Journal of Fluorine Chemistry 130 (2009) 225–230

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Study on the Langmuir aggregation of fluorinated surfactants on protein

Ling Li *, Zu Shun Xu, Gong Wu Song

Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Function Molecules, Hubei University, Xueyuan Road 11#, Wuchang, Wuhan City, Hubei Province 430062, People's Republic of China

ARTICLE INFO

Article history:
Received 7 July 2008
Received in revised form 16 October 2008
Accepted 17 October 2008
Available online 6 November 2008

Keywords:
Fluorinated surfactant
Human serum albumin
Langmuir aggregation

ABSTRACT

The microphase adsorption-spectral correction (MPASC) technique was described and applied to the study of the interactions of fluorinated surfactants such as potassium perfluorooctanesulfonate (PFOS) and potassium perfluorobutanesulfonate (PFBS) with human serum albumin (HSA). Sodium octanesulfonate (SOS) was also studied as non-fluorinated surfactant. The aggregation of PFOS, PFBS and SOS obeys the Langmuir monolayer adsorption. The results show that the adsorption ratios of surfactants to HSA are PFOS:HSA = 120:1, PFBS:HSA = 205:1 and SOS:HSA = 18:1. The adsorption constants are $K_{PFOS-HSA} = 5.01 \times 10^3$, $K_{PFBS-HSA} = 9.79 \times 10^2$ and $K_{SOS-HSA} = 4.03 \times 10^3$. The detection limits are 2.7 mg/L for BSA using PFOS, 3.1 mg/L using PFBS and 3.1 mg/L using SOS. It was found that fluorinated surfactant exhibited stronger interaction with protein than hydrogenated one, and fluorinated surfactant with long hydrophobic chain exhibited stronger interaction with protein than that with short hydrophobic chain.
Crown Copyright © 2008 Published by Elsevier B.V. All rights reserved.

1. Introduction

Surfactant is a group of amphipathic substance composed of both hydrophilic and hydrophobic groups. Surfactants are widely used in applications such as surfactants which can induce the unfolding of proteins, and in some special cases stabilize proteins at a very low concentration [1]. The proteins undergo changes in their natural state by the action of different surfactants, which are used as adsorbates in order to control the hydrophobic–hydrophilic character of protein surface [2]. Studying the structural and thermodynamic response of proteins in dependence on solvent conditions is one way to elucidate their stability, folding pathway and intermolecular aggregation behaviour [3]. The study of interaction of proteins with surfactants is fundamental from both the viewpoint of understanding and the application. Surfactants are used to extract proteins from cell membranes. Surfactant–protein interactions are comparable to some extent to lipid–protein interactions in the membranes of living cells [3,4] and can account for the transport of metabolites in body fluids [5].

There is one kind of special surfactants, fluorinated surfactants or fluorocarbon surfactants, whose interaction with proteins remained rarely studied. In fluorinated surfactants, the hydrogens in the hydrophobic tail are replaced by fluorine atoms. Lately, the use of fluorinated surfactants in pharmaceutical and medical applications demands a well-known knowledge about their interactions with proteins [6]. Fluorinated surfactants often occur together with proteins in the formulations of the chemical, biosciences, cosmetic and medical industries [7], and these applications of fluorinated surfactants demand the further understanding on their interactions with proteins [8]. Potassium perfluorooctanesulfonate (PFOS) and potassium perfluorobutanesulfonate (PFBS) are chosen as fluorinated surfactants in this experiment. Sodium octanesulfonate (SOS) is chosen as hydrogenated surfactant for the comparison.

In a protein molecule (P), many similar protonated imino (NH) groups tend to be arrayed on the same side to form a weak positively charged electrostatic film, and the negative carbonyl (CO) dipolar bonds tend to array on the opposite side to form a negative electrostatic film. Protein contains complex spatial structures, e.g. winding, folding, coil and helix, and these cause the double electrostatic films to cross to form many microelectrostatic fields. They can attract ions until kinetic equilibrium is achieved [9]. The microelectrostatic field is so narrow that surfactant molecules (S) are adsorbed in only a monolayer, because the adsorption described above depends on the electrostatic force. Then the Langmuir isotherm equation [10] is used:

$$\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{KNC_S} \quad (1)$$

where K is the equilibrium constant, C_S is the concentration of the excess S and γ is the mole ratio of the effective S adsorbed by P. With increase in S concentration, γ will approach a maximum,

* Corresponding author. Tel.: +86 2788662747; fax: +86 2788663043.
E-mail addresses: zbllanalyst@hubu.edu.cn, waitingll@yahoo.com (L. Li).

0022-1139/$ – see front matter. Crown Copyright © 2008 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.jfluchem.2008.10.007

called the adsorption ratio N. We calculated N and K by plotting γ^{-1} versus C_S^{-1} 1. Both C_S and γ are calculated by the relations [11]:

$$\gamma = \frac{\eta C_S 0}{C_P} \tag{2}$$
$$C_S = (1-\eta) C_S 0 \tag{3}$$

where

$$\eta = \frac{Ac - A}{A0} \tag{4}$$

C_P and C_S0 are the concentrations of P and S added initially and η indicates the effective fraction of S. Ac, A0 and A are the real absorbance of the P–S product, the measured absorbance of the reagent blank against water and that of the P–S solution against reagent blank measured directly at the peak wavelength λ_2, respectively. Ac is calculated by means of [12]

$$Ac = \frac{\Delta A - \beta \Delta A'}{1 - \alpha\beta} \tag{5}$$

where $\Delta A'$ indicates the absorbance of the P–S solution measured at the valley absorption wavelength λ_1. In general, α and β are the correction constants and they are calculated by directly measuring PS_N and S solutions [11,12].

Although there is a great deal of work on the study on Langmuir aggregation [9–12], an important lack can be observed on the characterization of the Langmuir aggregation of surfactants on proteins. The Langmuir adsorption technique provides a very helpful experimental strategy for the study of the aggregation of surfactants on protein. On the other hand, serum albumins are the most abundant proteins in plasma, which have been one of the most extensively studied proteins. They are the major soluble protein constituents of the circulatory system. They play a dominant role in the transport and deposition of endogenous and exogenous ligands in blood, since serum albumins often increase the apparent solubility of hydrophobic drugs in plasma and modulate their delivery to cells in vivo and in vitro [13]. In the current work, human serum albumin (HSA) is selected as protein model.

2. Experimental

2.1. Apparatus

Absorption spectra were recorded on a Hitachi UV3400 Spectrophotometer (Hitachi Co., Japan) with a 1 cm cell and individual absorbances were measured on PerkinElmer lambda 17 UV–VIS spectrophotometer (P-E Co., America). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution. Conductance was measured by using a DDS-11A conductivity meter (Shanghai Rex Instrumental Co., China). High performance particle sizer (Malvvern, America) was used to measure particle size. Transmission electron microscopy (TEM) micrographs were obtained by JEM-100SX electron microscope (JEOL, Japan).

2.2. Reagents

All reagents were of analytical-reagent grade, made in China. The working solution of PFOS, PFBS and SOS was 1.0×10^{-3} mol/L. The stock solution of HSA was prepared by dissolving commercially purchased HSA (Sino-American Biotechnology Company, China) in doubly distilled water at 0–4 °C. The working solution of the bovine serum albumin was 2.5×10^{-5} mol/L. Doubly distilled water was used throughout.

2.3. Method

Appropriate working solution of human serum albumin and surfactant solution were added to a 25 mL volumetric flask. The mixture was diluted to 10 mL with doubly distilled water and vortexes. Absorbance of PFOS–HSA system was measured at 250 and 275 nm, PFBS–HSA at 254 and 278 nm, and SOS–HSA system at 257 and 276 nm against the blank treated in the same way without protein.

3. Results and discussion

3.1. Analysis of absorption spectra

The absorption spectra of the PFOS–HSA, PFBS–HSA and SOS–HSA solution were shown in Figs. 1 and 2, so we can determine

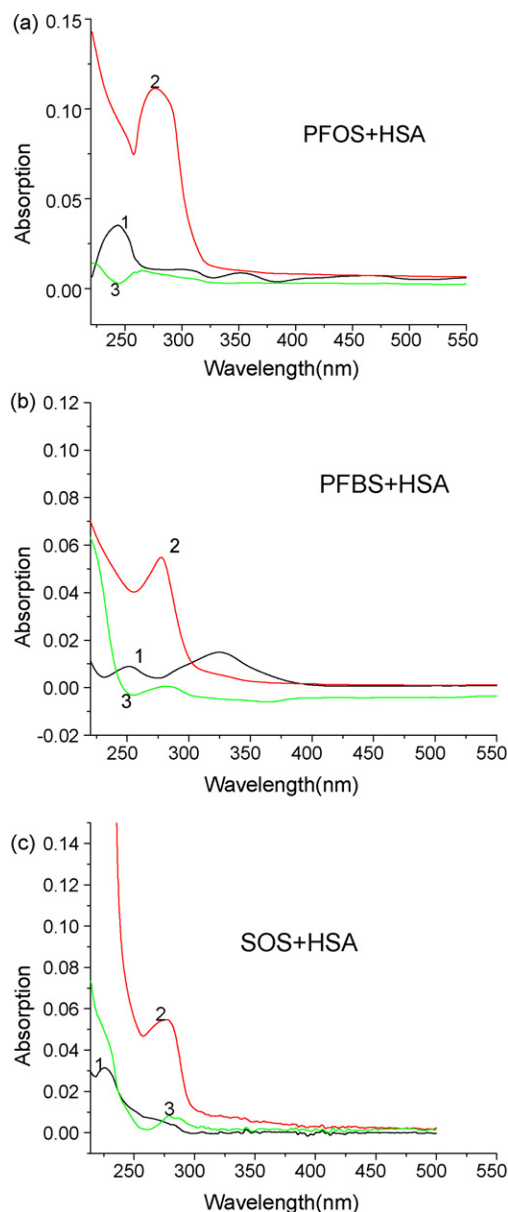

Fig. 1. Absorption spectra of surfactants–HSA. (a) 1, PFOS (3×10^{-4} mol/L); 2 and 3, PFOS (3×10^{-4} mol/L) + HAS (2.5×10^{-6} mol/L). (b) 1, PFBS (3×10^{-4} mol/L); 2 and 3, PFBS (3×10^{-4} mol/L) + HSA (1.5×10^{-6} mol/L). (c) 1, SOS (3×10^{-3} mol/L); 2 and 3, SOS (3×10^{-3} mol/L) + HSA (1.5×10^{-6} mol/L). 1 and 2, against water; 3, against reagent blank.

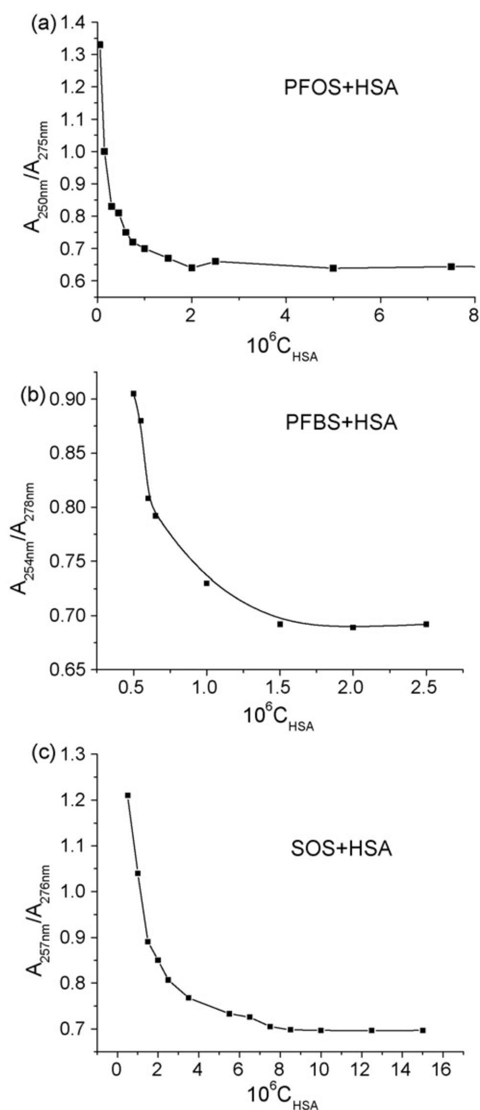

Fig. 2. Effect of the addition of HSA solution: (a) PFOS + HSA, (b) PFBS + HSA, and (c) SOS + HAS. PFOS: 1×10^{-4} mol/L; PFBS: 1×10^{-4} mol/L; SOS: 0.8×10^{-4} mol/L.

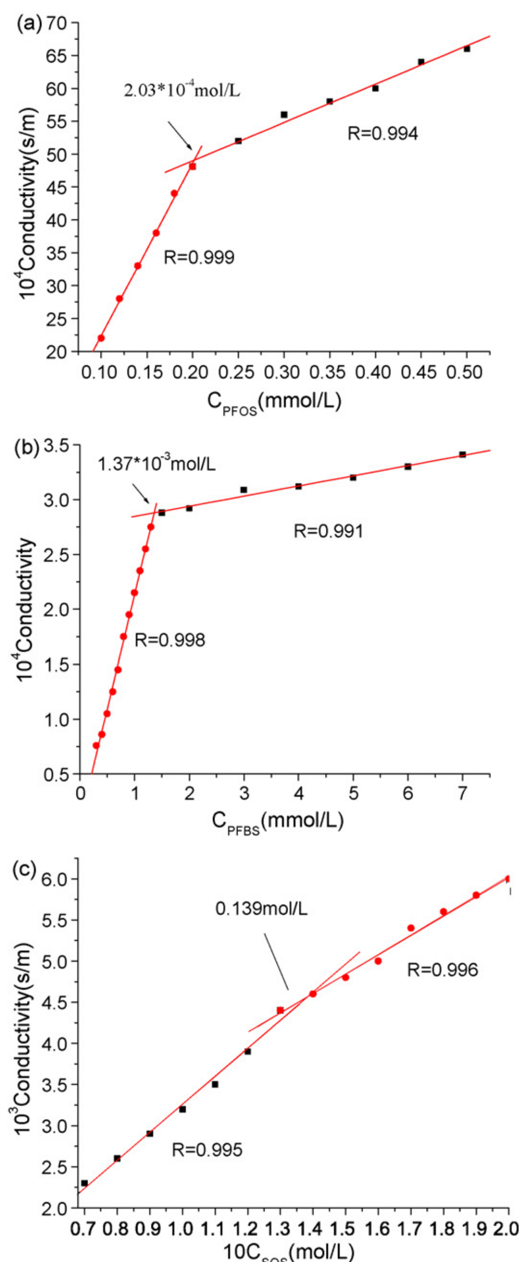

Fig. 3. Electrical conductivity of surfactants: (a) PFOS, (b) PFBS, and (c) SOS.

accurately and sensitively the aggregation of PFOS, PFBS and SOS on HSA by spectrophotometry. Fig. 1 shows the shift of spectral peak of PFOS–HSA, PFBS–HSA and SOS–HSA. It was clear that the spectral peak of PFOS is located at 250 nm from curve 1(a) and that of PFBS is located at 254 nm from curve 1(b), and SOS is at 226 nm.The spectral peak of the PFOS–HSA aggregate is located at 275 nm from curve 2(a) and that of PFBS–HSA is located at 278 nm from curve 2(b), and SOS is at 276 nm. So the red shift of the spectral peak is 25, 24 and 40 nm.

Fig. 3(a)–(c) shows the absorption of PFOS–HSA system, PFBS–HSA system and SOS–HSA system against reagent blank; then the valley absorption was obtained. The peak absorption is located at 275 nm and the valley absorption at 250 nm of PFOS–HSA system. The peak absorption is located at 278 nm and the valley absorption is at 254 nm of PFBS–HSA system. The peak absorption is located at 276 nm and the valley absorption is at 257 nm of SOS–HSA system. In order to obtain the maximal absorbance and minimal error in measurement of the solutions, the working wavelengths 250 and 275 nm may be used for the study on the aggregation of PFOS on HSA, and 254 and 278 nm for PFBS, and 257 and 276 nm for SOS.

Fig. 2(a) gave the change of the two absorbance ratios of solutions of various HSA concentrations, respectively, measured at 250 and 275 nm. Fig. 2(b) was obtained at 254 and 278 nm and Fig. 2(c) at 257 and 276 nm. From Fig. 2(a), the absorbance ratios approached a minimum and remained almost constant when HSA concentration was higher than 2.0×10^{-6} mol/L in a solution containing1.0×10^{-4} mol/L PFOS. So no free PFOS aggregate existed in such a solution and the correction coefficients were calculated to be $\alpha_{PFOS-HSA} = 0.639$ and $\beta_{PFOS-HSA} = 0.360$. From Fig. 2(b), the absorbance ratios approached a minimum and remained almost constant when HSA concentration was higher than 1.5×10^{-6} mol/L in a solution containing1.0×10^{-4} mol/L PFBS. So no free PFBS aggregate existed in such a solution and the correction coefficients were calculated to be $\alpha_{PFBS-HSA} = 0.692$ and $\beta_{PFBS-HSA} = 0.462$. Similarly, from Fig. 2(c), the absorbance ratios calculated approached a minimum and remained almost constant when HSA concentration was higher than 1.0×10^{-5} mol/L in a solution containing 0.8×10^{-4} mol/L SOS. The correction coefficients were to be

$\alpha_{SOS-HSA} = 0.697$ and $\beta_{SOS-HSA} = 0.250$. The equations $Ac = (\Delta A - 0.360\Delta A')/(1 - 0.639 \times 0.360)$, $Ac = (\Delta A - 0.462\Delta A')/(1 - 0.692 \times 0.462)$ and $Ac = (\Delta A - 0.250\Delta A')/(1 - 0.697 \times 0.250)$ were used in calculation of the real absorbance of the PFOS–HSA, PFBS–HSA and SOS–HSA, respectively.

3.2. Aggregation of PFOS, PFBS and SOS on HSA

Fig. 3 shows the plots of the conductivity against surfactants concentration. The inflection observed in all curves at a certain concentration of surfactants is considered to be the critical micelle concentration (CMC) of the micelles. It is obvious that the CMC of PFOS, PFBS and SOS is 2.03×10^{-4}, 1.37×10^{-3} and 0.0139 mol/L, respectively. All experiments for the Langmuir aggregation of surfactants on protein were conducted under the CMC of surfactants, which could be seen in Fig. 4, and all surfactant concentrations were lower than CMC in order that each surfactant in the solution was a monomer.

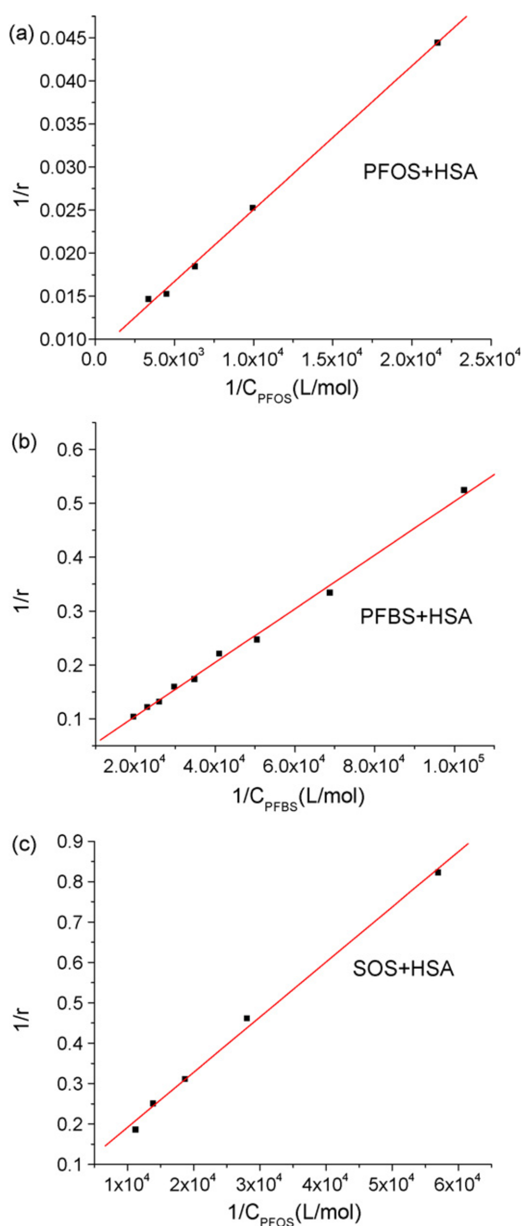

Fig. 4. γ^{-1} versus C_S^{-1}. HSA: 1.5×10^{-6} mol/L.

Table 1
Regression equations and relative constant.

Surfactant	Regression equation	Adsorption ratio (surfactant:HSA)	Adsorption constant
PFOS	$\gamma^{-1} = 0.00836 + 1.6696 \times 10^{-6}\, C_{PFOS}^{-1}$	120	5.01×10^3
PFBS	$\gamma^{-1} = 0.00488 + 4.9868 \times 10^{-6}\, C_{PFBS}^{-1}$	205	9.79×10^2
SOS	$\gamma^{-1} = 0.0550 + 1.3649 \times 10^{-5}\, C_{SOS}^{-1}$	18	4.03×10^2

By varying the surfactants concentration of the solution initially containing 1.5×10^{-6} mol/L of HSA, the absorbances were measured and γ of surfactants to HSA and C_S of surfactants calculated. Curve γ^{-1} versus C_S^{-1} is shown in Fig. 4. Each plot was found to be quite linear. Therefore, the aggregations of PFOS, PFBS and SOS on HSA obey the Langmuir isotherm adsorption. The regression equations are shown in Table 1.

From the intercepts, the adsorption ratio of each aggregate is calculated and the results are shown in Table 1. From the slopes, the adsorption constants of the aggregates are calculated and the results are listed as $K_{PFOS-HSA} = 5.01 \times 10^3$, $K_{PFBS-HSA} = 9.79 \times 10^2$ and $K_{SOS-HSA} = 4.03 \times 10^2$. Therefore, the recommended method is suitable to study the aggregation of a surfactant monomer on proteins.

It was clear that the adsorption constant of PFOS was more larger than that of both PFBS and SOS. For PFOS, PFBS and SOS which are anionic surfactants, it can be concluded that the driving forces are electrostatic interaction with HSA followed by thermodynamically favorable hydrophobic interaction. PFOS showed stronger interaction with HSA than SOS with similar hydrophobic chain length, because the rigidity of the C–F bond stiffened the perfluoroalkanoate chain and strengthened the binding to other molecules [3]. PFOS had stronger interaction than PFBS, for PFOS had long hydrophobic chain to enhance its hydrophobic nature.

3.3. Particle size of surfactants interaction with HSA

Fig. 5 shows the particle size of HSA with three different surfactants under their respective critical micelle concentration. The particle size of HSA with PFOS is the smallest, and that of HSA with SOS is the largest. If the size is smaller, then the surfactant binding to HSA is stronger. It could also be concluded that PFOS has the stronger interaction with HSA than PFBS and SOS. The conclusion is in accordance with the conclusion judging from adsorption constants (Table 1).

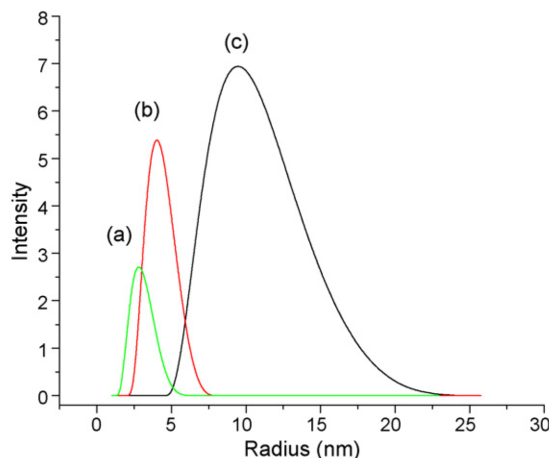

Fig. 5. Particle size distribution for fixed HSA concentration and different surfactants. (a) PFOS: 1.6×10^{-4} mol/L; (b) PFBS: 1.0×10^{-3} mol/L; (c) SOS: 0.096 mol/L. HSA: 1.2×10^{-5} mol/L.

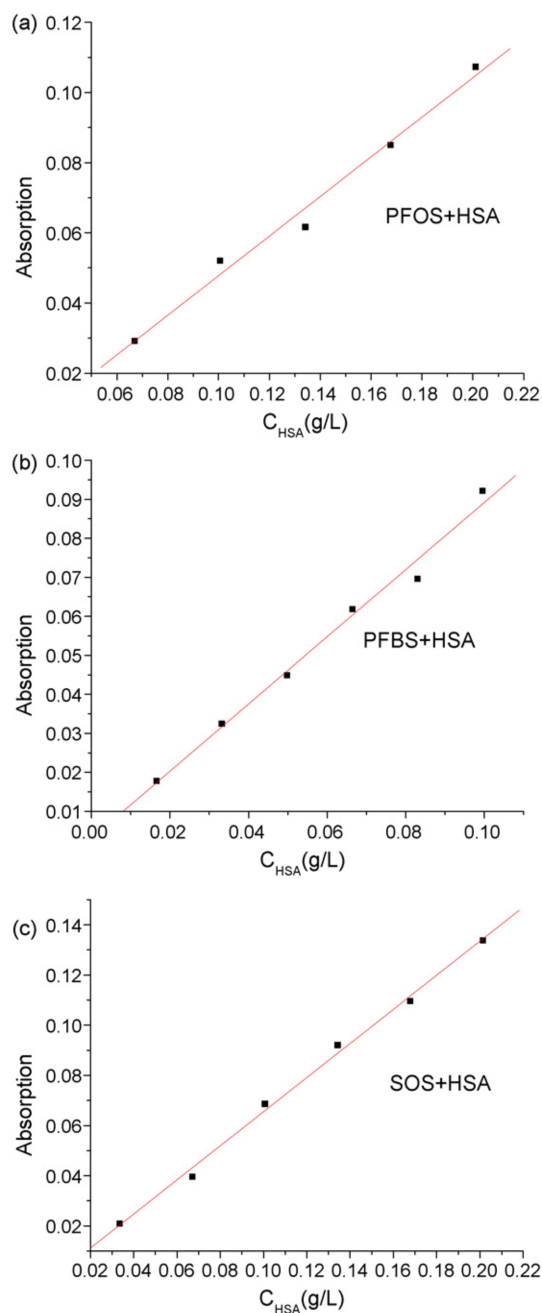

Fig. 6. Standard curves for the determination of protein. (a) PFOS: 1.0×10^{-4} mol/L; (b) PFBS: 4.0×10^{-5} mol/L; (c) SOS: 1.0×10^{-4} mol/L.

3.4. Calibration graph for the determination of HSA

The aggregation of surfactants on HSA can be used for the determination of HSA. Standard series of various protein solutions were prepared and measured according to the procedure. Ac of the aggregate in each solution is calculated and their curves are shown in Fig. 6.

Their regression equations are expressed as follows:

$Ac = 0.564C_{HSA} - 0.0086$ for PFOS–HSA, shown in Fig. 5(a)
$Ac = 0.862C_{HSA} + 0.0031$ for PFBS–HSA, shown in Fig. 5(b)
$Ac = 0.679C_{HSA} - 0.0023$ for PFBS–HSA, shown in Fig. 5(c)

The detection limit of protein was 2.7 mg/L, 3.1 mg/L and 4.2 mg/L.

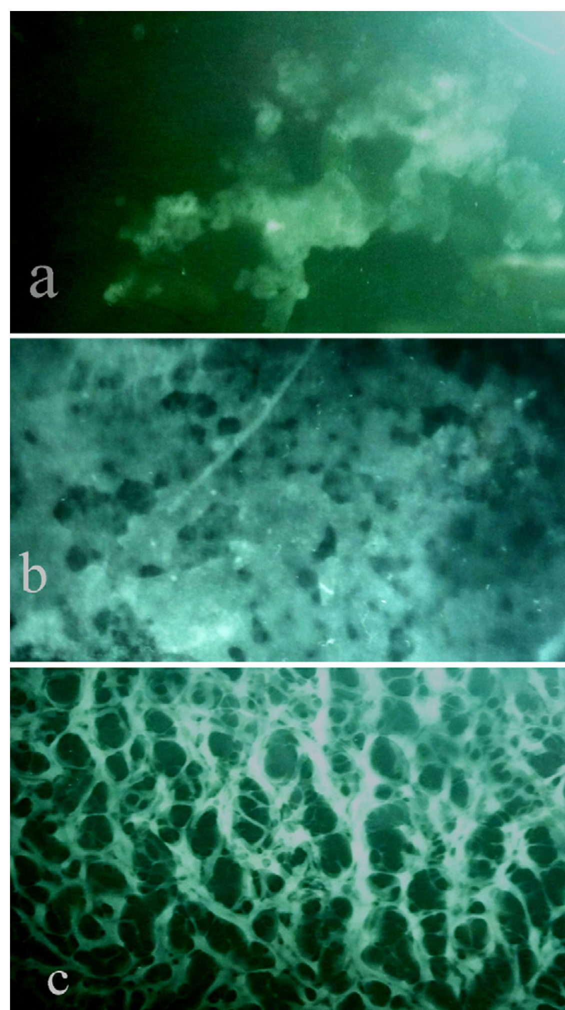

Fig. 7. TEM micrographs of surfactants binding to HSA. (a) PFOS: 5.0×10^{-6} mol/L; (b) PFBS: 5.0×10^{-5} mol/L; (c) SOS: 1.5×10^{-3} mol/L. HSA: 5.0×10^{-7} mol/L.

3.5. TEM micrographs of surfactants binding to HSA

The three different surfactants binding to HSA were studied by electron microscope. TEM micrographs are presented in Fig. 7.

The microcosmic appearance of PFOS binding to HSA is an aggregate formed of the amounts of particles with core shell structure (Fig. 7(a)), and that of both PFBS–HSA interaction and SOS–HSA interaction are aggregates too (Fig. 7(b) and (c)). The transmission electron microscopy analysis results showed that the aggregate of PFOS–HSA interaction has a more compact structure than that of both SOS–HSA and PFBS–HSA. It is very clear that in PFOS–HSA system, the structure is most uniformly closely packed. It can be concluded that the PFOS–HSA interaction is the strongest. Specific ionic interaction resulted in the surfactant absorbed by HSA, and non-specific hydrophobic interaction led to the aggregation. Because hydrophobic interaction played a major role in surfactant binding to HSA, the surfactant with stronger hydrophobic nature had stronger interaction with HSA. PFOS has a long hydrophobic chain than PFBS, and so PFOS is strongly hydrophobic in nature. In contrast to SOS, PFOS has fluorine atoms, which enhances its hydrophobic nature. The conclusion is also in accordance with the conclusion judging from adsorption constants (Table 1).

4. Conclusion

The investigation of the interactions of fluorinated surfactant (PFOS and PFBS) and hydrogenated surfactant (SOS) with HSA supports the Langmuir monolayer aggregation of PFOS, PFBS and SOS. Though the technique has not given higher sensitivity than other methods, it may meet precise and accurate criteria and offers the additional benefits of simplicity and versatility. The interactions of PFOS, PFBS and SOS with HSA were compared. The equilibrium constants of PFOS–HSA, PFBS–HSA and SOS–HSA are 5.01×10^3, 9.79×10^2 and 4.03×10^2, respectively. It indicated that PFOS shows stronger interaction with protein than PFBS, and PFOS also shows stronger interaction with protein than SOS.

The binding of surfactants to protein is driven by specific ionic interaction between the surfactant head group and the protein as well as by non-specific hydrophobic interaction [14]. Experiments exposed that fluorinated surfactant (PFOS) showed stronger interaction with HSA than hydrogenated surfactant (SOS) with similar hydrophobic chain length. In fluorinated surfactants, the hydrogens in the hydrophobic tail are replaced by fluorine atoms. Fluorinated surfactant has larger and highly electronegative fluorine atoms which enhance the hydrophobic nature, and the rigidity of the C–F bond is able to stiffen the perfluoroalkanoate chain; consequently, it binds strongly binding. In general, fluorinated surfactant has stronger hydrophobicity than hydrogenated ones with similar hydrophobic chain length. In the case that the fluorinated and hydrogenated surfactants have similar hydrophobic chain length, it could be expected that hydrophobic interactions became much stronger in systems of fluorinated surfactants and proteins due to the higher hydrophobicity of fluorine atoms. The conclusion actually proved that the hydrophobic interaction plays a major role in binding. Particle size and TEM micrographs also confirmed the conclusion.

Both PFOS and PFBS are fluorinated surfactants, but PFOS has long hydrophobic chain to enhance its hydrophobic nature. So fluorinated surfactant with the long hydrophobic chain (PFOS) has hydrophobic interaction and stronger ability to change protein structure than fluorinated surfactant with short one (PFBS). It can also be concluded that the hydrophobic forces played a major role in the binding. The influence of surfactants on proteins depends on the molecular structure of the surfactants, so higher the hydrophobicity of atoms, stronger the hydrophobic force is; longer the hydrophobic chain is, stronger the hydrophobic interaction is, which leads to stronger binding to HSA. Therefore, the classical spectrophotometry can still play an important role in studying the synergic mechanism of the fluorinated surfactant and the interaction of that with macromolecules.

Acknowledgements

The Natural Science Foundation of Hubei Province of China and Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules supported the work and we are also grateful to the financial support by Innovation Group Foundation and Elitist Foundation of the Provincial Science & Technology Department, Hubei, China. All the authors here express their deep thanks.

References

[1] K.P. Ananthapadmanabhan, in: E.D. Goddard, K.P. Ananthapadmanabhan (Eds.), In Interactions of Surfactants with Polymers and Proteins, 1st ed., CRC Press, London, 1993, p. 319.
[2] M.N. Jones, D. Chapman, Micelles, Monolayers and Biomembranes, Wiley-Liss, New York, 1995.
[3] G. Prieto, J. Sabín, J.M. Ruso, A. González-Pérez, Félix Sarmiento, Colloids Surf. A: Physicochem. Eng. Aspects 249 (2004) 51–55.
[4] S.J. Singer, G.L. Nicolson, Science 175 (1972) 720–732.
[5] A.L. Lehninger, D.L. Nelson, M.M. Cox, Principles of Biochemistry, CBS Publications, New Delhi, 1987.
[6] E. Kissa, Fluorinated Surfactants: Synthesis, Properties, Applications, Marcel Dekker, Inc., New York, 1994.
[7] E. Kissa, Fluorinated Surfactants and Repellents, Marcel Dekker, Inc., New York, 2001.
[8] J.G. Riess, Chem. Rev. 101 (2001) 2797–2805.
[9] H.W. Gao, J. Jiang, L.Q. Yu, Analyst 126 (2001) 528–534.
[10] I. Langmuir, J. Am. Chem. Soc. 40 (1918) 1361–1403.
[11] H.W. Gao, Recl. Trav. Chim. Pays-Bas 114 (1995) 61–64.
[12] H.W. Gao, Talanta 52 (2000) 817–823.
[13] D.C. Carter, J.X. Ho, Adv. Protein Chem. 45 (1994) 153–159.
[14] S. De, A. Girigoswami, S. Das, J. Colloid Interface Sci. 285 (2005) 562–573.