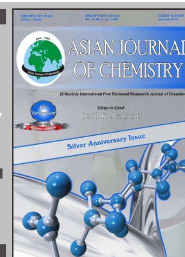
Asian Journal of Chemistry; Vol. 25, No. 6 (2013), 3068-3072



ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13521



Interaction Between Gelatin and Cationic Gemini Surfactant with Long Unsaturated Tail

XIAOMING CHEN[1,2,*] and LIN REN[1]

[1]School of Materials and Chemical Engineering, Anhui University of Architecture, Hefei 230601, P.R. China
[2]Anhui Key Laboratory of Advanced Building Materials, Hefei 230601, P.R. China

*Corresponding author: Tel: +86 551 3824970; E-mail: chenxmyn@hotmail.com

(*Received*: 28 January 2012; *Accepted*: 12 December 2012) AJC-12520

The interaction of cationic gemini surfactant 1,2-ethane-*bis*-[*N,N,N*-dimethyl-(Z-13-docosenyl)quaternary ammonium bromide] (hereafter noted 22-2-22) with gelatin above its isoelectric point were studied by pH measurements, viscosity and fluorescence spectroscopy. It has been found that three critical concentrations of 22-2-22, referred to as C_{inter}, C_{intra} and C_a respectively, can be detected by steady state fluorescence measurements. However, only C_{intra} can be noted effectively by viscosity measurement. The experimental results indicate that the polymer bound micelles act as transient cross-links resulting in the inter-polymer or intra-polymer association, which is depending on both surfactant and gelatin concentrations. When $C_{22-2-22} > C_{inter}$ micelles bound to more than one polymer strand act as transient cross-links between gelatin molecules, that is, inter-molecular association. As $C_{22-2-22}$ is greater than C_{intra}, more surfactant micelles tend progressively to envelop and "neutralize" the binding sites of polymer chains and break the inter-chain junctions. And relatively rich surfactant micelles made each gelatin strand accommodate surfactant micelle which bind with several sites of the same polymer chain, *i.e.*, the intra-polymer association occurs. Finally one gelatin chain may bind several surfactant micelles. However if $C_{gelatin}$ reaches the overlap concentration C* the intra-polymer association may be screened by the entanglement of polymer chains.

Key Words: Gelatin, Cationic gemini surfactant, Interaction.

INTRODUCTION

Interactions between surfactants and synthetic or natural polymers have long been subjected to world-wide theoretical and experimental investigations for the various industrial applications of such systems and were reviewed by Robb[1], Goddard[2] and later by Bárány[3]. Generally, the attractive interactions between polymers and surfactants such as electrostatic interaction and hydrophobic interaction introduce the formation of polymer-surfactant aggregates, resulting in the modification of various properties of polymer solution.

Because of the unique electrolytic character of proteins, phenomena involving protein-surfactant interactions are especially intriguing. Gelatin is a well characterized, denatured protein (collagen), which shows the typical properties of an amphoteric poly-electrolyte, *i.e.*, the existence of a pH-dependent net charge and of an isoelectric point. Complex formation between gelatin and ionic surfactants is of great interest for many physicochemical and biological phenomena. Many studies have been made on gelatin-surfactant interactions in past decades using viscosity[4,5], surface tension[6], NMR[7], circular dichroism spectra[6,8], fluorescence spectra[9,10] and dynamic light scattering[11,12]. The classical example keeping the stage for more than 50 years is the sodium dodecyl sulfate (SDS)-gelatin system. However relatively few studies have been done with cationic and nonionic surfactants.

To understand gelatin-surfactant interaction intensively, we should work with different types of surfactants. Gemini surfactant consists of two hydrophobic chains, two polar headgroups and a spacer linked at or near the head groups. It is superior to the conventional single-chain surfactant in many properties such as low Krafft temperature, low critical micelle concentration (CMC) and strong hydrophobic microdomain[6]. Not much is known on their solution properties in the presence of polymers or proteins.

The present results attend to make a contribution to clarify still unknown aspects of the above systems. Highly viscoelastic wormlike micelles formed by cationic single chain surfactant with long unsaturated tail has been reported by Raghavan *et al.*[13]. These micelles can thus function as thickening and rheology-control agents in aqueous systems, much like polymers. To our interest the interaction between gelatin and cationic Gemini surfactant 1,2-ethane-*bis*-(*N,N,N*-dimethyl-(Z-13- docosenyl) quaternary ammonium bromide) (22-2-22) is studied by pH measurements, viscosity and fluorescence spectroscopy.

EXPERIMENTAL

Gelatin was purchased from China National Pharmaceutical Group. This gelatin has an isoelectric point of 4.6 (determined viscometrically described in detail by Dreja *et al*.[14]). The intrinsic viscosity [η] of gelatin in aqueous solution at 30 ºC is 49 mL/g which was measured with a Ubbelohde capillary viscometer following a standard procedure. Therefore the overlap concentration C* of gelatin was about 0.02 g/mL, estimated from $C^*=1/[\eta]$[15].

Cationic gemini surfactant 1,2-ethane-*bis*-[*N,N,N*-dimethyl-(Z-13-docosenyl)quaternary ammonium bromide] (22-2-22), was kindly supplied by Qin F. and systhesis details were described[16] and its structure is displayed in Fig. 1. De-ionized distilled water was used in all experiments.

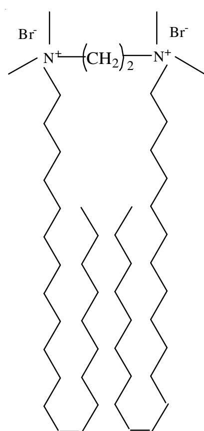


Fig. 1. Structure of 1,2-ethane-*bis*-[*N,N,N*-dimethyl-(Z-13-docosenyl)-quaternary ammonium bromide] (22-2-22)

Preparation of solutions: Two stock solutions were prepared, both containing the desired concentrations of gelatin. A high concentration of surfactant was also included in one of the solutions. Known quantities of the stock solutions were mixed to give any desired concentration of surfactant. This procedure was used for all experiments (pH, viscosity and fluorescence measurements) in which it was desired to vary the surfactant concentration systematically.

pH Measurements: Measurements of the sample pH values were carried out using a digital pH meter (Xianke Instruments Co. Ltd., Shanghai, China, model PHS-2C) at 30 ± 0.1 ºC maintained with a thermostatic water bath.

Viscometry: All viscosity measurements were carried out using a conventional ubbelohde capillary viscometer (inner diameter $\phi = 0.55$ mm) at 30 ± 0.1 ºC maintained with a thermostatic water bath. Measurements were initiated after approximately 5-10 min equilibrium time. Each flow time was determined by repeating at least three time measurements and kept long to neglect the kinetic corrections to the observed data. The precision of the measurements was 0.01 sec and the reproducibility was 0.2 sec. The relative viscosity η_r was calculated from $\eta_r = t/t_0$ with t and t_0 the flow time of the gelatin/22-2-22 mixed solution and the gelatin solution, respectively, neglecting the difference of the density between the solution and the solvent as performed in our previous studies[17-20]. The viscometer was thoroughly cleaned with concentrated chromic acid and de-ionized distilled water after each experiment.

Steady state fluorescence measurements: Intensities and spectra of fluorescence were carried out on a Hitachi F-4600 fluorescence spectrophoto-meter using 1.0 cm quartz cells. The emission spectra of gelatin were monitored with a fixed excitation wavelength at 280 nm.

RESULTS AND DISCUSSION

Considering that the net charge of gelatin is dependent on the pH value of solution, initially the pH value of mixed gelatin/22-2-22 solution were determined in the whole concentration range of our experiments as shown in Fig. 2.

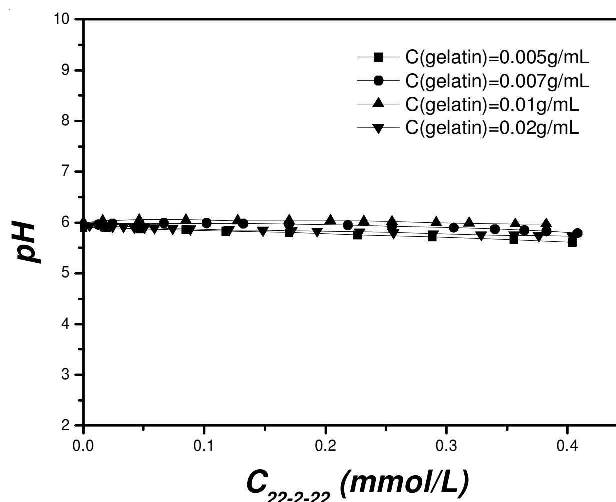


Fig. 2. pH Values of aqueous solutions of gelatin as a function of the 22-2-22 concentration for different gelatin concentration at 30 ºC

The results indicate that the pH value of mixed gelatin/22-2-22 solution were approximately 6 which is above its isoelectric point and almost do not change in all experiments, therefore no attempts were made to buffer the solutions because salt addition would have affected the charge conditions of gelatin which was suggested by Lips *et al*.[21] and later by Dreja *et al*.[14]. On such occasion we know that in our experiments gelatin has a net negative charge and the amount of net charge of gelatin does not change in the whole concentration range of our experiments.

Viscometry is convenient and particularly effective in probing conformational changes in macromolecular systems. Fig. 3 shows the relative viscosity η_r of 22-2-22 in gelatin aqueous solutions at 30 ºC when the concentration of gelatin, $C_{gelatin}$, is in the vicinity of and well below the overlap concentration C^*. The most salient feature in Fig. 3 is the maximum of η_r. Greener *et al*.[4] and Griffiths *et al*.[9] have ascribed the viscosity increase of gelatin-SDS solution to the inter-polymer association. However the concentration of polymer in their experiments, $C_{gelatin}$, were relatively high, most probably above the overlap concentration C^*. Through the electrostatic attractive interaction and the hydrophobic interactions 22-2-22 bind to gelatin chains and the hydrophobic aggregation of bound surfactant lead to the formation of polymer-bound micelles. As a result drastic changes are introduced in the conformation of gelatin molecule in solution. At low surfactant concentrations the marked increase of the relative viscosity is suggested to be the result of the formation of transient cross-links between

gelatin strands mediated by 22-2-22 micelles bound to more than one polymer strand, *i.e.*, inter-molecular association. With the increase of surfactant concentration 22-2-22 is relatively rich. More surfactant micelles tend progressively to envelop and "neutralize" the binding sites of polymer chains and break the inter-chain junctions. And relatively rich surfactant micelles made every gelatin strand have accommodate micelle which bind with several sites of the same polymer chain, *i.e.*, the intra-polymer association occurs, resulting in the marked decrease of the viscosity of gelatin solution. We call the critical concentration corresponding to the viscosity peak as C_{intra} here which indicates the beginning of intra-polymer association.

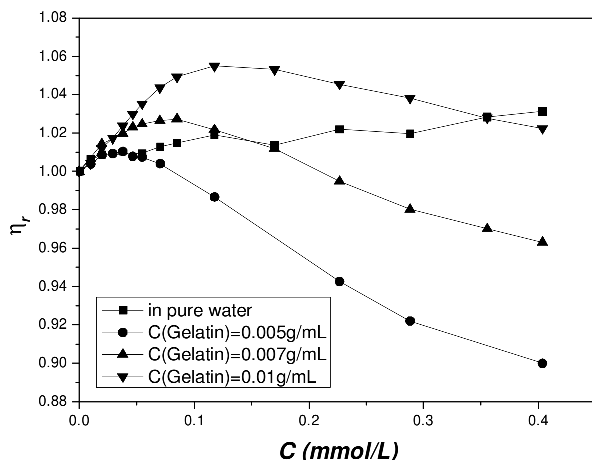


Fig. 3. Relative viscosity η_r of 22-2-22 as a function of the 22-2-22 concentration for different gelatin concentration at 30 ºC ($C_{gelatin} < C^*$)

To probe the association behaviour of gelatin in the presence of 22-2-22 the reduced viscosity of gelatin as a function of polymer concentration for different surfactant concentrations are determined (Fig. 4). The good linearity allows a successful determination of intrinsic viscosity by extrapolation of the reduced viscosity curves to zero polymer concentration. It can be seen that with the increase of surfactant concentration the intrinsic viscosity of gelatin decreases markedly, which is corresponding to the intra-polymer association of gelatin in extremely dilute solution.

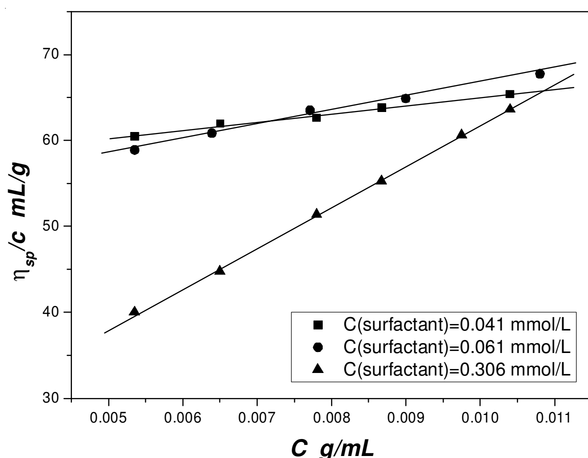


Fig. 4. Reduced viscosity of gelatin as a function of the gelatin concentration for different 22-2-22 concentration at 30 ºC

It is presumed that the entanglement of polymer chains will screened the intra-molecular association of polymers when $C_{gelatin}$ reaches the overlap concentration C*. As a result the solution viscosity may not decrease even the 22-2-22 concentration, $C_{22-2-22}$, is rich enough, therefore the viscosity maximum disappears (Fig. 5). It can be seen that when $C_{22-2-22} > 0.3$ mmol/L the relative viscosity of gelatin solution in which $C_{gelatin} = 0.02$ g/mL = C* increases only slightly.

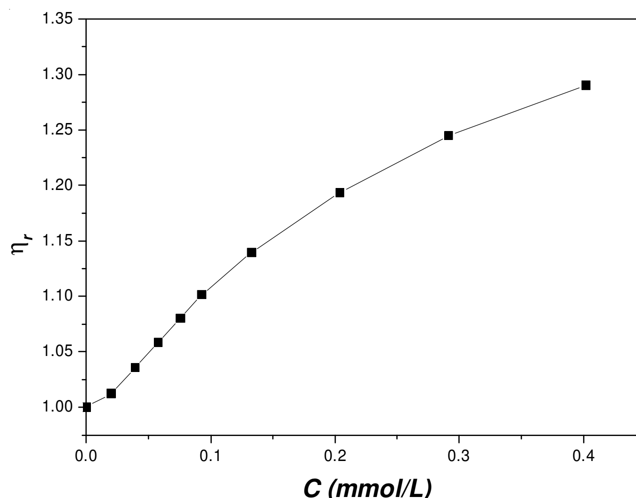


Fig. 5. Relative viscosity η_r of 22-2-22 as a function of the 22-2-22 concentration for $C_{gelatin} = 0.02$ g/mL = C* at 30 ºC

Fluorescence spectroscopy is widely used to monitor changes of the conformation of polymer induced by the interaction with ionic surfactants. These interactions can, in principle, produce changes in the position or orientation of fluorophores, altering their exposure to solvent, leading to alterations on the quantum yield[22]. As the binding of 22-2-22 on gelatin induces a strong change of polymer chain conformation which can be seen from viscosity results, it may be reflected by the influence on the intrinsic fluorescence spectra of gelatin.

Gelatin contains only two intrinsic fluorophores *viz.*, Tyr and Phe and has no Trp. Phe is not excited in most cases and its quantum yield in proteins is rather low[6], so the emission from this residue can be ignored. The intrinsic fluorescence of gelatin is almost completely contributed by Tyr. Fig. 6 shows fluorescence spectra of gelatin at different $C_{22-2-22}$ when $C_{22-2-22} < 0.01$ mmol/L and $C_{22-2-22} > 0.01$ mmol/L at 30 ºC. It can be seen that the addition of 22-2-22 to gelatin solution produces an increase in the intensity of gelatin fluorescence when $C_{22-2-22} < 0.01$ mmol/L and then a decrease when $C_{22-2-22} > 0.01$ mmol/L. However the wavelength of the maximum emission peak does not change in all cases.

Fig. 7 shows the fluorescence peak-intensity of gelatin as a function of $C_{22-2-22}$ for different $C_{gelatin}$ at 30 ºC when $C_{gelatin} <$ C*. The salient features of Fig. 7 are as following: (a) at $C_{22-2-22} = 0.01$ mmol/L the emission maximum intensity of gelatin reaches a maximum; (b) at $C_{22-2-22} > 0.01$ mmol/L fluorescence quenching occurs and a turning point of the plot can be determined at which $C_{22-2-22}$ is identical to the value of the viscosity peak position; (c) in case of $C_{gelatin} = 0.005$ g/mL when the $C_{22-2-22} > 0.25$ mmol/L the emission maximum intensity decreases more quickly.

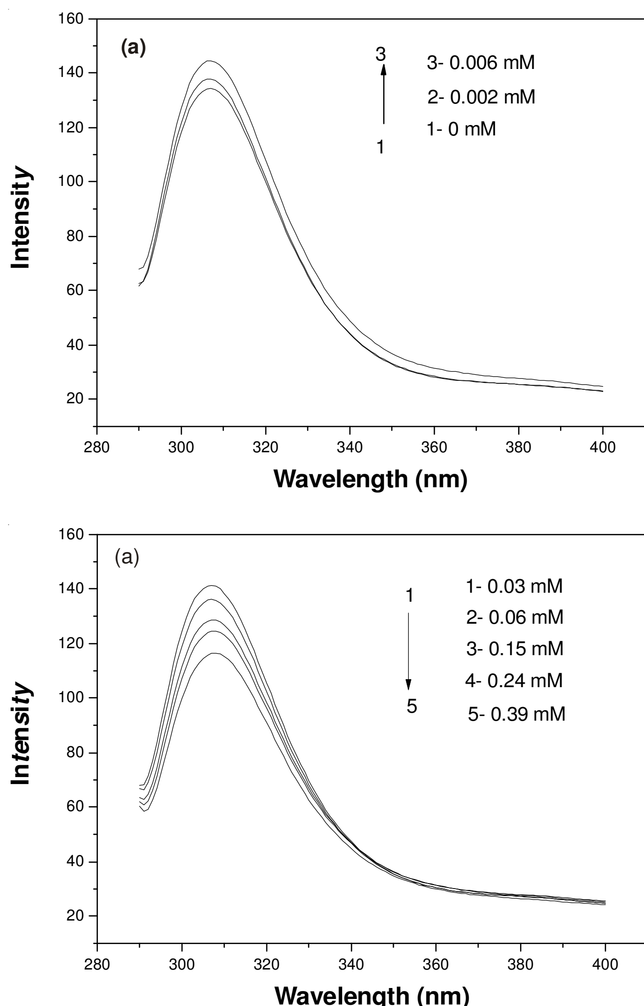


Fig. 6. Fluorescence spectra of gelatin at different 22-2-22 concentrations ($C_{gelatin}$ = 0.01 g/mL, $C_{22\text{-}2\text{-}22}$ < 0.01 mmol/L, $C_{22\text{-}2\text{-}22}$ > 0.01 mmol/L) at 30 ºC

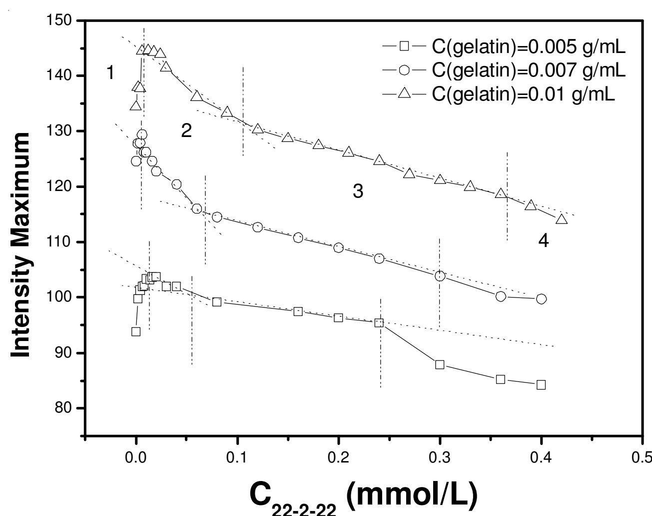


Fig. 7. Fluorescence maximum intensity of gelatin as a function of the 22-2-22 concentration for different gelatin concentration at 30 ºC ($C_{gelatin}$ < C*)

It is well known that the interaction between the fluorophores and the rest amino-acid residues on protein chains may cause the fluorescence quenching of proteins[23]. For gelatin there are hydrophobic microdomains formed by the aggregation of the hydrophobic amino-acid residues. The hydrophilic polymer chain will then surround these hydrophobic regions in a highly coiled conformation to protect them from the water. The binding of surfactant to gelatin chain allows the solubilization of the hydrophobe and its protection from water by the surfactant headgroup. This in turn liberates the hydrophilic polymer chain, enabling them to rearrange into a more extended state, *i.e.*, the partially denaturing of gelatin. As a result the quencher groups for fluorophores are removed and therefore the fluorescence peak-intensity increases until $C_{22\text{-}2\text{-}22}$ = 0.01 mmol/L. With the further addition of 22-2-22 the fluorescence maximum intensity decreases, suggesting that the fluorescence quenching occurs. A most probably reason is the aggregation of gelatin which cause the fluorophores and the quencher group become closer. Considering the viscosity results, the critical concentration corresponding to the maximum of the fluorescence peak-intensity may be the initiation of the inter-polymer association. We call this as critical concentration C_{inter}. Therefore the viscosity increase of viscosity when $C_{22\text{-}2\text{-}22} < C_{intra}$ is caused by firstly the unfolding of gelatin and secondly the inter-polymer association both due to the binding of 22-2-22 onto the gelatin chains.

When $C_{22\text{-}2\text{-}22} > C_{inter}$ at low surfactant concentrations inter-polymer association mediated by the surfactant aggregates dominates. One surfactant aggregates may bind more than one polymer strands. As a result the fluorescence peak-intensity decreases more quickly. While at high surfactant concentrations the fluorescence peak-intensity decreases more slowly due to the intra-polymer association.

It is expected that if the polymer concentration is low enough ($C_{gelatin}$ = 0.005 g/mL) with the increase of $C_{22\text{-}2\text{-}22}$ one polymer strand may accommodate several surfactant micelles. As a result the fluorescence maximum intensity decreases more quickly once again. The turning point here we noted C_a. Look back to the viscosity results, it can be seen that when $C_{22\text{-}2\text{-}22} > C_a$ the viscosity diminishes more slowly, suggesting the decrease of intra-polymer association and subsequently may be the case in which one polymer strand may accommodate several surfactant micelles. The schematic illustration of such results of gelatin/22-2-22 interactions is shown in Fig. 8.

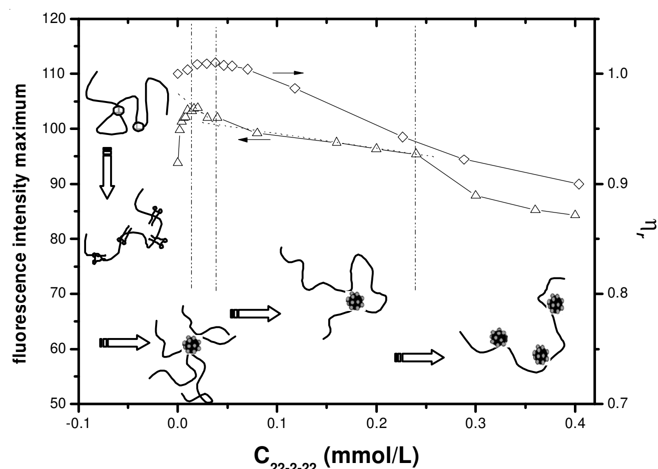


Fig. 8. Schematic illustration of the interaction between gelatin and 22-2-22

Fig. 9 shows the fluorescence peak-intensity of gelatin as a function of the 22-2-22 concentration for $C_{gelatin}$ = 0.02 g/mL=C* at 30 ºC. The most different feature between Fig. 9 and Fig. 7 which is corresponding to $C_{gelatin}$ < C* is that the turning point of the plot during the decrease of the fluorescence peak-intensity of gelatin disappears. This suggests that the intra-polymer association may be screened, corresponding with the viscosity results.

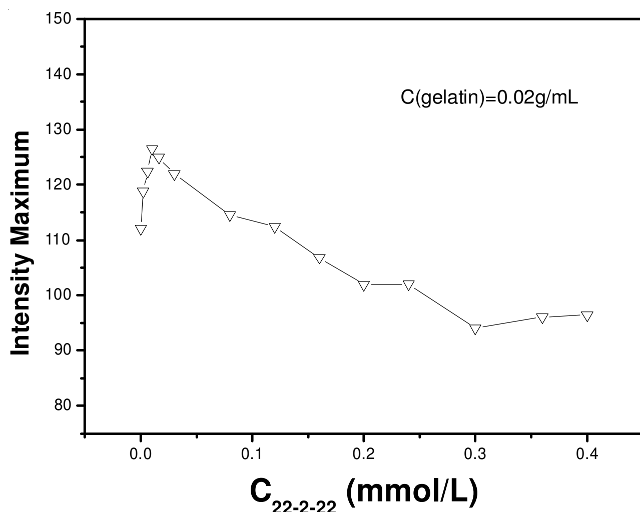


Fig. 9. Fluorescence maximum intensity of gelatin as a function of the 22-2-22 concentration for different gelatin concentration at 30 ºC ($C_{gelatin}$ =0.02 g/mL = C*)

Conclusion

The interaction of cationic gemini surfactant 1,2-ethane-*bis*-[*N, N, N*-dimethyl-(Z-13-docosenyl)quaternary ammonium bromide] (noted 22-2-22) with gelatin above its isoelectric point were studied by pH measurements, viscosity and fluorescence spectroscopy. It has been found that two critical concentrations of 22-2-22, referred to as C_{inter}, C_{intra} respectively, can be detected by steady state fluorescence measurements. However, only C_{intra} can be noted effectively by viscosity measurement. The experimental results indicate that the polymer bound micelles act as transient cross-links resulting in the inter-polymer or intra-polymer association which is depending on both surfactant and gelatin concentrations.

ACKNOWLEDGEMENTS

The project was supported by the Doctor Research Foundation of Anhui University of Architecture (K02541), the Students Scientific Innovation Program of Anhui University of Architecture (C11039), the Postdoctoral Science Foundation of Anhui University of Architecture (20110101) and Key Project of Anhui Provincial College Natural Science (KJ2011A070).

REFERENCES

1. I.D. Robb, in ed.: E.H.L. Reyenders, In Anionic Surfactants, Physical Chemistry of Surfactant Action; Surfactant Science Series, Marcel, Dekker: New York, vol. 10, p. 109 (1981).
2. E.D. Goddard, *Colloids Surf.*, **19**, 1301 (1986).
3. S. Bárány, *Macromol. Symp.*, **166**, 71 (2001).
4. J. Greener, B.A. Contestable and M.D. Bale, *Macromolecules*, **20**, 2490 (1987).
5. H. Fruhner and G. Kretzschmar, *Colloid Polym. Sci.*, **270**, 177 (1992).
6. D. Wu, G. Xu, Y. Sun, H. Zhang, H. Mao and Y. Feng, *Biomacromolecules*, **8**, 708 (2007).
7. P.C. Griffiths, P. Stilbs, A.M. Howe and T.H. Whitesides, *Langmuir*, **12**, 5302 (1996).
8. R. Wüstneck, R. Wetzel, E. Buder and H. Hermel, *Colloid Polym. Sci.*, **266**, 1061 (1988).
9. P.C. Griffiths, J.A. Roe, B.L. Bales, A.R. Pitt and A.M. Howe, *Langmuir*, **16**, 8248 (2000).
10. T.H. Whitesides and D.D. Miller, *Langmuir*, **10**, 2899 (1994).
11. A. Saxena, T. Antony and H.B. Bohidar, *J. Phys. Chem. B*, **102**, 5063 (1998).
12. M.A. Abed and H.B. Bohidar, *Int. J. Biol. Macromol.*, **34**, 49 (2004).
13. S.R. Raghavan and K.W. Kaler, *Langmuir*, **17**, 300 (2001).
14. M. Dreja, K. Heine, B. Tieke and G. Junkers, *Colloid Polym. Sci.*, **274**, 1044 (1996).
15. P. Hormnirum, A. Sirivat and M.A. Jamieson, *Polymer*, **41**, 2127 (2000).
16. F. Qin, Master Degree Dissertation, China, Hefei: University of Science and Technology of China, p. 18 (2008).
17. J. Li, H. Li, H. Yang, P. Zhu and P. He, *Chin. J. Polym. Sci.*, **26**, 31 (2008).
18. Y. Yan, H. Li, H. Yang, J. Qian, P. Zhu and P. He, *Chin. J. Chem. Phys.*, **21**, 169 (2008).
19. X. Chen, H. Yang, X. You, P. Zhu and P. He, *Chin. J. Polym. Sci.*, **24**, 437 (2006).
20. H. Yang, Y. Yan, P. Zhu, H. Li, Q. Zhu and C. Fan, *Eur. Polym. J.*, **41**, 329 (2005).
21. A. Lips, T. Evans and I. Evans, in ed.: K. Schwenke and R. Mothes, Food Proteins: Structure and Functionality. VCH, Weinheim, pp. 331-340 (1993).
22. E.L. Gelamo and M. Tabak, *Spectrochim. Acta A*, **56**, 2255 (2000).
23. D. Zhu, J. Chen and S. Zhu, Analysis Method for Molecular Luminescence. China, Shanghai: Fudan University Press, p. 17 (1985).