Articles

Langmuir Aggregation of Fluorescein Isothiocyanate (FITC) on Cetyl Trimethylammonium Bromide (CTAB) and Application to Determination of Anionic Detergent in Sewage

Hong-Wen Gao* and De-Yan Zhou†

School of Chemistry and Chemical Engineering, Anhui University, Hefei-230039, P.R. China
†School of Anhui Agricultural Mechanization, Hefei-230031, P.R. China
Received May 21, 2001

The microphase adsorption - spectral correction (MPASC) technique was described and applied to study the interaction of fluorescein isothiocyanate (FITC) with cetyl trimethylammonium bromide (CTAB). The synergism mechanism of micelle was analyzed and discussed. The aggregation of FITC on CTAB obeys the Langmuir monolayer adsorption. Results showed that the monomer and micellar aggregate is FITC-$CTAB_3$ and $(FITC\text{-}CTAB_3)_{29}$, respectively at pH 9.62. The adsorption constant of the CTAB-FITC aggregate was determined to be 2.10×10^5. Interestingly, it was observed for the addition of an anionic surfactant to desorb FITC from its CTAB aggregate and this has been applied to the quantitative determination of trace amounts of anionic detergent (AD) in sewage.

Keywords : MPASC technique, Fluorescein isothiocyanate (FITC), Microelectrostatic attraction, Langmuir aggregation, Determination of anionic detergent (AD).

Introduction

A surfactant is often quite useful in present supersensitive determination of trace amounts of component due to its solubilization, stabilization, raising sensitivity and so on. Some earlier models were proposed to explain the synergism mechanism *e.g.* synergism perturbation,[1] hydrogen bond formation,[2] micelle catalysis,[3] rigid asymmetric micro-environment[4] and others.[5]

The interaction between a surfactant and a chromophore often occurs just like a precipitation-dye adsorption. A surfactant molecule has usually long chain and various aggregation forms in aqueous solution *e.g.* spherical, worm-like, tubules and lamellae.[6] Recently, the study of the surfactant molecular aggregation is still very active.[7-9] Understanding the aggregation of micelle and its assembly with small ions and organic stains is very significant to synthesize the new-type efficient detergent. In a surfactant (S) solution, the aggregation of S molecules will form an electrostatic global micelle (Fig. 1-left) when S is more than the critical micellar concentration (CMC). So, the electrostatic attraction of a ligand (L) with opposite charge occurs [10] until the kinetic equilibrium (Fig. 1-right). Because of the electrostatic attraction, the solubilization of L occurs in the S solution. Similarly, the same electrostatic adsorption of L on S monomer surface can occur when S is less than CMC (Fig. 1-(1)). The aggregation of L on S surface is in only a monolayer like biomacromolecule.[11] It obeys the Langmuir adsorption[12] and the following equilibrium occurs: L (aqueous phase, C_L) $\Longleftrightarrow$ SL_N (surfactant phase, C_S) in L-S solution. The Langmuir equation is used:

$$\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{KNC_L} \tag{1}$$

where K is the equilibrium constant and C_L the concentration of the excess L and γ is the molar ratio of L adsorbed to S. Within increase in L concentration, γ will approach a maximum, called the adsorption ratio **N**. The γ^{-1} *vs.* C_L^{-1} is linear and from this we may calculate N and K. Both C_L and γ are calculated by means of[13]:

$$\gamma = \eta \times \frac{C_{L0}}{C_S} \tag{2}$$

$$C_L = (1 - \eta) C_{L0} \tag{3}$$

where

$$\eta = \frac{A_C - \Delta A}{A_o} \tag{4}$$

where both C_S and C_{L0} are the concentration of the S and L added initially and η indicates the effective fraction of L. A_c, $\boldsymbol{A_0}$ and ΔA are the real absorbance of the S-L product, the measurement absorbance of the reagent blank against water and that of the S-L solution against reagent blank directly measured at the peak wavelength λ_2, respectively. The A_c is calculated by means of [14]:

*To whom correspondence should be addressed. Fax: +86-551-510-6110; e-mail: gaohongw@mail.hf.ah.cn

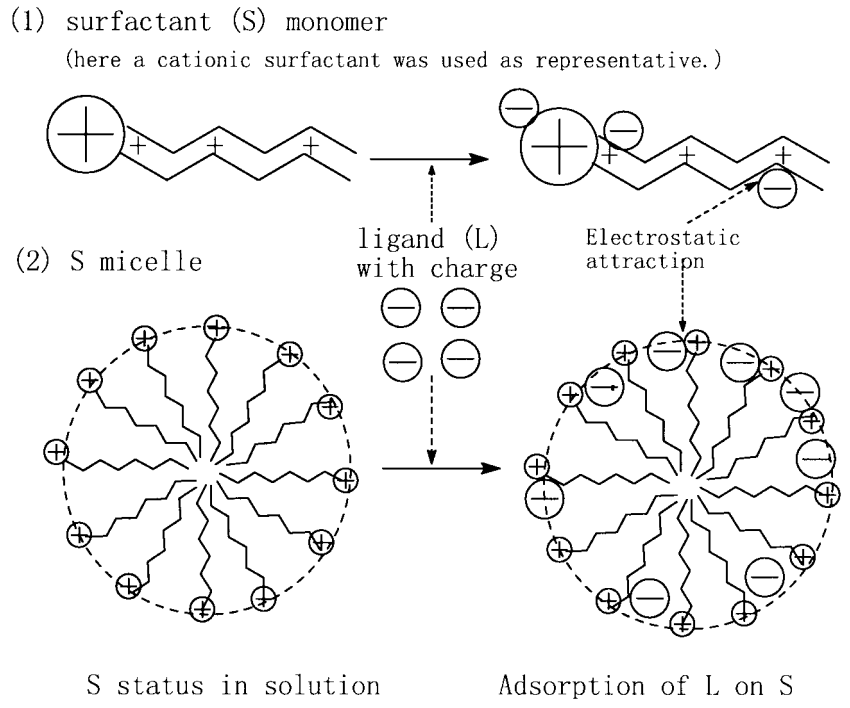


Figure 1. The aggregation of L on S monomer and micelle.

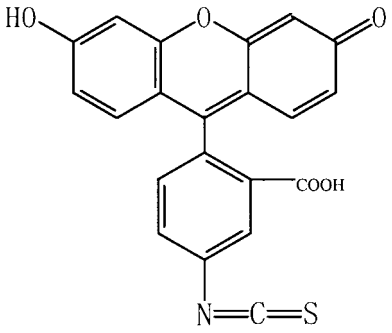
$$A_c = \frac{\Delta A - \beta \Delta A'}{1 - \alpha\beta} \quad (5)$$

where $\Delta A'$ indicates the absorbance of the S-L solution measured respectively at the valley absorption wavelength λ_1. In general, α and β are the correction constants and they are calculated by measuring directly $\mathbf{SL_N}$ and **L** solutions.[13-14] In addition, the molar absorptivity (real $\varepsilon_r^{\lambda 2}$ not apparent $\varepsilon_a^{\lambda 2}$) of the adsorption product $\mathbf{SL_N}$ at λ_2 is also directly calculated by the following equation:

$$\varepsilon_r^{\lambda_2} = \frac{NA_c}{\delta \gamma C_s} \quad (6)$$

where δ is the cell thickness (cm).

The cooperation of both the Langmuir adsorption and the spectral correction (MPASC) technique provides a very helpful experimental strategy for study of aggregation of chromophore on surfactant micelle. In the present article, we have studied the aggregation of fluorescein isothiocyanate



Fluorescein Isothiocyanate (FITC)

(FITC) on CTAB micelles and its application to sensitive determination of trace amounts of AD. The reagent was earlier used in the volumetric determination. The structure of the chromophore is given below:

The reagent was earlier synthesized as a labelling agent for proteins[15-17] and applied to identification of pathogents.[18] It forms anions at pH 9.62 and they can be adsorbed on CTAB molecular surface to form the CTAB-FITC aggregate. The aggregation of FITC on CTAB obeys the Langmuir monolayer adsorption. Results showed that the maximal adsorption ratio of CTAB to FITC is 3 : 1 at pH 9.62 and its adsorption constant $K_{CTAB\text{-}FITC} = 2.10 \times 10^5$. The analysis of the samples gave that the recovery of added SDS was between 92.5 and 110% with RSD 6.67%.

Experimental Section

Materials and instruments. Absorption spectra were recorded on a TU1901 Spectrophotometer (PGeneral, Beijing) with 1-cm cell. The conductivity meter, Model DDS-11A (Tianjin Sec. Anal. Instrument.) was used to measure conductivity together with Model DJS-1 conductivity immersion electrode (electrode constant 0.98, Shanghai Tienkuang Devices) in production of deionized water between 0.5 and 1 $(\mu\Omega cm)^{-1}$. pH of solution was measured on pHS-2C acidity meter (Leici Instruments, Shanghai) and Model 630D pH Pen (Shanghai Ren's Electronic). The temperature was adjusted and remained constant in electric heated thermostat bath, Model 116R (Changjiang Test Instruments, China).

Preparation of solutions. The standard stock solution of CTAB (10.00 mmol/L) was prepared by dissolving cetyl trimethylammonium bromide (CTAB) (Shanghai Chemical

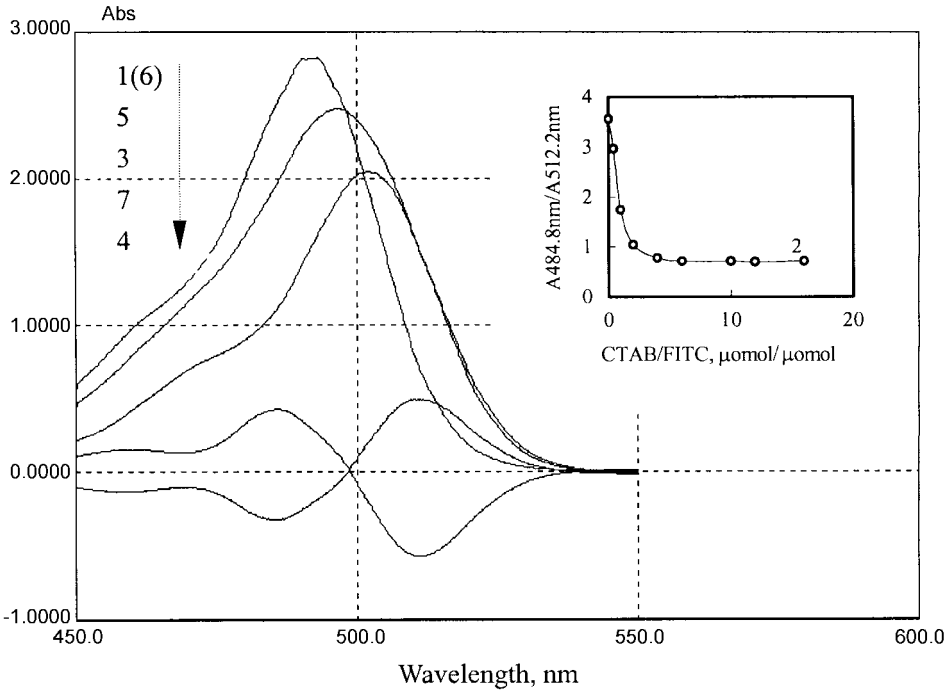


Figure 2. Absorption spectra of FITC, FITC-CTAB and SDS-FITC-CTAB solutions at pH 9.62: 1- FITC (1.00 μmol) solution against water; 2- Change of $A_{484.8nm}/A_{512.2nm}$ of the FITC (0.50 μmol)-CTAB (between 0 and 8.0 μmol) solutions against water; 3- FITC (0.40 μmol)-CTAB (2.00 μmol) solution no longer containing free FITC, against water; 4- FITC (0.40 μmol)-CTAB (0.50 μmol) solution against the FITC (0.40 μmol) solution; 5- FITC (0.40 μmol)-CTAB (0.80 μmol) solution against water, where the FITC-CTAB aggregate and free FITC both co-exist. 6- FITC (0.40 μmol)-CTAB (0.80 μmol)-SDS (1000 μg) solution no longer containing free FITC-CTAB aggregate, against water and 7- FITC (0.40 μmol)-CTAB (0.80 μmol)-SDS (200 μg) solution against the FITC (0.40 μmol)-CTAB (0.80 μmol) solution.

Reagents Centre) in deionized water at 40 °C and then 1.00 mmol/L CTAB was prepared daily by diluting the stock solution. FITC solution (1.000 mmol/L) was prepared by dissolving 0.2170 g of fluorescein isothiocyanate (FITC, content 90%, Shanghai Zhengxiang Chemical Reagents Institute) in 500 mL of acetone. Britton-Robinson buffer solutions (pH 5.72-13) were used to control the acidity of solution. 2 M NaCl was used to adjust the ionic strength of the aqueous solutions. Na2-EDTA solution (5%) was prepared to mask the foreign metallic ions co-existed possibly in the practical samples. The CTAB-FITC mixture solution was prepared for the determination of AD in water by mixing 50.0 mL of 1.00 mmol/L FITC, 10.0 mL of 10.0 mmo/L CTAB and 10 mL of pH 9.62 buffer solution. All reagents were of analytical grade and used without further purification.

Methods. For the aggregation of FITC on CTAB, into a 10 mL calibrated flask were added an appropriate working solution of CTAB, 1 mL of buffer solution (pH 9.62) and 0.500 mL of 1.00 mmol/L FITC. The mixture was diluted to 10 ml with deionized water and mixed thoroughly. After 5 min, absorbances were measured at 484.8 and 512.2 nm, respectively against the blank treated in the same way without CTAB.

For the determination of AD, 5.0 mL of a sample was taken in a 10 mL flask. Added 1.0 mL of pH 9.62 buffer solution, 0.5 mL of EDTA solution and 0.8 mL of the CTAB-FITC mixture solution. Diluted to 10 mL and mixed well. After 5 min, measured absorbances at 512.2 and 484.8 nm, respectively against reagent blank (AD concentration is 0) and calculated the correction absorbance A_β of solution.

Results and Discussion

Spectral analysis. The absorption spectra of the CTAB-FITC solutions are shown in Figure 2. Curve 1 indicates that the spectral peak of FITC solution is located at 493.2 nm. Curve 2 shows change of the absorbances ratios of the FITC solutions at 512.2 and 484.8 nm containing various CTAB concentrations. We see that the ratio reaches minimum and remains almost constant when CTAB is over 4 times FITC in molarity. Therefore, the solution containing 2.00 μmol of CTAB and 0.400 μmol of FITC will contain none of free FITC. Curve 3 gives its spectrum and its peak is located at 502.2 nm so the spectral peak red shift of the adsorption product is about 10 nm. However, from the relative spectral curve 4, its peak and valley are all located at 512.2 and 484.8 nm, respectively. Therefore, the two wavelengths were used in this work. From curves 1 and 3, the correction coefficients were calculated to be $\beta_{FITC} = 0.282$ and $\alpha_{CTAB\text{-}FITC} = 0.704$. So the formula $A_c = 1.25(\Delta A - 0.282\Delta A')$ was used for calculation of real absorbance of the CTAB-FITC aggregate.

Because curves 1 and 6 are coincidence, the anionic surfactant SDS may desorb FITC from its CTAB micellar aggregate. This is attributed that the mixture of the cationic and anionic surfactants will form the mixed micelles.[19] The electrostatic attraction of CTAB was strongly weakened

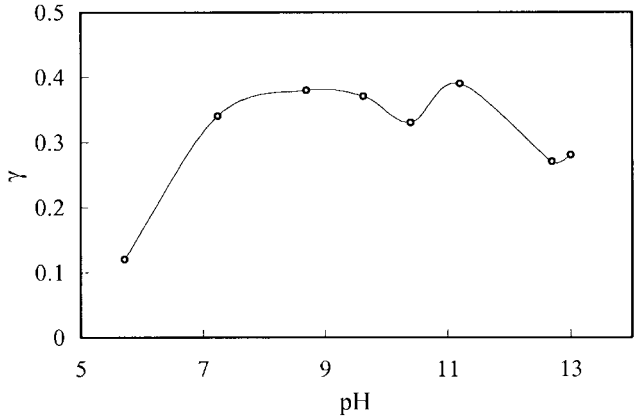


Figure 3. Effect of pH on the adsorption ratio (γ) of FITC to CTAB in solution initially containing 0.50 μmol of FITC and 1.00 μmol of CTAB.

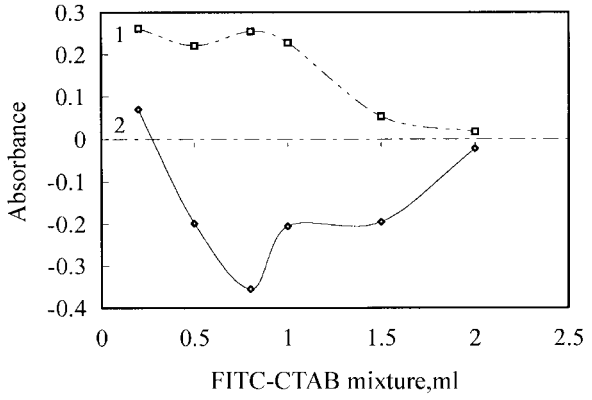


Figure 5. Effect of ionic strength (1) and temperature (2) on absorbance of the FITC-CTAB solution initially containing 0.500 μmol of FITC and: 1- 1.00 μmol of CTAB and 2- 2.00 μmol of CTAB. Both were measured at 512.2 nm against reagent blank.

until complete loss and the desorption of $FITC^{2-}$ occurred. This desorption becomes insensitive if CTAB concentration is much more than SDS one. Therefore, the recommended preparation was suggested, described in "Experimental Section". Curve 7 gives the relative spectrum of the FITC-CTAB-SDS solution, which is just opposite to curve 4 so the absorption peak is located at 484.8 nm and the valley at 512.2 nm. From curve 3, the correction coefficient was calculated to be $\beta_{CTAB\text{-}FITC} = 0.704$ so the correction absorbance of FITC desorbed: $A_\beta = \Delta A\text{-}0.704\Delta A'$ was used in determination of AD.

Effect of pH on aggregation of FITC. In various pH solutions, the absorption of the CTAB-FITC solutions was measured and its effect on the adsorption ratio of FITC to CTAB is shown in Figure 3. We observe that the increase of acidity of solution causes decrease of the ratio. Between pH 7 and 11, the adsorption ratio remains a maximum. This is attributed that $FITC^{2-}$ was formed in basic solution and it is attracted easily and closely on CTAB.

Variation of FITC concentration and determination of characteristic constant of aggregate. For The aggregation of FITC on CTAB, by varying the addition of FITC, the absorption of the solutions was measured. Calculated C_L and γ of each solution and their relationship is shown in Figure 4. We find that C_L^{-1} *vs.* γ^{-1} is linear and the regression equation is $\gamma^{-1} = 3.31 + 15.73 \times 10^{-6} C_L^{-1}$. Therefore, the aggregation of FITC on CTAB obeys the Langmuir monolayer adsorption. From the intercept, the adsorption ratio of FITC to CTAB is calculated to be 0.30. Therefore, the monomer aggregate $FITC \cdot CTAB_3$ and micellar aggregate $(FITC \cdot CTAB_3)_{29}$ is fomred respectively when CTAB is less or more than its CMC 0.96 mM. From the lslope, the equilibrium constant of the aggregate was calculated to be $K = 2.10 \times 10^5$. Additionally, from Equation 6 the real (not apparent) molar absorptivity of the micellar aggregate was calculated to be $\varepsilon_r^{512.2nm} = 1.38 \times 10^6$ L mol^{-1}cm^{-1}. In the determination of the property constants of the aggregate, the spectral correction technique is advanced in operation and principle by comparing the classical methods such as Scatchard model,[20] molar ratios,[21] continuous variations[22] and equilibrium movement.[23]

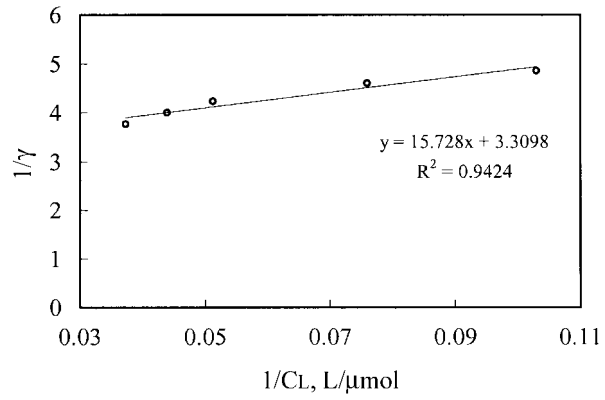


Figure 4. Relationship between the adsorption ratio (γ) and free FITC molarity (C_L, mmol/l): γ^{-1} *vs.* C_L^{-1}.

Effect of ionic strength and temperature on aggregation of FITC. In order to inquire the effect of total ionic concentration of solution, the addition of NaCl was carried out. From curve 1 in Figure 5, the adsorption ratio of FITC to CTAB decrease with increase in ionic strength. This is attributed that more Cl^- may be adsorbed on CTAB to take up limited surface. Curve 2 gives the effect of temperature of solution on the adsorption ration of FITC to CTAB. We observe that the ratio decreases with increase in temperature between 20 and 80 °C. This phenomenon accords with the common nature of a surface adsorption.

At room temperature (20 °C), the effect of the interaction time shows the interaction of FITC with CTAB is complete in 3 min. So the aggregation of FITC on CTAB is rapider than a common chemical reaction. The absorbance measurement of the adsorption solution was made after 5 min.

Figure 6 gives the absorbance variation of the FITC-CTAB-SDS solutions with change of the FITC-CTAB aggregate. We observe that the absorbances approach a maximum at the addition of 0.80 mL of the FITC-CTAB mixture. So the addition volume was used in the quantitative determination of AD in samples.

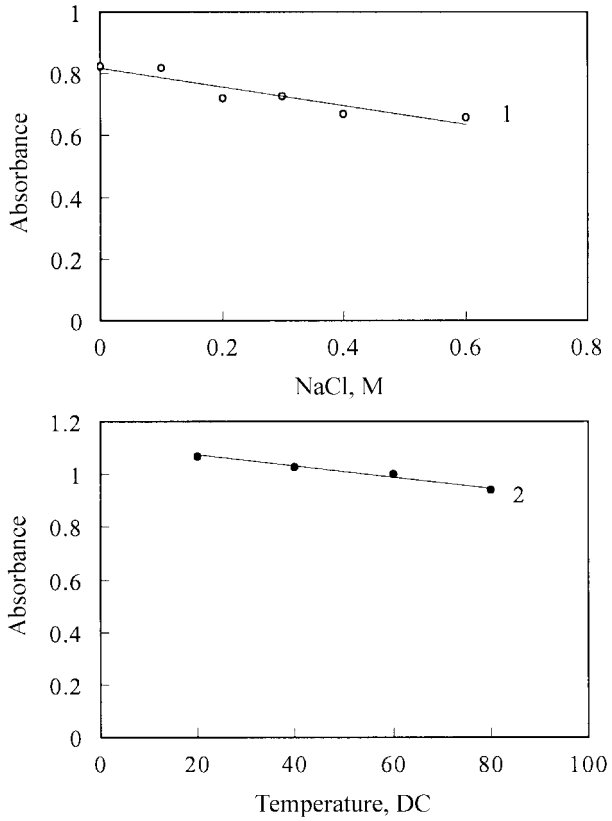


Figure 6. Effect of addition of the FITC (0.500 mM)-CTAB (1.0 mM) mixture on absorbance of solutions initially containing 100 μg of SDS: 1-measured at 484.8 nm and 2- at 512.2 nm against reagent blank.

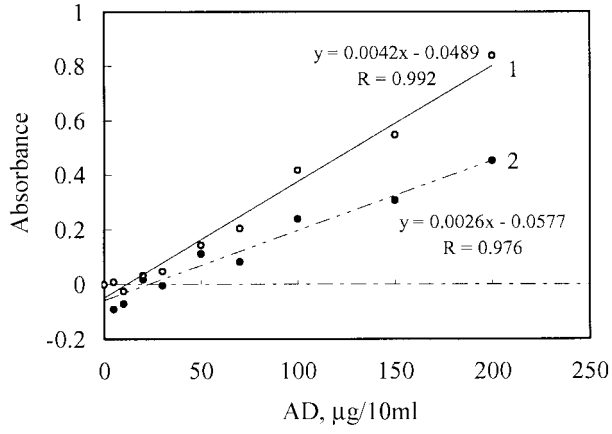


Figure 7. Standard curves for the determination of AD with FITC at pH 9.62 at 484.8 nm. 1- real absorbance (A_c) and 2- measured absorbance (ΔA).

Calibration graph and precision for determination of AD. According to the recommended procedures, the standard series of SDS solutions were prepared and measured. The standard curves are shown in Figure 7. Curve 1 is more linear with higher slope than curve 2. The regression equation of curve 3 is: $A_\beta = 0.0042x - 0.05$ (x- μg of SDS or AD) and it was used in the quantitative determination of AD in samples. The detection limit was only 10 μg in 10 mL of solution. Six replicated determinations of 100 μg of SDS gave the mean 106 ± 4 μg by the spectral correction method (from curve 1) and 89.6 ± 12 μg by the single wavelength spectrophotometry (from curve 2). So, the spectral correction method is more accuracy and precise than the ordinary that.

Table 1. Determination of AD in samples

Sample No.	Added	Found AD (μg)
1 #	5 mL of sample	15.0 ± 1.0 RSD: 6.67%
	30.0 μg of SDS + 5 mL of sample	48.0 ± 1.2 rec. 110%
2 #	5 mL of sample	30.2 ± 1.6
	20.0 μg of SDS + 5 mL of sample	49.0 ± 2.1 rec. 96.0%
3 #	5 mL of sample	22.4 ± 1.3
	20.0 μg of SDS + 5 mL of sample	40.9 ± 1.5 rec. 92.5%

Effect of foreign ions in determination of AD. By adding 5% EDTA in the determination of AD, the influence of foreign substances including ions and organic compounds, on the determination of CTAB was tested at pH 9.62. None of the following ions affected the direct determination of 100.0 μg of SDS (less than 10% error): 1 mg of SO_3^{2-}, $C_2O_4^{2-}$, glucose, humic acid, amino acid, Mg(II), SDBS; 0.5 mg of F^-, Al(III), Mn(II), Ni(II), Zn(II), Pb(II); 0.2 mg of Cu(II), Co(II), Cd(II), Fe(III) and Hg(II).

Determination of AD in samples. Three samples were determined. Sample 1 (1#) was sampled from Huaihe River and sample 2 (#) from local sewage pipe. Sample 3 (3#) was prepared by adding PO_4^{3-}, Mn(II), Zn(II), Fe(II, III), Pb(II) and Co(II) in drinking water background solution. The analytical results of samples are given in Table 1. The recovery of standard SDS is between 92.5 and 110% with RSD 6.67%.

Conclusions

The investigations to the interaction of FITC with CTAB support the monolayer aggregation of stain on surfactant. Though MPASC technique has not given the higher sensitivity than other methods such as RLS.[24] However, it may meet precision and accuracy criteria and offers the additional benefits of simplicity and versatility. We describe the basic physics behind MPASC technique, survey some ongoing research on application to different micellar and macromolecular solution. We understand the classical method can still play important role in studying the synergism mechanism of surfactant micelle.

Acknowledgment. Financial support is gratefully acknowledged from the Anhui Provincial Science and Technology Foundation for Excellent Youths. I thank my colleagues Fu-Xin Xie, Xiang-Tai Kong and Jia-Xiang Yang for providing the technical and external assistance.

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