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Use of Gemini surfactant in a one-step ellagic acid assay by resonance light scattering technique

Zhanguang Chen[a,*], Yurui Peng[a], Shaobin Wang[b], Xi Chen[c], Tianhe Song[a], Sihua Qian[a], Meizhen Chen[d], Qinrong Wang[a]

[a] Department of Chemistry, Shantou University, Daxue Road, Shantou 515063, Guangdong, China
[b] The First Affiliated Hospital of Shantou University Medical College, Shantou 515041, China
[c] Guangdong Pharmaceutical University, Guangzhou 510006, China
[d] Department of Biology, Shantou University, Shantou 515063, China

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ABSTRACT

Ellagic acid (EA) reacted with Gemini zwitterionic surfactant, phosphodiesters quaternary ammonium salt (PQAS), and formed fine particles which produced strong enhancement in intensity of resonance light scattering (RLS). The effects of several factors on the RLS signal, such as pH, ionic strength, PQAS concentration and so on, were optimized. The relationship between enhanced RLS intensity and EA concentration was constructed. A novel and rapid method for the determination of EA was built. The linear range of this method was 0.016–4.0 $\mu g\,mL^{-1}$ and the detection limit was 13.9 $ng\,mL^{-1}$. Under the optimum conditions, the proposed method was applied to determine EA in body fluids with the results of quantitative recoveries between 98.4–101.4% in human serum samples and 99.1–102% in human urine samples. This method characterized by low limit detection is very sensitive and the cost is low, and constitutes a fast one-step procedure which requires only measuring the RLS intensities. The mechanism of the reaction was also studied. This investigation could contribute to the research on the delivery and release of bioactive molecules by Gemini surfactants.

1. Introduction

Phenolic phytochemicals are important components of fruits and vegetables and are partly responsible for their beneficial health effects against oxidation-linked chronic diseases such as cancer and cardiovascular diseases. It is believed that phytochemicals function either by countering the negative effects of oxidative stress by directly acting as an antioxidant or by activating/inducing cellular antioxidant enzyme systems [1]. One of such compounds is ellagic acid (EA) (the structure is shown in Fig. 1(a)), dilactone of hexahydroxydiphenic acid, found in raspberries, strawberries, cranberries, walnuts, pecans, and other plant foods. EA is currently being investigated for its antimutagetic, anticancer activity in the bacterial and mammalian system [2–7]. Some researches suggested that the extent to which EA inhibited mutagenesis and carcinogenesis correlated strongly with its affinity towards the oligonucleotide. EA has aroused great interest worldwide owing to its beneficial health effects [8–12]. Poor absorption of EA in small animals and human bodies due to its low solubility in water has been reported [13]. Both chemical and biochemical factors that affect the absorption and metabolism of EA were reviewed [1]. The large variations in bioavailability observed among EA and individuals were explained by Scalbert and Williamson [14]. EA, as a kind of polyphenol, also attracted great attention to its bioavailability studies in vivo and in vitro [15–17].

HPLC with UV detector has been used to investigate the bioavailability and pharmacokinetics of EA in body fluids [13,18–22]. HPLC, a separation technique, required large amounts of high purity organic solvents, long system stabilization time and special sample preparation protocols. Finding a new and favorable method for the determination of EA is on the focus of the researchers as UV techniques currently in use have poor sensitivities.

Gemini surfactants, as the name implies, consist of two surfactant moieties, linked together by a spacer unit. Gemini surfactants, with excellent foaming and wetting properties, have attracted substantial interest in the academic as well as the industrial communities [23]. Furthermore, there is a special department to study the Gemini surfactants as potential vehicles for the transport of bioactive molecules, and several Gemini surfactants have been successfully used to deliver DNA into mammalian cells [24]. Since Gemini surfactants are relatively new, few species have been reported or disclosed in the previous report. Phosphodiesters quaternary ammonium salt (PQAS) was a kind of new Gemini surfactants synthesized recently with the general properties of Gemini

* Corresponding author. Tel.: +86 75482903330; fax: +86 75482902767.
E-mail address: kqlu@stu.edu.cn (Z. Chen).

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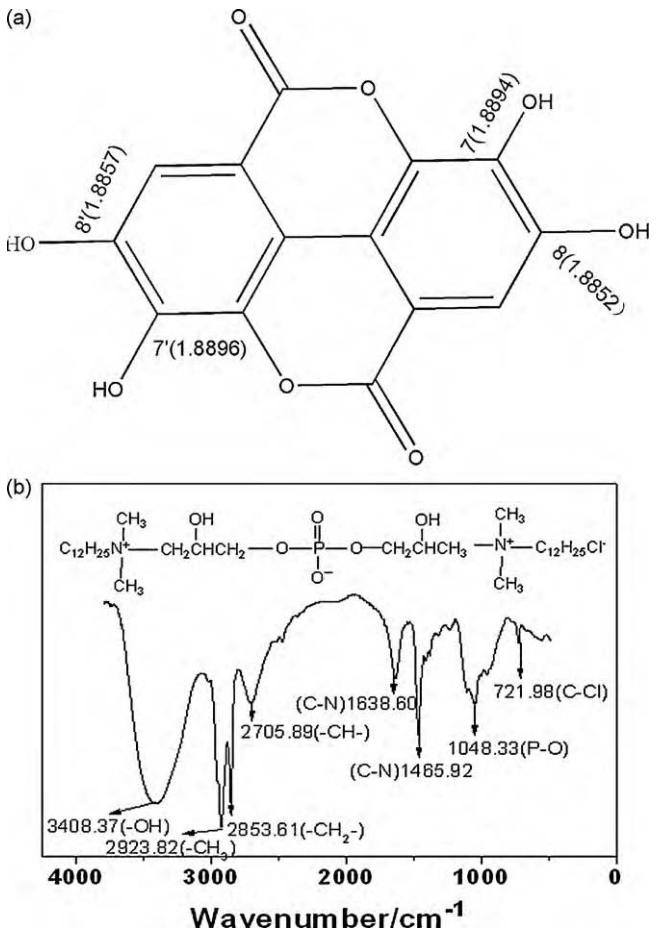

Fig. 1. (a) Charge distribution of EA molecule (EAH_4). (b) IR spectra and structure of PQAS.

surfactants, and without toxic effects [25]. Its structure was given in Fig. 1(b). The investigation of the functional substance, which was bound to PQAS for the determination of bioactive compound, was not explored.

Resonance light scattering (RLS) is a scattering–absorption–rescattering process related to the molecular structure, size, shape, charge distribution and binding constant. When the incident beam is near or within an absorption band of the scattering molecule, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. The intensive absorption of light energy of the electron occurs, and then rescattering takes place and the scattering light intensity at some wavelengths becomes much higher than that of usual scattering light [26–28]. This phenomenon is called resonance light scattering (RLS). In 1993, based on this theory, Pasternak et al. firstly studied the aggregations of porphyrins by using a common fluorescence spectrometry [29,30]. Huang et al. have successfully used RLS technique for quantitative analysis to determine biomacromolecule [31,32]. Due to the distinct advantages of speed, convenience and sensitivity, the researchers, in recent years, have developed progressively for the assay purposes [33–37]. Great attention was paid to the determination of DNA [38–42], protein [43–46], medicines [47,48], amino acid [49], dextrin [50], polysaccharide [51] and so on by the RLS technique.

In this paper, the bioavailability of EA in human body was studied based on the interaction between EA and PQAS by the RLS technique. The mode of the interaction was also studied. EA which is a neutral hydrophobic molecule is more soluble in aqueous solution of Gemini surfactants, and can be safely delivered into cells and then into the nucleus. PQAS, without toxic side effects, would be used as pharmaceutical material because of its safe delivery and release of functional EA in the body. The proposed method characterized by its high sensitivity and good selectivity, and lower cost was applied to the determination of micromolecule EA, which has not yet been reported in the literature. Therefore, this study has a promising progress in the biochemical analyses and can provide the theoretical basis for the drug design.

2. Materials and methods

2.1. Apparatus and reagents

All the RLS intensity and spectra were recorded on the LS-55 spectrofluorometer (PerkinElmer) with a 1 cm × 1 cm quartz cuvette. The Agilent 8453 Model ultraviolet spectrophotometer was used to record the absorption spectra. The acidity of the solution was determined on the pHS-3 Model instrument. FT-NIR Spectrometer (PerkinElmer) combined with Opus 5.5 software was used for analysis of the structures of PQAS–EA, EA, PQAS. The spectra generated over a range of wave numbers from 12,000 to 4000 cm^{-1} were interpreted based on the overtones of different functional groups in the product.

Ellagic acid (EA) was purchased from Sigma Chemical Co. and 0.0200 g of EA was weighed accurately and dissolved in 100 mL DMF in a flask. The stock solution of EA was diluted for the working solution of 20.0 μg mL^{-1}. Phosphodiesters quaternary ammonium salt (PQAS) was synthesized according to the procedure described in Ref. [23]. At pH 4.2 (in pH 6.8 solution), the EA having negative charges reacted with positively charged PQAS by electrostatic effect.

0.328 g PQAS was weighed accurately and was dissolved directly in 250 mL of freshly obtained doubly distilled water. The 2.0×10^{-3} mol L^{-1} of PQAS working solution was obtained. All the solutions above were stored at 1–4 °C in the refrigerator.

The acidity of assay system was controlled by sodium hydrogen phosphate–citric acid buffer (Na_2HPO_4–$C_6H_8O_7$). Na_2HPO_4–$C_6H_8O_7$ buffer of different pH were made up of 0.2 mol L^{-1} $Na_2HPO_4{\cdot}2H_2O$ and 0.1 mol L^{-1} $C_4H_2O_7{\cdot}H_2O$. 0.01 mol L^{-1} NaCl was applied to adjust the ionic strength of the solution. All other reagents and solvents used were of analytical grade or the best grade commercially available and the doubly distilled water was used throughout.

2.2. General procedure

Into a 10-mL calibrated tube with plug was added 1.0 mL Na_2HPO_4–$C_6H_8O_7$ buffer solution, 1.0 mL PQAS and appropriate EA or samples and stirred after each solution added. The mixture was diluted to the mark with doubly distilled water and mixed thoroughly for determinations.

All RLS spectra were obtained by scanning in the range of 250.0–700.0 nm with the excitation and emission slit widths of 5.0 nm. The RLS intensity was determined at 394.5 nm. The enhanced RLS intensity of the system by adding EA is represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, where ΔI_{RLS} is the enhancement of RLS intensity, I_{RLS} and I^0_{RLS} are the RLS intensity of the system with and without EA, respectively.

3. Results and discussion

3.1. Features of spectra

A series of UV–Vis absorption spectra were depicted in Fig. 2(a). It can be seen that there was no absorption of PQAS, and EA has

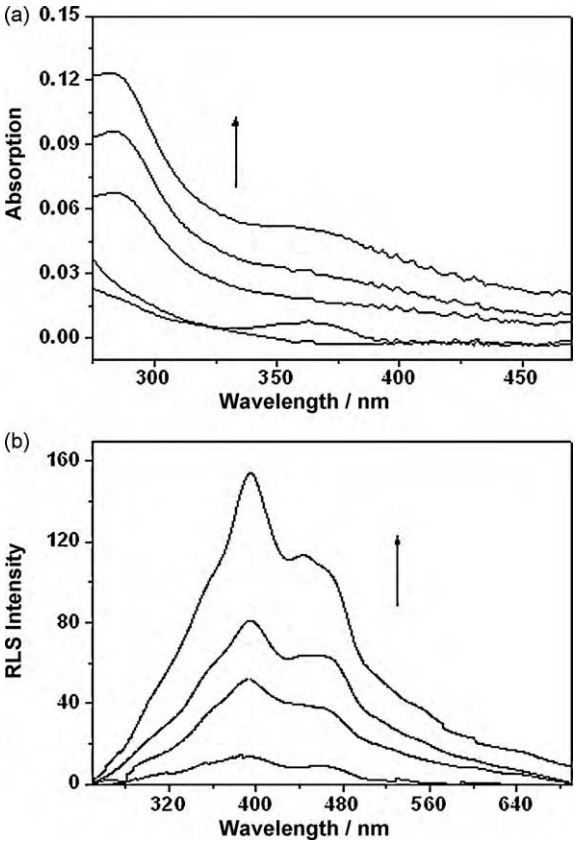

Fig. 2. (a) Absorption spectra of PQAS–EA system. From bottom to top or in the sense of the arrow: PQAS 2×10^{-4} mol L^{-1}, pH 4.2, EA ($\mu g\,mL^{-1}$): 0.0, 1.0 (without PQAS), 0.5, 1.0, and 2.0. (b) RLS spectra of the PQAS–EA system. From bottom to top or in the sense of the arrow: PQAS 2×10^{-4} mol L^{-1}, pH 4.2, EA ($\mu g\,mL^{-1}$): 0.0, 0.5, 1.0, and 2.0.

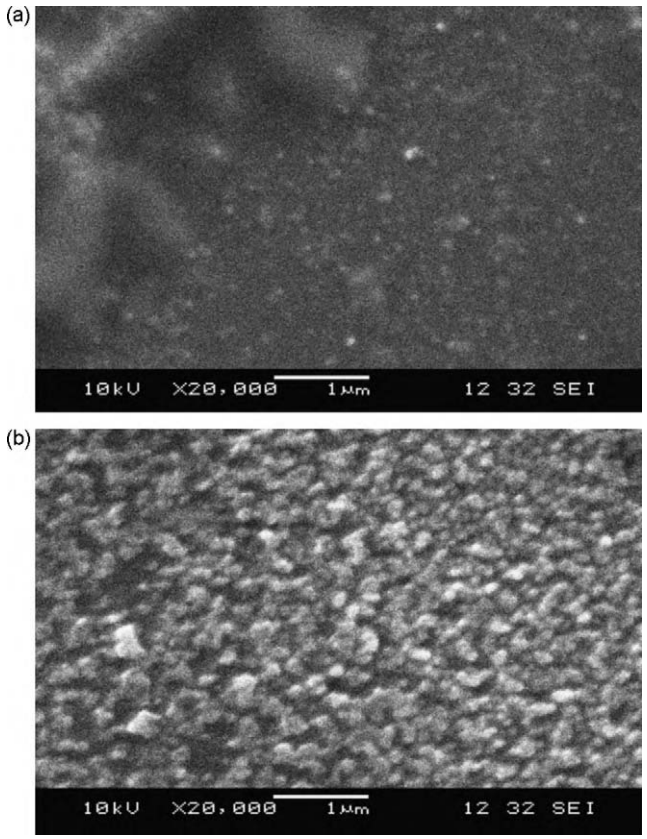

Fig. 3. Scanning electron micrograph of EA in the absence of PQAS (a) and in the presence of PQAS (b). Conditions: EA, 20.0 $\mu g\,mL^{-1}$; pH 4.2; PQAS, 2.0×10^{-3} mol L^{-1}.

a weak spectra at 363.0 nm which could be assigned π–π^* transition in the plane of the EA chromophore. However, in the PQAS–EA system, the absorption of EA at 363.0 nm disappeared and the new peak at 284.0 nm showed up. This indicated that a reaction had occurred between PQAS and EA. With the concentration of EA increasing, the intensity of the absorption of the system at 284.0 nm was increased gradually with a slight blue shift. This phenomenon would be due to the effect of the hydrogen bond and hydrophobic interactions on the structure of new complex produced by PQAS and EA.

The RLS spectra are studied and the results were shown in Fig. 2(b). The RLS intensity of PQAS without EA was very weak and enhanced evidently in the presence of EA at 394.5 nm. With the increase of EA, the enhanced RLS intensity of assay system increased and was proportional to the EA concentration. The characteristics indicated that the interaction between PQAS and EA occurred and the new complex was produced. The complex can result in the enhanced RLS spectra owing to its appropriate particle size [26,27]. This deduction can be confirmed by the scanning electron microscopy (Fig. 3). From Fig. 3, it can be seen that the size of EA with PQAS (b) is much larger than that without PQAS (a). This work also indicates that the enhanced RLS intensity results from the increscent size of the particles.

3.2. Effect of acidity

The effect of acidity on the RLS intensity was studied and the results were shown in Fig. 4(a). It was found that the enhanced RLS intensity of PQAS–EA system increased with the increasing pH value and reached a maximum at pH 4.2 (the pH value of the assay solution is 6.8, however, the pH 4.2 refers to the pH value of buffer which is used to control the acidity of the solution.), and then reduced with the pH value further increasing. EA has four phenolic OH groups with a benzopyranone structure and the pK_a values corresponding to the various prototropic change equilibriums could be presented as

$$EG(OH)_4 \overset{pK_{a_1}}{\rightleftharpoons} EG(OH)_3(O^-) + H^+ \quad (1)$$

$$EG(OH)_4 \overset{pK_{a_2}}{\rightleftharpoons} EG(OH)_2(O^-)_2 + 2H^+ \quad (2)$$

$$EG(OH)_4 \overset{pK_{a_3}}{\rightleftharpoons} EG(OH)(O^-)_3 + 3H^+ \quad (3)$$

$$EG(OH)_4 \overset{pK_{a_4}}{\rightleftharpoons} EG(OH)(O^-)_4 + 4H^+ \quad (4)$$

pK_a values could be estimated between 6.3 and 11.2 in the reported literature [52].

When the pH was over 4.2, the acidity of the solution affected the charges of PQAS and the structure of EA, which reduced the combination between EA and PQAS, resulting in RLS intensity of the assay system decreasing. pH 4.2 of Na_2HPO_4–$C_6H_8O_7$ buffer was applied to adjust the acidity of the solution.

3.3. Effect of PQAS concentration

Purification was performed and the structure of PQAS was confirmed by the Infrared Spectroscopy technique. The result obtained was described in Fig. 1(b). As marked in Fig. 1(b), functional groups of PQAS at the wave numbers were in good agreement with declared content. The effect of PQAS concentration on the RLS intensity of assay system was studied and the result was summarized in Fig. 4(b). With the PQAS concentration increasing, RLS intensity of assay system without EA was very weak and remained stable. However, when EA was added, the RLS intensity increased sharply and

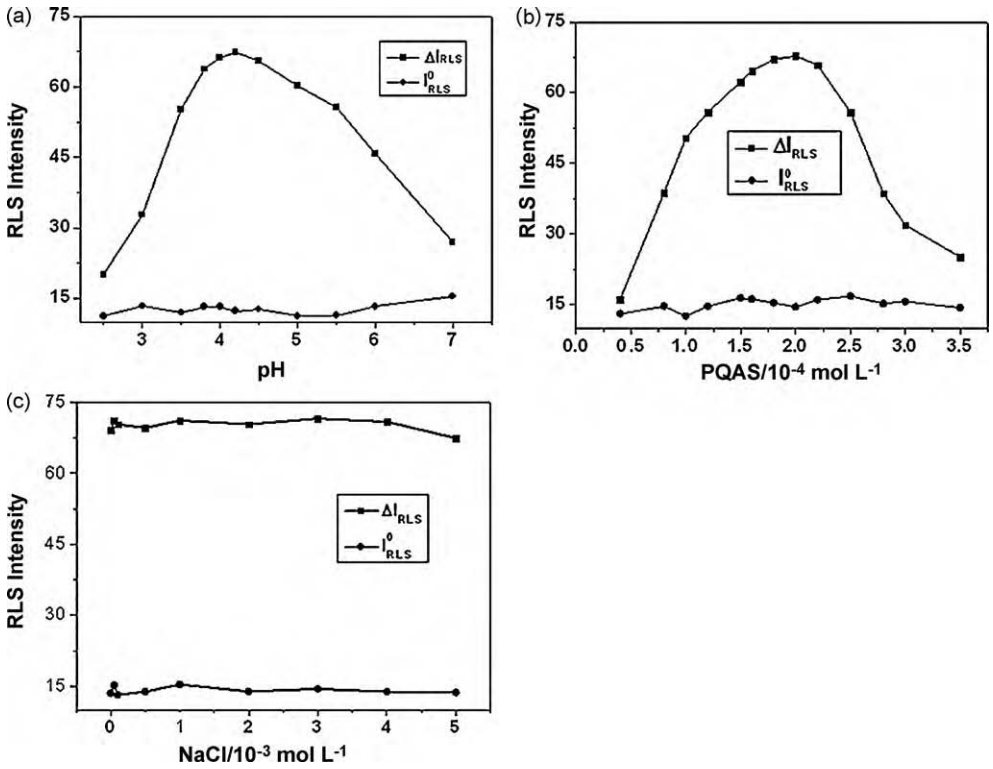

Fig. 4. (a) Effect of pH on the RLS intensity. Conditions: PQAS 2.0×10^{-4} mol L^{-1}, EA 1.0 μg mL^{-1}. (b) Effect of PQAS concentration on the RLS intensity. Conditions: EA 1.0 μg mL^{-1}, pH 4.2. (c) Effect of ionic strength on the RLS intensity. Conditions: PQAS 2.0×10^{-4} mol L^{-1}, EA 1.0 μg mL^{-1}, pH 4.2.

reached a maximum at 2.0×10^{-4} mol L^{-1} of PQAS. The concentration of PQAS was 2.0×10^{-4} mol L^{-1} in this assay.

3.4. Effect of ionic strength

The effect of the ionic strength on the RLS intensity of the system was investigated. The results were shown in Fig. 4(c). With the concentration of NaCl increasing, the RLS intensity remained relatively stable. The reaction between PQAS and EA, which is mainly by the hydrogen bond and hydrophobic interaction, was not affected by ionic strength and the RLS intensity remained stable. Thus, the effect of electrolyte on the RLS intensity should be ignored.

3.5. Incubation and stability

Under the optimal conditions, the incubation and stability of the reaction between PQAS and EA were studied by determining the RLS intensity once every 2 min after mixing. The results indicated that PQAS and EA reacted rapidly at room temperature. The RLS intensity of the assay system reached a maximum in about 6 min and remained stable for 80 min. Therefore, the proposed method does not need crucial timing.

3.6. Tolerance of foreign substances

The effect on the I_{RLS} of the foreign substances was estimated with 1.0 μg mL^{-1} EA and the results were summarized in Table 1. There were no co-existing substances and ions interfering with the determination of EA. From the results described, it can be seen that common metal ions such as Na^+, Mg^{2+}, K^+, Zn^{2+}, Mn^{2+}, and Ca^{2+} can be tolerated at high concentrations. The common organic solvent, such as DMF and ethanol, can be tolerated about 25% and 15% (v/v), respectively. In this study, DMF used to resolve EA in the working system was 10% (v/v), so it did not influence the determination of EA. The most serious interference was Bi^{3+}. However, the concentration of Bi^{3+} was much low in human serum. Furthermore, dilution 100–1000 times with water can minimize all these interferences in the analysis of samples. Thus, this method can be used practically in the EA determination.

Table 1
Effects of the potentially interfering substances on the determination of 1.0 μg mL^{-1} EA.

Interfering substances	Concentration	Change in I_{RLS} ($n = 3$, %)
$MgSO_4$	5000 μmol L^{-1}	−0.6
KCl	5000 μmol L^{-1}	1.3
$ZnSO_4$	4000 μmol L^{-1}	−5.1
$Mn(SO_4)_2$	3000 μmol L^{-1}	−4.8
$CaCl_2$	500 μmol L^{-1}	−5.6
$FeSO_4$	6.0 μmol L^{-1}	0.4
$Pb(NO_3)_2$	5.0 μmol L^{-1}	5.8
$Al_2(SO_4)_3$	4.0 μmol L^{-1}	4.7
$CuSO_4$	2.0 μmol L^{-1}	1.6
$Bi(NO_3)_3$	0.05 μmol L^{-1}	4.7
Ethanol	15% (v/v)	3.2
DMF[a]	25% (v/v)	0.1
Urine	0.2 mol L^{-1}	4.1
Glucose	5000 μg mL^{-1}	−4.3
Fructose	500 μg mL^{-1}	1.6
Sucrose	500 μg mL^{-1}	7.7
Lysine	5000 μg mL^{-1}	−5.8
Cysteine	500 μg mL^{-1}	−7.1
Arginine	50 μg mL^{-1}	1.1
Glutamine	50 μg mL^{-1}	7.9
Vitamin A	20 μg mL^{-1}	5.3
Vitamin B6	20 μg mL^{-1}	2.6
Vitamin C	20 μg mL^{-1}	3.4
Gallic acid	5.0 μg mL^{-1}	2.8
RF[b]	2.0 μg mL^{-1}	6.6
BSA[c]	1.0 μg mL^{-1}	4.0
HSA[d]	1.0 μg mL^{-1}	2.1

[a] N,N-dimethylformamide.
[b] Riboflavin.
[c] Bovine serum albumin.
[d] Human serum albumin.

Table 2
Comparisons with other assays of EA.

Method	Linear range ($\mu g\,mL^{-1}$)	Detection limit (3σ, $ng\,mL^{-1}$)	References
UV[a]	0.5–8.0	200	[18]
HPLC[b]	0.5–8.0	100	[18]
HPLC–UV	1.0–15.6	31.9	[13]
HPLC–UV	0.1–10	100	[19]
IP-RP-HPLC–UV[c]	5.0–100	1400	[20]
Spectrophotometric	–	1000	[21]
RLS	0.016–4.0	13.96	This work

[a] Ultra-violet.
[b] High-performance liquid chromatography.
[c] Isocratic ion-pair high-performance liquid chromatographic with UV detector.

3.7. Calibration and detection limit

The enhanced RLS intensity of the assay system was determined by adding different volumes of EA according to the experimental procedure, and the calibration curve for quantification of EA was linear over the concentration range from 0.016 to 4.0 $\mu g\,mL^{-1}$. The linear regression equation was $\Delta I_{RLS} = 4.33 + 68.46C$ ($\mu g\,mL^{-1}$, $n = 10$) with a correlation coefficient of $r = 0.9992$. The detection limit was 13.96 $ng\,mL^{-1}$. The limit of detection was given by $3S_0/S$, where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the 15 blank determinations, and S is the sensitivity of the calibration graph. Comparisons with other methods of determination of EA were shown in Table 2. It can be found that this method has the advantages of high sensitivity, low detection limit and wide linear range.

4. Analytical applications

4.1. Sample collection

Volunteer (from Shantou University) could not take any kind of medicine during three days before the testing day. The 10 mL fresh blood and urine of the volunteer who had eaten a certain amount of EA for 2 h were taken into two different tubes and stored at 37 °C.

4.2. Human serum samples

The blood sample was kept at 37 °C in a water bath for about 0.5 h and the blood coagulation was preconditioned. The sample without blood clot was deproteinized by adding approximate 10 mL acetonitrile and centrifuging (40 min at 4000 rpm) at the room temperature. Then the acetonitrile was removed under reduced pressure on a rotary evaporator in a 60 °C water bath. The human serum without proteins was dissolved by water in a 100 mL volumetric flask and diluted to the mark with doubly distilled water and stored at 4 °C. A suitable volume of the solution appropriately spiked with the standard EA solution was taken and three different samples with different EA concentrations were obtained for analysis. The results were summarized in Table 3. In all cases quantitative recoveries between 98.4% and 101.4% were obtained.

4.3. Human urine samples

The urine sample was pre-treated by heating and filtration. The filtration was done according to the procedure described above in *human serum samples* section and the processed sample was dissolved in a 100 mL volumetric flask. The sample was diluted to the mark with doubly distilled water and stored at 4 °C. According to the experimental procedure, the samples described above appropriately and spiked with the standard EA solution were taken for assay and the results were shown in Table 3. In all cases quantitative recoveries between 99.1 and 101.9% were obtained. The determination results of the present work have been compared with that of HPLC and the results are also described in Table 3.

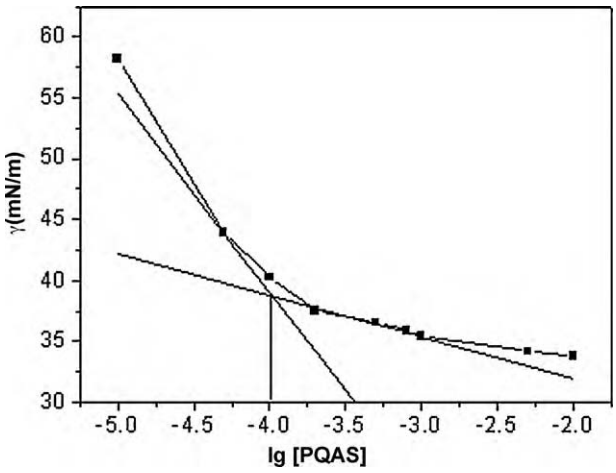

Fig. 5. Effect on the surface tension by the PQAS concentration. Conditions: pH 4.2, PQAS (10^{-4} mol L^{-1}): 0.01, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 5.0, and 10.0 in the *X*-coordinate.

4.4. Mechanism of the reaction

The critical micelle concentration (CMC) value for the Gemini surfactant PQAS was determined from surface tension vs. log concentration plots (Fig. 5) to be 1.0×10^{-4} mol L^{-1}. In the determination system, the concentration of RLS probe PQAS was 2.0×10^{-4} mol L^{-1} which exceeded CMC (1.0×10^{-4} mol L^{-1}). Therefore, the micelle was formed by the tail-tail hydrophobic interactions of PQAS. On the surface of micelle, many positive charges contained by N^+ in the PQAS molecule contributed to the reaction between EA with negative charge and micelle of PQAS.

The reaction between PQAS and EA was also studied by IR spectrum and the result was shown in Fig. 6. The results showed that the peak (–OH) at 3408.37 cm^{-1} becomes widened as result of the hydrogen bond formed between EA and PQAS. The peak at 1684 cm^{-1} which is produced by lactone ring and the peak at 1102.84 cm^{-1} which is produced due to C–O–C remained almost unchanged between B and C spectra curve. The data suggested that the lactone rings did not open both in the EA and the PQAS–EA systems at pH 4.2 (in the pH 6.8 solution), which is consistent with the reported literature [53–56]. Based on the previous studies, the mechanism of reaction can be described in Fig. 7. In the

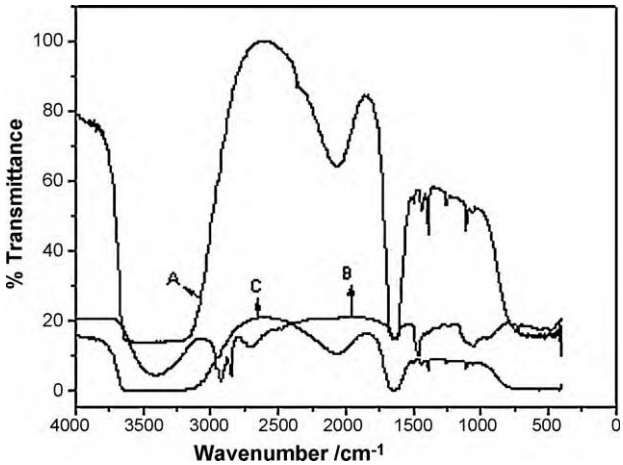

Fig. 6. IR spectra of PQAS + EA (A), EA (B) and PQAS (C).

Table 3
Analytical results of real samples[a].

Sample	HPLC method	Present method				
	Found ± values (μg mL^{-1})	Found (μg mL^{-1})	Added (μg mL^{-1})	Total found (μg mL^{-1})	Recovery (%)	RSD (%)
Human serum sample						
1	0.20 ± 0.02	0.22	0.6	0.82	100.0	5.1
2	0.19 ± 0.02	0.21	1.0	1.19	98.4	2.9
3	0.25 ± 0.03	0.23	1.6	1.85	101.4	3.0
Human urine sample						
1	0.31 ± 0.01	0.31	1.00	1.32	101.6	2.5
2	0.40 ± 0.06	0.44	1.00	1.43	99.1	3.1
3	0.62 ± 0.04	0.61	1.00	1.63	101.9	4.6

[a] Average of five determinations.

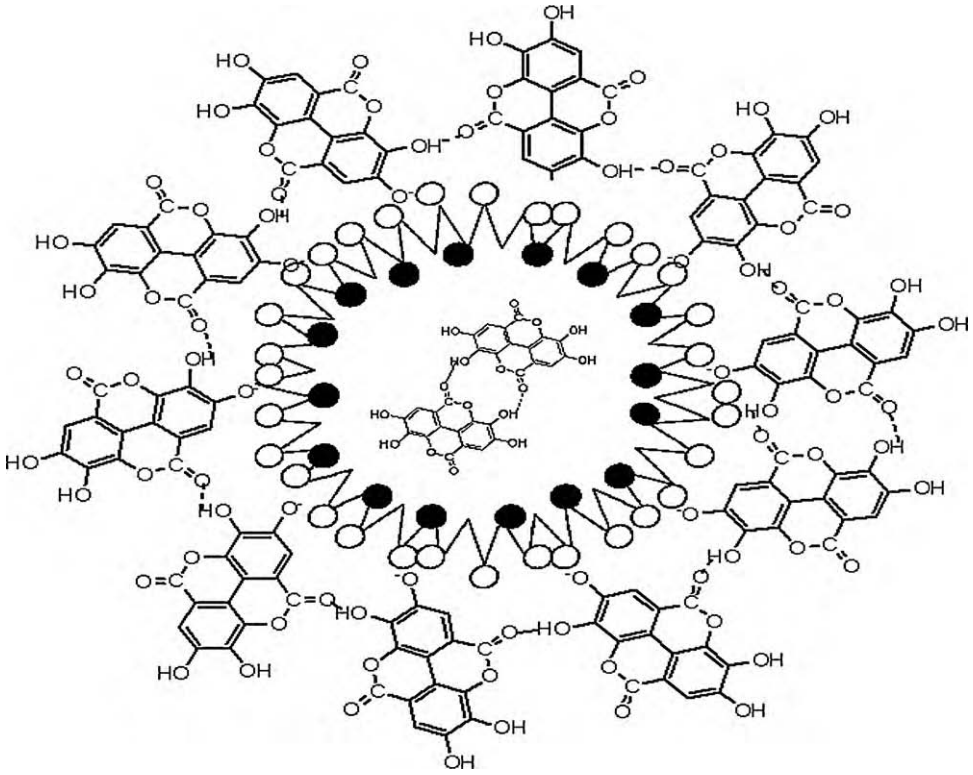

Fig. 7. Proposed model for the complex formation between EA and PQAS. P (●) and N (○).

assay system, the Gemini surfactant PQAS (2.0×10^{-4} mol L^{-1}) is in the formation of micelle which is positively charged. Because the π electron density on 7-OH (1.8894) is larger than that on 8-OH (1.8852), the structure $(8\text{-EAH}_3)^-$ is the deprotonated species in the assay system [53–56]. The EA molecules can be contained in the micelle of PQAS by hydrophobic interaction. Moreover, the EA molecules with negative charges also can stack on the surface of the micelle of PQAS by electrostatic and hydrogen bond force (Fig. 7). The schematic illustrated the reaction between PQAS and EA, and the large particle was formed which resulted in the enhanced RLS signal in the assay system. The result was unanimous in that from provided SEM in Fig. 3.

5. Conclusion

In this contribution, the reaction between a new kind of Gemini surfactant PQAS and EA was investigated and the effect of the factors (pH, ionic strength, PQAS concentration and so on) was also studied. In the pH 4.2 Na_2HPO_4–$C_6H_8O_7$ buffer, PQAS was combined with EA and the complex was produced. The new complex with fine particle size can produce intense RLS signal (at 394.5 nm). With the addition of EA, the RLS intensity of the assay system increased and enhanced RLS intensity was proportional to the EA concentration. The novel method was established to determine EA in human serum and urine. The results obtained were satisfactory.

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References

[1] D.A. Vattem, K. Shetty, J. Food Biochem. 29 (2005) 234–266.
[2] S. Benedetti, S. Drusch, S. Mannion, Talanta 78 (2009) 1266–1271.
[3] M. Freitas, G. Porto, J.L.F.C. Lima, E. Fernandes, Talanta 78 (2009) 1476–1483.
[4] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C. Thomas, C.W.W. Beecher, H.H.S. Fong, Science 275 (1997) 218–220.
[5] L. Jurd, I.P. Plant, J. Am. Chem. Soc. 78 (1956) 3445–3448.
[6] K. Ippoushi, A. Takeuchi, K. Azuma, Food Chem. 112 (2009) 185–188.

[7] A.C. Whitley, G.D. Stoner, M.V. Darby, T. Walle, Biochem. Pharmacol. 66 (2003) 907–915.
[8] S.U. Mertens-Talcott, J.H. Lee, S.S. Percival, S.T. Talcott, J. Agric. Food Chem. 54 (2006) 5336–5343.
[9] R.W. Teel, Cancer Lett. 30 (1986) 329–336.
[10] A. Strati, Z. Papoutsi, E. Lianidou, P. Moutsatsou, Clin. Biochem., doi:10.1016/j.clinbiochem.2009.05.017.
[11] N. Suzuki, A. Masamune, K. Kikuta, T. Watanabe, K. Satoh, T. Shimosegawa, Dig. Dis. Sci. 54 (2009) 802–810.
[12] C. Girish, B.C. Koner, S. Jayanthi, K.R. Rao, B. Rajesh, S.C. Pradhan, Fundam. Clin. Pharmacol. 23 (2009) 735–745.
[13] N.P. Seeram, R. Lee, D. Heber, Clin. Chim. Acta 348 (2004) 63–68.
[14] A. Scalbert, G. Williamson, J. Nutr. 130 (2000) 2073–2085.
[15] A. Robledo, A. Aguilera-Carbo, R. Rodriguez, J.L. Martinez, Y. Garza, C.N. Aguilar, J. Ind. Microbiol. 35 (2008) 507–513.
[16] A. Aguilera-Carbo, C. Augur, L.A. Prado-Barragan, E. Favela-Torres, C.N. Aguilar, Appl. Microbiol. Biotechnol. 78 (2008) 189–199.
[17] A. Aguilera-Carbo, J.S. Hernandez, C. Augur, L.A. Prado-Barragan, E. Favela-Torres, C.N. Aguilar, Food Bioprocess. Technol. 2 (2009) 208–212.
[18] I. Bala, V. Bhardwaj, S. Hariharan, M.N.V. Ravi Kumar, J. Pharm. Biomed. Anal. 40 (2006) 206–210.
[19] S. Shahrzad, I. Bitsch, J. Chromatogr. A 741 (1996) 223–231.
[20] P. García del Moral, M.J. Arín, J.A. Resines, M.T. Díez, J. Chromatogr. B 855 (2007) 276–279.
[21] T.C. Wilson, A.E. Hagerman, J. Agric. Food Chem. 38 (1990) 1678–1683.
[22] O. Nara, Anal. Chim. Acta 338 (1997) 247–253.
[23] S.K. Hait, S.P. Moulik, Curr. Sci. 82 (2002) 1101–1111.
[24] European Network on Gemini surfactants (1994–1998), Available at URL address: http://www.ch.cam.ac.uk/resources/misc/ENGEMS.html.
[25] G.M. Yu, W.M. Song, Q.G. Deng, Y.B. Wu, J. Qiqihar Univ. 23 (2007) 7–13.
[26] C.Z. Huang, Y.F. Li, Anal. Chim. Acta 500 (2003) 105–117.
[27] J. Yguerabide, E.E. Yguerabide, J. Cell. Biochem. 37 (2001) 71–81.
[28] J. Yguerabide, E.E. Yguerabide, Anal. Biochem. 262 (1998) 157–176.
[29] R.F. Pasternack, C. Bustamante, P.J. Collings, L.A. Giannetto, E.J. Gibbs, J. Am. Chem. Soc. 115 (1993) 5393–5399.
[30] R.F. Pasternack, P.J. Collings, Science 269 (1995) 935–939.
[31] C.Z. Huang, K.A. Li, S.Y. Tong, Anal. Chem. 68 (1996) 2259–2263.
[32] C.Z. Huang, K.A. Li, S.Y. Tong, Anal. Chem. 69 (1997) 514–520.
[33] W. Lu, B.S. Fernández Band, Y. Yu, Q.G. Li, J.C. Shang, C. Wang, Y. Fang, R. Tian, L.P. Zhou, L.L. Sun, Y. Tang, S.H. Jing, W. Huang, J.P. Zhang, Microchim. Acta 158 (2007) 29–58.
[34] J. Ling, C.Z. Huang, Y.F. Li, Y.F. Long, Q.G. Liao, Appl. Spectrosc. 42 (2007) 177–201.
[35] J. Ling, C.Z. Huang, Y.F. Li, L. Zhang, L.Q. Chen, S.J. Zhen, Anal. Chem. 28 (2009) 447–453.
[36] H.Y. Wang, Y.F. Li, C.Z. Huang, Talanta 72 (2007) 1698–1703.
[37] M.H. Xiang, X. Xu, D.X. Li, F. Liu, N. Li, K. Li, Talanta 71 (2008) 1207–1211.
[38] P. Bao, A.G. Frutos, C. Greef, J. Lahiri, U. Muller, T.C. Peterson, L. Warden, X.Y. Xie, Anal. Chem. 74 (2002) 1792–1797.
[39] Z.G. Chen, W.F. Ding, F.L. Ren, J.B. Liu, Y.Z. Liang, Anal. Chim. Acta 550 (2005) 204–209.
[40] H. He, J.C. Ren, Talanta 77 (2008) 166–171.
[41] Y.Q. Cheng, Z.P. Li, Y.Q. Su, Y.S. Fan, Talanta 71 (2007) 1757–1761.
[42] H.P. Zhou, X. Wu, J.H. Yang, Talanta 78 (2009) 809–813.
[43] Z.G. Chen, G.L. Liu, M.H. Chen, Y.R. Peng, M.Y. Wu, Anal. Biochem. 384 (2009) 337–342.
[44] Z.G. Chen, J.B. Liu, Y.L. Han, Talanta 71 (2007) 1246–1251.
[45] L.J. Dong, X.G. Chen, Z.D. Hu, Talanta 71 (2007) 555–560.
[46] H.C. Pan, X.C. Tao, C.J. Mao, J.J. Zhu, F.P. Liang, Talanta 71 (2007) 276–281.
[47] Z.G. Chen, Y.R. Peng, J.H. Chen, L. Zhu, J. Pharm. Biomed. Anal. 48 (2008) 946–950.
[48] Z.G. Chen, G.L. Liu, M.Z. Chen, B.J. Xu, Y.R. Peng, M.H. Chen, M.Y. Wu, Talanta 77 (2009) 1365–1369.
[49] Z.G. Chen, J.B. Liu, Y.L. Han, L. Zhu, Anal. Chim. Acta 570 (2006) 109–115.
[50] Z.G. Chen, L. Zhu, T.H. Song, J.H. Chen, J.H. Huang, Y.L. Han, Anal. Chim. Acta 635 (2009) 202–206.
[51] Z.G. Chen, G.L. Liu, M.H. Chen, M.Y. Wu, Talanta 79 (2009) 171–176.
[52] K.I. Priyadarsini, S.M. Khopde, S.S. Kumar, H. Mohan, J. Agric. Food Chem. 50 (2002) 2200–2206.
[53] M. Hasegawa, M. Terauchi, Y. Kikuchi, A. Nakao, J. Okubo, T. Yoshinaga, H. Hiratsuka, M. Kobayashi, T. Hoshi, Monatsh. Chem. 134 (2003) 811–821.
[54] R.E. Press, D. Hardcastle, J. Appl. Chem. 19 (1969) 247–251.
[55] L. Jurd, J. Am. Chem. Soc. 81 (1959) 4610–4615.
[56] T. Sato, M. Kataoka, J. Heterocycl. Chem. 34 (1997) 665–667.