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Biophysical characterization of complexation of DNA with oppositely charged Gemini surfactant 12-3-12

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ABSTRACT

The interaction between DNA and cationic gemini surfactant trimethylene-1, 3-bis (dodecyldimethylammonium bromide) (12-3-12) has been investigated by the measurements of fluorescence, surface tension, UV spectrum and circular dichroism (CD). Micelle-like structure of 12-3-12 induced by DNA appears at critical aggregation concentration (CAC), which is much lower than critical micelle concentration (CMC) of 12-3-12 in DNA-free solution. CAC is independent of DNA concentration, but the CMC of the mixed solutions of DNA and 12-3-12(CMC_{mix}) increases with the increasing of DNA concentration. The surface tensions of the mixed system are higher than that of the pure surfactant solution, much different from the so-called synergistic lowering of the surface tension for other polymer-surfactant systems. Phase separation occurs after the neutralization point and the precipitate redissolves with superfluous 12-3-12. Cationic surfactant 12-3-12 can exclude ethidium bromide (EB) from the DNA/EB complex, and this process does not depend on the DNA concentration but on the charge ratio of 12-3-12 to DNA. The binding constant of EB to DNA decreases sharply at the charge ratio from 0.5 to 1.0. Circular dichroism (CD) spectra show that DNA undergoes a conformational transition from native B-form to chiral ψ-phase with increasing of 12-3-12.

1. Introduction

The study of the interaction between surfactants and polymers is an active field of research in colloidal science [1,2]. DNA is not only an important biological material but also an interesting anionic polymer with a unique double helical structure. The interactions of DNA with cationic surfactants are of interest in many applications, for example, the development of methods for DNA extraction and purification, and lately, the potential use of these systems as vehicles for gene delivery and gene transfection [3,4]. The unique structure of DNA also can serve as a good template to fabricate the ordered or hierarchical DNA based molecular assemblies [5]. Numbers of investigations have been presented on the DNA/cationic surfactant complex formation, precipitation, thermodynamics, and microstructure by variety of methods [6–15]. Similar to the interaction between oppositely charged surfactants and other polyelectrolyte, complexation of cationic surfactant and DNA occurs at very low concentration which is usually a few orders of magnitude lower than the critical micellization concentration (CMC) of the surfactant. This is attributed primarily to the strong electrostatic interaction between surfactant and the phosphate site of DNA [14,16]. The binding of surfactant to DNA was shown to involve a two-step process by the binding isotherms, first, binding to an isolated phosphate site on DNA strand, and second, a highly cooperative binding event which is contributed by the hydrophobic interactions between surfactant alkyl chains [7]. The phase diagrams of DNA-cationic surfactant systems show a strongly associative behavior [13]. The phase separation starts at very low concentration of DNA or surfactant and is enhanced as the alkyl chain length of the surfactant increases, but the salt effect on the phase behavior is complex since the phase separation could be either enhanced or decreased depending on experimental conditions. The discrete coil-globule transition of large DNA molecules, consisting of several kilo base pairs, can be induced by cationic surfactant, and the coexistence of random coils and compact globules can be observed by the fluorescence microscopy [6,8,9]. Recent researches on the DNA with relatively low molecular weight revealed that the addition of cationic surfactant can cause changes in the aggregated form of DNA from loosely packed spherical to rodlike via toroidal structure [17].

Most of the studies on the interaction between DNA and surfactant were focused on the systems containing conventional surfactant with single alkyl chain and single hydrophilic head group. Recently, some researchers reported the interactions of between DNA and novel surfactants such as gemini surfactant [18–24], double-tailed surfactant and bola surfactant etc. It is shown that the spacer length of gemini surfactant plays an important role in determining the properties of monolayers at the air–water interface formed by the DNA/gemini surfactant complex [19], and the counterion has a marked influence on both micellization and aggregation of gemini surfactant in the presence of DNA [22]. While many details on the aggregation of gemini surfactant on DNA are not very clear, further investigation on the system containing gemini surfactant and DNA is still needed.

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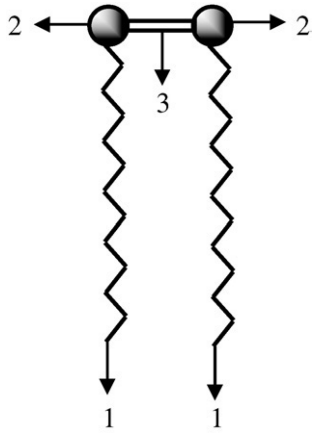

Scheme 1. Schematic representation of gemini surfactant. 1 – hydrophobic tail; 2 – hydrophilic head; 3 – spacer.

As a new family of surfactant shown in Scheme 1, gemini surfactants consist of two hydrocarbon chains and two polar groups linked with a spacer, which have stimulated extensive interest with stronger surface activity, better solubilizing, wetting, foaming, and lime-soap dispersing capability than the conventional surfactant [25–27]. Gemini surfactants allow for expanded structural diversity by changing the length of hydrophobic chains, the polarity of head groups, the structure of spacer, and the counterions.

In this work, we investigated the biophysical characters of complexation of DNA and cationic gemini surfactant trimethylene-1, 3-bis (dodecyldimethylammonium bromide) (12-3-12). As a simple and effective method of studying the mixtures of two components, of which one is highly active and the other relatively inactive at the air/water interface, surface tension is often used to investigate the interaction between surfactant and polymer [1,2], while, it is seldom used in the system of DNA and surfactant. We measured surface tension of DNA and surfactant mixed solutions and obtained some interesting results that much different from others' work. The competitive binding of ethidium bromide and cationic species to DNA was utilized to study the interaction of DNA and cationic surfactant by measuring the UV–vis absorption and fluorescence emission. To determine the conformational change of DNA upon binding with cationic surfactant, circular dichroism experiments were performed. The phase diagram of system containing DNA and 12-3-12 was obtained by visual observation. And the fluorescence spectroscopy of pyrene was also measured to detect the formation of the DNA/12-3-12 complex.

2. Materials and methods

2.1. Materials

Gemini surfactant 12-3-12 was prepared as described in Ref. [27]. Salmon sperm DNA was purchased from Sigma and used without further treatment. The concentration of DNA phosphate groups in the solution was determined by UV absorbance at 260 nm, the corresponding molar extinction coefficient was 6600 $L{\cdot}M^{-1}$ cm^{-1}. Pyrene and ethidium bromide (EB) were obtained from Aldrich chemicals and Sigma, respectively. Concentration of EB was determined by spectrophotometer assuming a molar extinction coefficient of 5600 $L\ M^{-1}$ cm^{-1} at 480 nm. Deionized water was treated with $KMnO_4$ to remove oxidable impurities and redistilled.

2.2. Methods

10 mM NaBr aqueous solution was used as solvent in all samples. DNA and 12-3-12 stock solutions were prepared, respectively, and then mixed up to obtain DNA/surfactant samples with various content. The pH of all the solutions was 6.0 and was not adjusted after mixing. Fluorescence spectrum was recorded by F4500 fluorescence spectrophotometer (HITACHI). For the measurement of fluorescence emission of pyrene, the redistilled water was saturated by pyrene first and then used to prepare the samples. The typical emission spectrum of pyrene (λ_{EX}=335 nm) has five peaks at 373, 379, 384, 390, and 397 nm, and the ratio of the first to third vibronic peaks I_1/I_3 is used to detect the microenvironment of pyrene. To study the displacement of EB from DNA/EB complex by surfactant, DNA/EB mixed solutions were obtained first, and then the 12-3-12 stock solution was added to the solutions step by step. The excitation and emission wavelengths of solutions with EB were 535 and 595 nm, respectively. The phase behavior of the mixtures of DNA and 12-3-12 was determined by the preparation of a number of samples with step changes in surfactant or DNA concentration over the range of interest. These samples were equilibrated in a thermostated water bath at 25 °C for up to 24 h. The surface tensions were measured on tensiometer of Thermo Cahn using the ring method. All the solutions were kept for 30 min until surface tension did not change with time. The average values of equilibrium surface tension were obtained by repeating three times. Circular dichroism (CD) experiments were carried out with a Jasco spectropolarimeter J-810 (Jacso). In a 1 cm path length quartz cuvette, spectra were measured as the average of three scans from 220 to 350 nm at a scan rate of 50 nm/min.

3. Results and discussion

3.1. Micropolarity

Pyrene has been widely used as a probe of interactions between surfactant and polymers [28,29]. The shape and intensity of the fluorescence emission of this probe are sensitive to its microenvironment in solutions. The intensity ratio of the first to the third vibronic bands I_1/I_3 can be taken as a measure of the polarity of the environment, being high in polar media and low in hydrophobic environments. Fig. 1 shows the plots of I_1/I_3 for pyrene solubilized in surfactant with different concentration of DNA (0, 0.03 mM, 0.06 mM and 0.1 mM). Compared with solution containing 12-3-12 alone, the I_1/I_3 ratio for DNA/12-3-12 mixed system decrease sharply when surfactant concentration is very low; indicating the formation of hydrophobic environment in the solution, and this concentration is defined as critical aggregation concentration (CAC). Owing to the electrostatic attraction between the oppositely charged surfactant molecules and DNA, the effective concentration of surfactant around DNA is higher than that in bulk phase, so the CAC is much lower than the CMC for the DNA-free solutions. In the case of different DNA concentrations, The CAC value

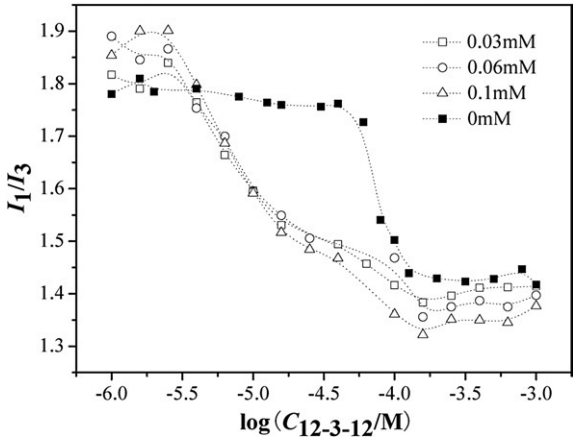

Fig. 1. Dependence of I_1/I_3 on 12-3-12 concentration with different DNA concentrations.

keeps almost constant at about 2.5×10^{-6} M, which suggests that DNA behaves like a separate phase when contact with surfactant.

3.2. Surface tension

Surface tension measurements provide a simple and informative method of studying mixtures of two components, of which one is highly active and the other relatively inactive at air/water interface. The surface tension of mixed system of DNA and gemini surfactant 12-3-12 are shown in Fig. 2. The DNA concentrations are kept constant (0.03 mM, 0.06 mM and 0.1 mM, respectively), while the surfactant concentration is varied by several order of magnitudes. The pure DNA solution exhibits no surface activity, i.e. its surface tension is nearly equal to that of water. It is seen that, in all cases, the surface tensions of the mixed solutions are higher than that of the pure surfactant solution, and it is different from the so-called synergistic lowering of the surface tension in the references [1,2]. Indeed, the value of surface tension depends on the interplay between the self-assembly of surfactant at the interface and the binding process of surfactant with DNA in the solution and at the interface. The cause of the higher surface tension for mixed solution is probably due to the fact that the added DNA draws the surfactant molecules from surface to bulk phase and form surface-inactive complexes.

The surface tension plots of DNA/12-3-12 systems display two turning points before which the surface tension changes quite sharply, designated as C_1 and C_2. C_1 is the low-concentration end of the first plateau and is typically referred as the critical aggregation concentration (CAC) for the system, which corresponds to micellization of the surfactants on to DNA chains in bulk phase. The values of C_1 are almost independent on DNA concentration, and accounting for the experiment error, this result is in good agreement with the result of the micropolarity measurement. Increasing 12-3-12 concentration, the surfactants continue binding onto DNA and the surface tensions keep constant until the sites on DNA are saturated. Further adding surfactant to the solution can therefore lower the surface tension and the curves eventually coincide with that of the DNA-free surfactant system at C_2, where the micelle in bulk phase is formed. C_2 can be defined as the critical micelle concentration of the mixed solution (CMC_{mix}), and its value increases with the increasing of DNA concentration because much more 12-3-12 molecules are needed to saturate the sites on DNA.

3.3. Cationic surfactants-DNA phase behavior

Taking the application of the complexes of DNA and surfactant in gene delivery and other aspects into account, we found it is important to study the phase behavior of the two components. Fig. 3 shows the phase behavior obtained by visual observation for DNA and 12-3-12 mixed system. The electrostatic interactions between the components are obviously strong and lead to an intense association. Surfactants aggregate around DNA, which reduces the charges of the complex and the intermolecular repulsions, and then the cloud-like precipitate would be visually observed. When surfactant concentration is in excess, the redissolving of the precipitate may occur like some other reported polyelectrolyte-surfactant systems, giving rise to the concept that a second layer of bound surfactant ions with cationic groups pointing "outwards" was attached to the first layer through association of the hydrocarbon chains [1]. The excess positive charge conferred by the second layer transformed the complex into a cationic aggregation, which can be validated by the zeta potential data of this system [30].

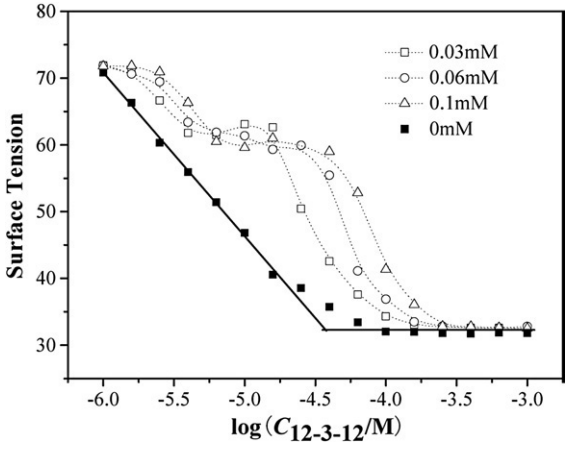

Fig. 2. Dependence of surface tension on 12-3-12 concentration with different DNA concentrations.

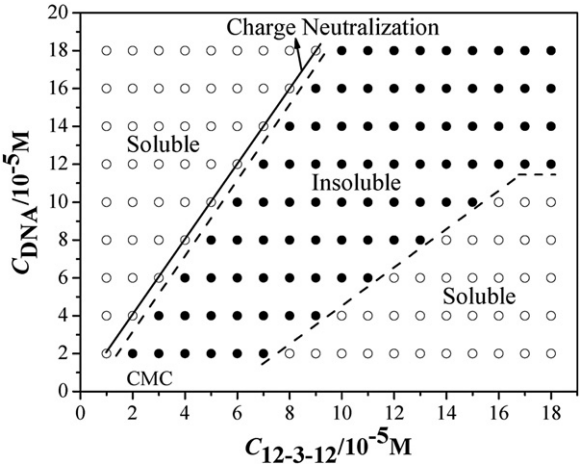

Fig. 3. Phase behavior of DNA-12-3-12 system.

An interesting point is that the precipitate region all lies beyond the neutralization line, i.e. an excess amount of surfactant is needed to induce the phase separation. It is good evidence that the electrostatic interaction plays an important role in the formation of complex before phase separation. However, the existence of NaBr in the solution screens the electrostatic interactions between DNA and 12-3-12 and stabilize the DNA/12-3-12 complexes, so the precipitation of the system delays. As for the redissolving of the precipitation, the hydrophobic interaction is the main driving force, so the higher DNA concentration, the much more surfactants are needed.

3.4. EB exclusion

EB is a cationic dye widely used as a probe for native DNA [14,31,32]. When the ethidium ion intercalates into DNA molecules, a red-shift of λ_{max} in UV–vis spectrum and the enhancement of its fluorescence intensity can be detected [31]. The spectral change of EB upon its binding to DNA can be utilized to study the interaction of DNA with other positively charged species, such as small cationic ions, polypeptides polyamines and surfactants, which may competitively displace the dye from DNA to solution.

Fig. 4A shows the fluorescence change of DNA/EB/12-3-12 systems with different concentrations of DNA corresponding to 0.03 mM, 0.06 mM and 0.1 mM respectively. Upon adding 12-3-12 to the DNA/EB solution, initially only a few 12-3-12 molecules bind with DNA and most EB molecules stay closely with DNA, so the fluorescence decay is negligible. Afterwards, the cooperative binding of 12-3-12 to DNA occurs due to the electrostatic and hydrophobic interactions, leading to a rapid quenching in intensity. It can be seen from Fig. 4A that the curves are shifted to higher surfactant concentrations when DNA concentration is increased. This can be easily explained by the fact that

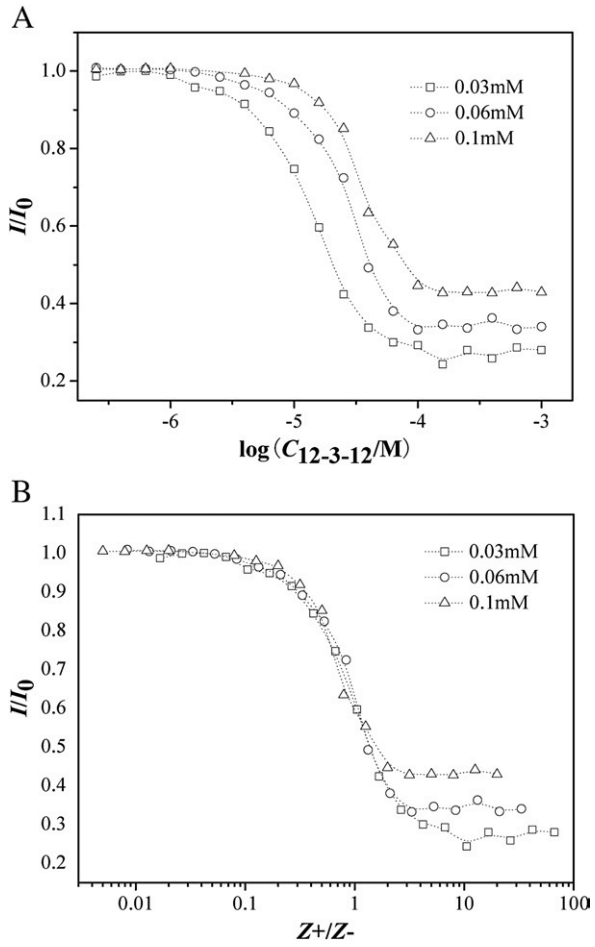

Fig. 4. A. Dependence of relative fluorescence intensity I/I_0 of DNA/EB on 12-3-12 concentration; B. Dependence of relative fluorescence intensity I/I_0 of DNA/EB on cationic to negative charge ratio Z+/Z−.

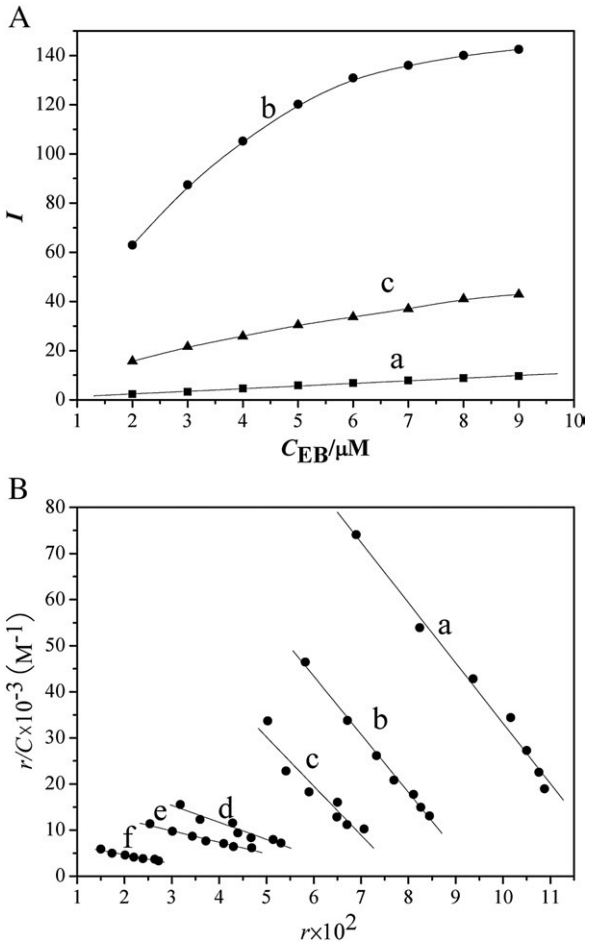

Fig. 5. A. Dependence of the fluorescence intensity of EB on its concentration in 10 mM NaBr solution (a); in the presence of 0.03 mM DNA (b); in the presence of both 0.03 mM DNA and 100 μM 12-3-12 (c); B. Fluorescence Scatchard plots for the binding of EB to DNA in the presence of 0, 8, 12, 16, 20, 100 μM 12-3-12 respectively (from a to f).

when the number of DNA chains increases in solution, more DNA/EB complexes are formed and more surfactant molecules are needed to exclude EB from DNA. Fig. 4B represents the same values of the fluorescence change as a function of the charge ratio of surfactant to DNA, instead of the surfactant concentration shown in Fig. 4A. More interestingly, all the data points before the second plateau overlap to one single curve, which indicates that the relative fluorescence intensity is independent of the DNA concentration but the charge ratio. This result is different from the interaction between DNA and conventional surfactant dodecyltrimethylammonium bromide (DTAB) [14]. Izumrudov et al. found that the transition regions of the fluorescence intensity are not determined by the charge ratio but by the surfactant concentration, which indicates the onset of polyanion-induced surfactant aggregation. In the present work, the gemini surfactant 12-3-12 has the same hydrocarbon chains with DTAB, but the two cationic headgroups are linked by a spacer, so the electronic attraction between DNA and 12-3-12 is much stronger than DTAB. For DTAB, the fluorescence does not decay until the charge ratio >10, where the hydrophobic interaction is the main motivity. While, for 12-3-12, the distinct fluorescence quenching occurs as charge ratio <1, which demonstrates the electronic attraction plays the important role, so the result is dependent on the charge ratio but not the surfactant concentration.

The effect of 12-3-12 on the fluorescence intensity of EB in the presence of DNA has also been followed by adding increasing amounts of EB to a preformed DNA-12-3-12 complex containing 0.03 mM DNA and 0–100 μM 12-3-12. As shown in Fig. 5A, the fluorescence in **c** (with 100 μM 12-3-12) is much lower than that without 12-3-12 (**b**) but higher than EB alone (**a**), indicating that a small quantity of EB can still bind to DNA in the presence of a large amount of 12-3-12, but the fluorescence quantum yield is much lower than that in the presence of DNA itself.

The Scatchard analysis [33,34] is a method to investigate the equilibrium between free and bound molecules when small molecules bind independently to a set of equivalent sites on a polymer. The binding constant (K_{EB}) and the number of EB molecules intercalated to DNA (n) in the presence of 12-3-12 were calculated using Eq. (1). The fluorescence Scatchard plots were obtained for binding of EB to DNA in the presence of various 12-3-12 concentrations.

$$\frac{r_{EB}}{C_{EB}} = K_{EB}n - K_{EB}r_{EB} \quad (1)$$

Where, in the present case, r_{EB} is the ratio of bound EB per nucleic acid phosphate; C_{EB} is the concentration of free EB; n is the number of

Table 1
Binding parameters of EB to DNA in the presence of 12-3-12 (C_{DNA}=0.03 mM)

$C_S \times 10^5$(M)	Z+/Z−	$K_{EB} \times 10^{-5}$ (M^{-1})	n
0	0	13.08	0.130
0.8	0.53	12.59	0.094
1.2	0.80	10.65	0.078
1.6	1.07	3.69	0.072
2.0	1.33	2.47	0.070
10.0	6.67	1.91	0.044

binding sites per nucleic acid phosphate; K_{EB} is the intrinsic binding constant to a site. Using fluorescence to determine r_{EB}, the corresponding Scatchard plots were constructed in Fig. 5B and the values of K_{EB} and n are listed in Table 1. As shown in Fig. 5B and Table 1, with the addition of 12-3-12, both the slop i.e. K_{EB} and the intercept on the abscissa i.e. n decrease, indicating noncompetitive inhibition of EB binding in the presence of 12-3-12. Actually, before the charge ratio = 0.5, the surfactant decreases the number of EB virtual sites on DNA, but the binding constant K_{EB} changes little, which indicate that the12-3-12 molecules bind on DNA through electrostatic interaction at lower charge ratio and no cooperative binding occurs. At the ratio between 0.5 and 1.0, the value of K_{EB} has a sharp decrease and the number of binding sites n decreases further, due to the cooperative binding of 12-3-12 to DNA by the hydrophobic interaction and the disassembly of DNA/EB complex. Further increase of 12-3-12 shows less influence on both the K_{EB} and n, meaning that there is still some binding which is rather independent of changes in surfactant concentration.

3.5. CD measurements

To determine the conformational change of DNA upon binding with cationic surfactant, CD experiments were performed. In the absence of 12-3-12 surfactant, CD spectrum exhibits a positive band near 277 nm and a negative band near 245 nm as shown in Fig. 6, indicating a typical B-form of DNA. After adding 12-3-12, the spectrum shifts to a longer wavelength. With the increase of 12-3-12, the negative band is enhanced gradually and the positive band becomes flat with the appearance of a long "tail". These chirooptical features, known as ψ-anomalies, have been ascribed to the supramolecular chiral order of a cholestric-like phase, or called ψ-phase. In such a phase, DNA molecules are supposed to be tightly packed together to form highly condensed structures that exhibit negative CD signals because of a left-handed tertiary conformation [35–37].

This result can be connected with the work by Sakai's group who used the Cryo-TEM to determine the aggregated form of DNA with cetyltrimethylammonium bromide (CTAB) and concluded that the DNA morphology change from loosely packed spherical to rodlike via toroidal structure by the addition of CTAB [17]. DNA molecules take on a spaciously expanded random coil form in good solvents or at high temperatures because of the strong electrostatic repulsion between the negatively charged phosphate groups, whereas they can be in a spherical, toroidal, rodlike, and highly folded solid-like form in poor solvents or at low temperatures because of the suppressed electrostatic repulsion. Upon adding 12-3-12, the DNA/12-3-12 complexes are formed due to the electrostatic interaction, thereby reducing the surface charge, so the DNA molecules will take a tight conformation, as deduced by the CD spectrum.

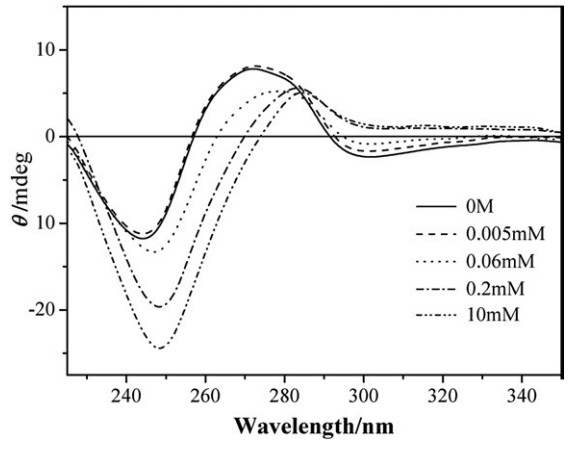

Fig. 6. CD spectra of 0.1 mM DNA in the presence of 0, 0.005, 0.06, 0.2 and 10 mM 12-3-12 respectively.

4. Conclusion

Gemini surfactant 12-3-12 can interact with DNA through electrostatic and hydrophobic forces at low surfactant concentration. The surface tensions of DNA/12-3-12 mixed system are higher than that of the pure surfactant, which is different from the so-called synergistic lowering of the surfactant tension for other polymer-surfactant systems. The cause of the surface tension increase is probably due to the fact that the added DNA removes surfactant molecules from surface to bulk phase and form surface-inactive complexes. The CAC value is independent of the concentration of DNA, but the CMC_{mix} increase with the increasing of DNA concentration. The study on the phase behavior shows that the electrostatic interaction plays an important role in the formation of complex before phase separation, and the redissolving of the precipitation depends on the hydrophobic interaction. The process of EB exclusion from DNA by 12-3-12 depends on the charge ratio of surfactant to DNA and the binding constant of EB to DNA decreases sharply at the ratio from 0.5 to 1.0. With increasing of 12-3-12, the conformation of DNA can transit from native B-form to chiral ψ-phase, in which the DNA molecules are tightly packed together to form highly condensed structure.

Acknowledgments

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