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Determination of nucleic acid based on increased resonance light-scattering of fluorinated surfactants

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ABSTRACT

Two novel surfactants perfluoroalkanesulfonyl quaternary ammonium iodides (FC134) and potassium perfluorooctanesulfonate (FC95) were successfully used as new probes for detection of DNA by resonance light-scattering (RLS) technique. Resonance light-scattering characteristics of the binding of fluorinated surfactants FC134 and FC95 to calf thymus nucleic acid (ctDNA) were studied. After DNA was added, aggregation of FC134 on the molecular surface of DNA in the pH 3.0–6.0 and aggregation of FC95 on the surface of DNA in the pH 3.5–6.0 occurred, both of which resulted in an enhanced resonance light-scattering peak at 370 nm. The intensity of resonance light-scattering was found to be proportional to the concentration of DNA. The determination limits were 3.5 and 20.0 $\mu g\,L^{-1}$, respectively. UV–vis spectra and IR-spectra both proved the binding of fluorinated surfactants to DNA.

1. Introduction

The nature and dynamics of binding small molecules to biopolymers represents an area of active investigation [1]. DNA is an interesting anionicpolyelectrolyte and an important biological material with a unique double helical rodlike structure. There has been considerable interest in the design of molecules probe that can recognize and react at DNA sites in a sequence-defined fashion [2,3] since the last century. The development of novel methods and new probe of DNA determination is very important in both clinical and laboratory tests [4,5].

Surfactant is a group of amphipathic substance composed of both hydrophilic and hydrophobic groups. Surfactants are widely used in both consumer and industrial applications: food processing, medicines, and pharmaceuticals. Recently, the interactions between DNA and cationic surfactants have attracted immense interest in the separation and purification of DNA [6]. In fluorinated surfactants, all or most hydrogens in the hydrophobic tail are replaced by fluorine atoms. Although, there is a great deal of work on the study of the interactions between DNA and hydrogenated surfactants [7–11], an important lack can be observed on characterization of the interactions between DNA and fluorinated surfactants. Furthermore, as cationic surfactants can induce DNA to cross the cell or nuclear membrane and sequentially interact with the target, the research on the interaction between cationic surfactant and DNA is very important. Therefore, perfluoroalkylsulfonyl quaternary ammonium iodides (FC134) were selected. In order to compare with the interaction between cationic fluorinated surfactant and DNA, anionic fluorinated surfactant potassium perfluorooctanesulfonate (FC95) was selected. FC134 and FC95 have the same fluorocarbon chain, so the comparing is meaningful.

On the other hand, the resonance light-scattering (RLS) technique has been developed as a sensitive instrumental analysis method in the determination of DNA [12,13] in the recent years. The resonance light-scattering technique was used to determine DNA by Pasternack [12,13] first. The RLS spectrum was obtained by scanning simultaneously the excitation and emission wavelengths through a monochromator of a common spectrofluorometer with $\Delta\lambda = 0$ nm. It was found in the RLS spectrum that when DNA was added, the intensity of RLS enhanced, and there was a linear relationship between the enhanced intensity and the concentration of nucleic acid. It was concluded that the resonance light-scattering technique was based on the long range assembly of probe molecules on the surface of nucleic acid, which resulted in enhanced RLS intensity.

Therefore, the aim of this work is first to give experimental results of the determination of DNA with fluorinated surfactant perfluoroalkylsulfonyl quaternary ammonium iodides (FC134) and potassium perfluorooctanesulfonate (FC95) by resonance light-scattering technique. It might also be helpful for the development of the application of fluorinated surfactants for biomedical purpose.

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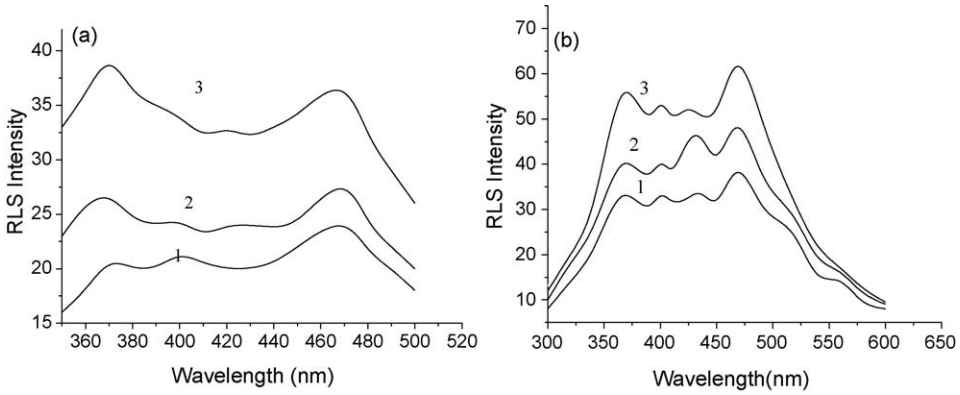


Fig. 1. Resonance light-scanning of fluorinated surfactant–DNA system. (a) FC134: 1.1×10^{-5} mol L^{-1}; DNA(1-3): 0, 0.4, 0.8 mg L^{-1}; pH 3.50. (b) FC95: 3.2×10^{-6} mol L^{-1}; DNA(1-3): 0, 1.44, 2.88 mg L^{-1}, pH 5.50.

2. Results and discussion

2.1. Spectral characteristics

Fig. 1 was obtained according to the standard procedure. Fig. 1(a) showed that FC134 had a weak RLS peak at 470 nm. When ctDNA was added, enhanced RLS peaks could be observed at 370 and 470 nm. Fig. 1(b) showed that FC95 had four weak RLS peaks at 370, 400, 445 and 470 nm, respectively. When ctDNA was added, enhanced RLS peaks could be observed at 370, 400, 445 and 470 nm. The phenomena mainly resulted from the long range assembly of FC134 and FC95 on the molecular surface of nucleic acid [14]. The extent to which a particle absorbs and scatters light depends on its size, shape, and index of refraction relative to the surrounding medium, and scattering. Scattering in each sphere is proportional to the square of the volume.

According to the following formula of RLS [15,16]:

$$I_{\mathrm{RLS}} = \frac{32\pi^3 V^2 n^2 N}{3\lambda_0^4}[(\delta_n)^2 + (\delta_k)^2]$$

where n is the refractive index of the medium, N is the molarity of the solution, λ_0 is the wavelength of the incident and scattered light, V is the square of molecular volume, δ_n and δ_k are the fluctuations in the real and imaginary components of the refractive index of the particle, respectively. When other factors are constant, I_{RLS} is related to the size of the formed particle and directly proportional to the square of molecular volume. Therefore, with the increase of molecular volume of the ion-association, I is enhanced obviously.

Therefore, the amount of scattering is directly proportional to the volume of each sphere. Thus, the larger the aggregation, the greater the scattering. FC134 is a positive ion in solution, according to literature [14,17,18], as a positively charged molecule, it has a condensing effect on nucleic acids. When the molar ratio of the FC134 to nucleic acid is rather high at a lower ionic strength, the FC134 molecule assembles and aggregates on the molecular surface of nucleic acid. This leads to long range assembly, which likely induces the formation of suprahelical structures of nucleic acids. FC95 is negative ion in solution, as the strong hydrophobic nature, it can still bind to DNA. Hydrophobic force can make FC95 molecule assemble and aggregate on the molecular surface of nucleic acid and lead to long range assembly. When incident light shines on the suprahelical structures of nucleic acids, resonance occurs. Therefore, since the aggregation of FC134 and FC95 on the molecular surfaces of nucleic acids produces large particles in size, strong enhanced resonance light-scattering can be observed.

2.2. Effect of pH

Using Tris–HCl (0.01 mol L^{-1}) solution to control the acidity according to the procedure, the intensity of RLS in different acidity was determined. Fig. 2(a and b) showed that the RLS intensity of FC134 and FC95 changed little with different acidities. However, the RLS intensity enhanced much when DNA was added. The conformation of DNA changed with acidity of solution, which resulted in the change in RLS intensity. The RLS intensity of FC134–DNA system was weak and not very stable at pH < 3.0 or pH > 7.0, but was stable in the pH range of 3.0–6.0, seen in Fig. 2(a). The maximum enhancement existed in the pH range of 3.5–4.8. Therefore, pH 3.5 was selected. The RLS intensity of FC95–DNA

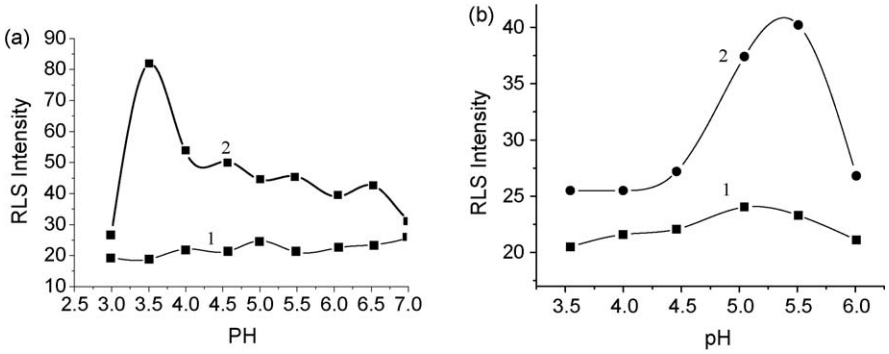


Fig. 2. Effect of pH on the RLS intensity of fluorinated surfactant–DNA system. (a) FC134: 1.143×10^{-5} mol L^{-1}; DNA(1-2): 0, 0.80 mg L^{-1}. (b) FC95: 3.2×10^{-6} mol L^{-1}; DNA(1-2): 0, 0.72 mg L^{-1}.

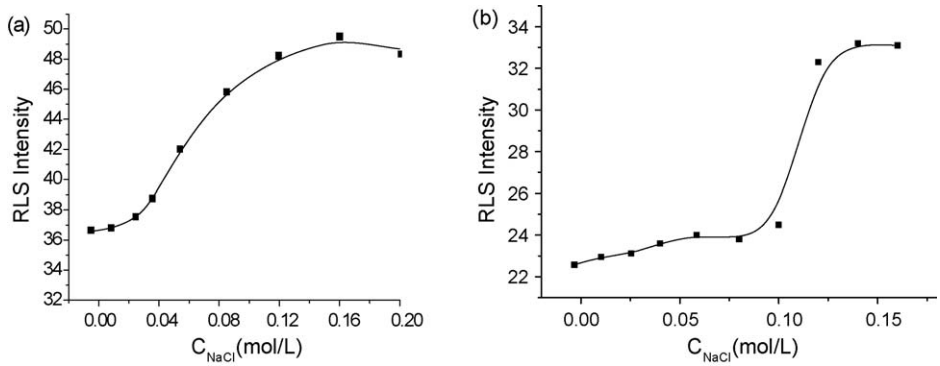


Fig. 3. Effect of NaCl on the RLS intensity of fluorinated surfactant–DNA system. (a) FC134: 1.143×10^{-5} mol L^{-1}; DNA: 0.8 mg L^{-1}; pH 3.5. (b) FC95: 3.2×10^{-6} mol L^{-1}; DNA: 0.72 mg L^{-1}; pH 5.5.

system was weak and not very stable at pH < 3.5 or pH > 6.0, but was stable in the pH range of 4.5–6.0, seen in Fig. 2(b). The maximum enhancement existed in the pH range of 5.0–5.5. Therefore, pH 5.5 was selected.

2.3. Effect of ionic strength

1.0 mol L^{-1} NaCl solution was used to adjust the ionic strength of the system. It could be seen from Fig. 3(a) that when ionic strength was lower than 0.04 mol L^{-1}, the system was scarcely affected. Fig. 3(b) showed that when ionic strength was lower than 0.10 mol L^{-1}, the system was scarcely affected. But with the increase of ionic strength, the RLS intensity enhanced.

Suppose fluorinated surfactant binds to phosphate backbone of DNA by electrostatic binding mode, then with increasing concentration of NaCl, Na^+ will be adsorbed on the phosphate backbone of DNA by electrostatic attraction, which certainly affected the interaction between fluorinated surfactant and DNA, and the RLS intensity decreased. If fluorinated surfactant binds to DNA base pairs by interaction binding mode, Na^+ will not take effect on the binding, but it will still be adsorbed on the phosphate backbone of DNA. With the increasing NaCl concentration, Na^+ make the particle size bigger, so the RLS intensity will increase.

FC134 is cationic surfactant, and it can bind to DNA through electrostatic force. Besides, it is a fluorinated surfactant, which has strong hydrophobic nature, so it can bind to DNA through hydrophobic force. The RLS intensity increasing proved the binding mode was not electrostatic binding mode but interaction binding mode, which exposed that hydrophobic force played a major role in the binding. FC134 bound to DNA base pairs by interaction binding mode mainly induced by hydrophobic force, associated with electrostatic force. FC95 only can bind to DNA through hydrophobic force so it bound to DNA base pairs by interaction binding mode induced by hydrophobic force.

Table 1
Effect of concentration of FC134 and FC95 on the linear relationship.

Fluorinated surfactant	Concentration (mol L^{-1})	Linear range of DNA (mg L^{-1})	Linear regression equation	Correlation coefficient
FC134	2.2×10^{-6}	0.2–0.8	$I = 6.75C + 20.133$	0.9563
	4.4×10^{-6}	0.08–0.8	$I = 19.63C + 24.017$	0.9756
	6.6×10^{-6}	0.034–1.0	$I = 41.83C + 20.819$	0.9993
	8.8×10^{-6}	0.038–1.0	$I = 40.16C + 22.457$	0.9983
FC95	3.0×10^{-6}	0.63–9.0	$I = 2.61C + 19.02$	0.9985
	4.0×10^{-6}	0.54–9.0	$I = 3.02C + 18.95$	0.9865
	5.0×10^{-6}	0.45–9.0	$I = 4.41C + 15.667$	0.9993
	6.0×10^{-6}	3.6–9.0	$I = 3.40C + 13.88$	0.9913

2.4. Effect of concentration of FC134 and FC95

The experiment showed that the enhanced intensity of RLS took on an excellent linear relationship when the concentration of DNA was lower than 1.0 mg L^{-1} and concentration of FC134 was in the range of 2.2×10^{-6} to 1.1×10^{-5} mol L^{-1} for FC134–DNA system; when the concentration of DNA was lower than 9.0 mg L^{-1} and concentration of PC95 was in the range of 3.0×10^{-6} to 6.0×10^{-6} mol L^{-1} for FC95–DNA system. The effect of different concentrations of FC134 and FC95 on the linear relationship in the range was displayed in Table 1. It is clear that when the concentration of FC134 was 6.6×10^{-6} mol L^{-1}, the linear range and the correlation coefficient of the similar linear regression equation were both the best. When the concentration of FC95 was

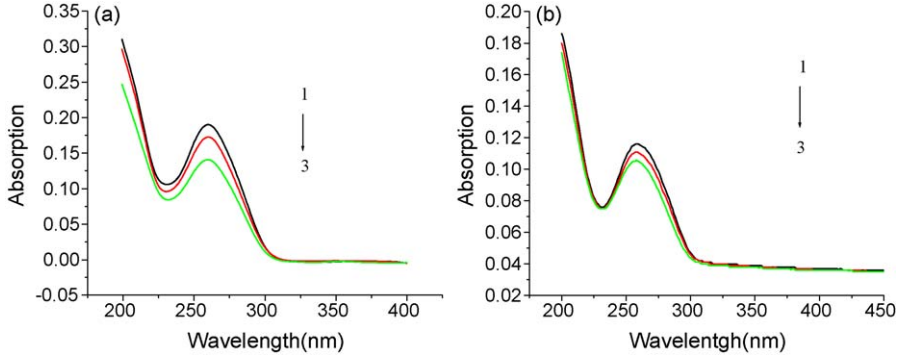


Fig. 4. UV–vis spectra of fluorinated surfactants–DNA complex. (a) DNA: 7.2 mg L^{-1}; FC134(1-3): 0, 1.1×10^{-5}, 2.2×10^{-5} mol L^{-1}; (b) DNA: 4.5 mg L^{-1}; FC95(1-3): 0, 1×10^{-5}, 1.8×10^{-5} mol L^{-1}.

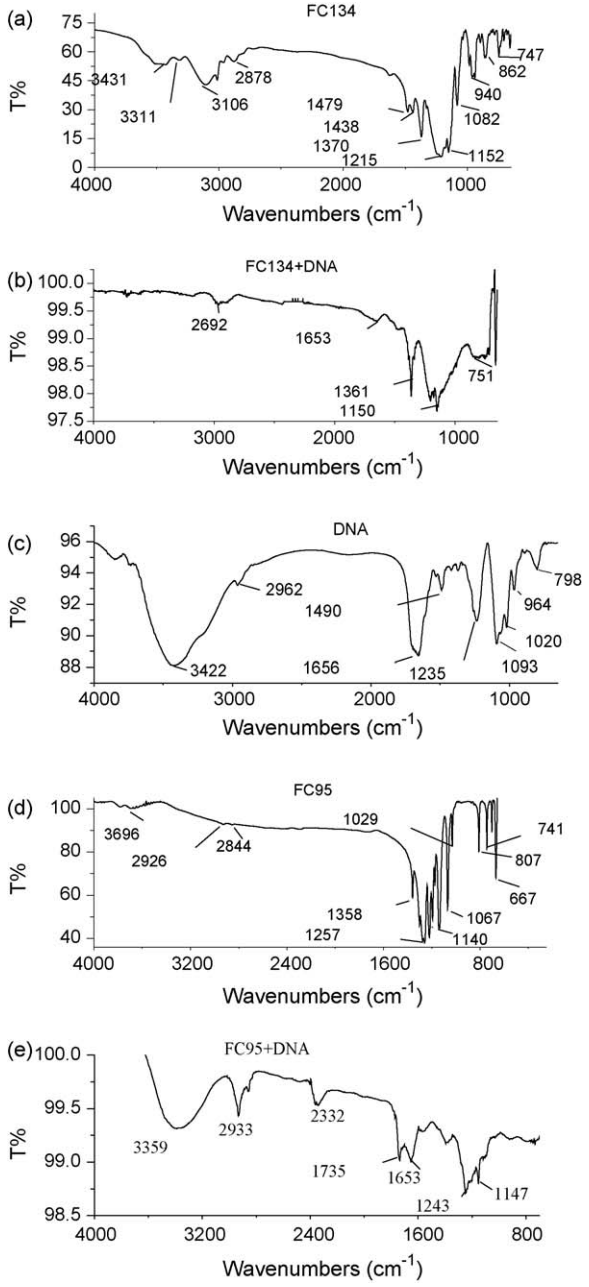


Fig. 5. The FT-IR spectra of fluorinated surfactants–DNA complex. (a) FC134 (b) FC134–DNA (c) DNA (d) FC95 (e) FC95–DNA.

5.0×10^{-6} mol L^{-1}, the linear range and the correlation coefficient of the similar linear regression equation were both the best.

2.5. Calibration curve

The calibration curve was obtained according to the above standard procedure. There was linear relationship between the RLS intensity and the concentration of DNA when the concentration of DNA was in the range of 0.068–1.0 mg L^{-1} using FC134. The linear regression equation is

$$I = 20.819 + 41.83C\,(\mathrm{DNA}); \quad r = 0.9993$$

The limit of determination (3σ) was 3.5 μg L^{-1}.

There was linear relationship when the concentration of DNA was in the range of 0.45–9.0 mg L^{-1} using FC95. The linear regression equation is

$$I = 4.41C\,(\mathrm{DNA}) + 15.667; \quad r = 0.9993$$

The limit of determination (3σ) was 20.0 μg L^{-1}.

2.6. UV–vis spectra

Fig. 4 showed that the light absorption of a DNA system decreased at a wavelength of 260 nm when fluorinated surfactants was added. With the addition of FC134, DNA generated obvious hypochromic effect, seen in Fig. 4(a), and with the addition of FC95, DNA also generated obvious hypochromic effect, seen in Fig. 4(b). According to the literature [19], this may be explained by the fact that fluorinated surfactants aggregated on the surface of the nucleic acid, therefore, the concentration of monomer decreased because of the aggregation, which resulted in hypochromism. This is a convincing proof of fluorinated surfactants binding to DNA.

2.7. IR-spectra

Fig. 5 demonstrated the FT-IR spectra of DNA, FC134, FC95, FC134–DNA complex and FC95–DNA complex. The absorbance band at 1656 cm^{-1} of DNA shifted to 1653 cm^{-1} in FC134–DNA and FC95–DNA complex, confirming the interaction between FC134 and DNA, FC95 and DNA had happened, seen in Fig. 5(b, c and e). The disappearance of the absorption band at 1020, 1093, 1235,1490 cm^{-1} in the FC134–DNA and FC95–DNA complex was an evidence that the interaction had altered the conformation of DNA [20].

As for FC134, the absorbance band at 1370 cm^{-1} shifted to 1361 cm^{-1} in FC134–DNA complex, and the disappearance of the absorption band at 862, 940, 1082, 2878, 3106, 3311,3431 cm^{-1} in the FC134–DNA complex proving the binding of FC134 to DNA. As for FC95, the absorbance band at 1257 cm^{-1} shifted to 1243 cm^{-1}

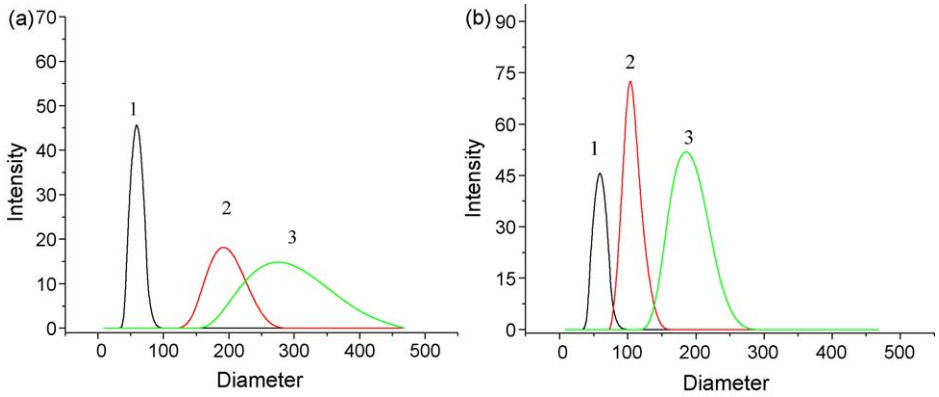


Fig. 6. Particle size distribution for fixed DNA concentration and surfactants. (a) DNA: 2.7 mg L^{-1}; FC134(1-3): 0, 3.3×10^{-5}, 6.6×10^{-5} mol L^{-1}; (b) DNA: 2.7 mg L^{-1}; FC95(1-3): 0, 3.0×10^{-5}, 9.0×10^{-5} mol L^{-1}.

in FC95–DNA complex, FC95 and the disappearance of the absorption band at 807, 1067 cm^{-1} in the FC95–DNA complex proving the binding of FC95 to DNA.

2.8. Particle size

Fig. 6 showed the alteration of particle size of DNA with different surfactants. The results revealed that the particle diameter increased when surfactant was added. The phenomena can be explained that the surfactant ions bind to DNA, inducing surfactant aggregation, which made DNA change the conformation and made particle size bigger, so the RLS intensity enhanced.

3. Conclusion

The resonance light-scattering technique is useful and sensitive for the determination of trace substances. In this study, due to the hydrophobic force and the interaction between fluorinated surfactants (FC134 and FC95) and DNA, the large particles were produced. UV–vis spectra, IR-spectra and particle size proved FC134 and FC95 binding to DNA. The large particles resulted in the enhancement of the RLS intensity which was proportional to the concentration of the DNA in a certain range [21,22]. Based on the linear relationship, the method to determine the trace of DNA was established. The proposed method proved to be rapid, sensitive and selective; the experiment procedure was very simple and the operation was just performed on a common spectrofluorometer.

Compared with the common fluorimetric method in a spectrofluorometer, the limit of detection was 10 $\mu g\ L^{-1}$ using ethidium bromide [23], and 0.5 $\mu g\ L^{-1}$ using ToTo or YoYo [24]. The limit of detection was 3.5 $\mu g\ L^{-1}$ using FC134 and 20 $\mu g\ L^{-1}$ using FC95. It was clear that the technique using FC134 is rather sensible.

Compared with other substances analyzed by the same light-scattering technique reported, the detection limit was 65.0 $\mu g\ L^{-1}$ using brilliant green [25], 10.5 $\mu g\ L^{-1}$ using phenosafranine [26] and 10.0 $\mu g\ L^{-1}$ using mixed complex La(bpy)(phen)Cl_3 [27]. It could be concluded that using FC134 or FC95 is applicable to the determination of DNA, and FC134 is more sensitive. The reason of that is probably FC134 is cation in solution, it can bind to DNA through electrostatic force beside hydrophobic force, so it can bind to DNA stronger than FC95. Therefore, using FC134 in resonance light-scattering technique may have wide applications in the quantification of nucleic acids.

4. Experimental

4.1. Reagents and chemicals

All reagents were of analytical-reagent grade, made in China. The working solutions of Fluoro Surfactant FC134 and FC95 were 1.1×10^{-3} and 1.0×10^{-3} mol L^{-1} respectively, and the molecular structure of FC134 and FC95 molecules were shown in Fig. 7.

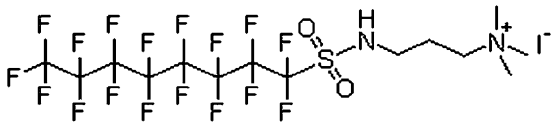
The stock solution of DNA was prepared by dissolving commercially purchased DNA (Sino-American biotechnology company, China,) in doubly distilled water at 0–4 °C. The working solution of the DNA was 90 mg L^{-1}. Doubly distilled water was used throughout.

0.01 mol L^{-1} Tris–HCl solution was used to control the acidity, and 0.1 mol L^{-1} NaCl was used to adjust the ionic strength of the aqueous solutions.

Doubly distilled water was used throughout.

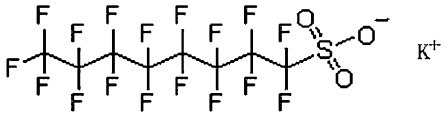
4.2. Apparatus

The resonance light-scattering spectrum and the intensity of resonance light-scattering were measured with a Shimadzu RF-540 spectrofluorometer (Kyoto, Japan). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution, and a PHB-4 pH meter was used to measure the pH value of the solution. The FT-IR spectra of FC134–DNA complex and FC95–DNA were obtained on PerkinElmer Spectrum One after the complex was dried at 25 °C under vacuum, the solid FC134, FC95 and DNA were used directly to obtain FT-IR spectra. High performance particle sizer (Malvvern, America) was used to measure particle size.



Perfluoroalkylsulfonyl quaternary ammonium iodides

(FC134)



Potassium perfluorooctanesulfonate

(FC95)

Fig. 7. Structure of perfluoroalkylsulfonyl quaternary ammonium iodides (FC134).

4.3. Standard procedure

Appropriate working DNA and fluorinated surfactant solution were added to a 25 mL volumetric flask. The mixture was diluted to 10 mL with doubly distilled water and vortexed. Five minutes later, all the absorption and RLS measurements were obtained against the blank treated in the same way without DNA.

The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromator of the RF-540 spectrofluorometer through the wavelength range 300–600 nm with $\Delta\lambda = 0$ nm. The RLS intensity was measured at the maximum wavelength 370 nm.

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