1   **Interaction of a Biosurfactant, Surfactin with a Cationic**
2   **Gemini Surfactant in Aqueous Solution**

3   Lei Jin,^{†,1} Vasil M. Garamus,^{†,2} Fang Liu,¹ JingwenXiao,¹ HelmutEckerlebe²,
4   RegineWillumeit-Römer,² BozhongMu,¹ andAihuaZou^{*,1}

5

6   ¹Shanghai Key Laboratory of Functional Materials Chemistry, State Key
7   Laboratory of Bioreactor Engineering and Institute of Applied Chemistry, School of
8   Chemistry and Molecular Engineering, East China University of Science and
9   Technology, Shanghai 200237, P. R. China,

10   ²Helmholtz-ZentrumGeesthacht, Centre for Materials and Coastal Research,
11   Institute of Materials Research, Max-Planck-Strasse 1, 21502 Geesthacht, Germany

12

13

14

15

16

17

18

19

20

21

22

23

---

24   * To whom correspondence should be addressed.

25   Tel/Fax.: +86-21-64252231.

26   E-mail: aihuazou@ecust.edu.cn.

27   ¹East China University of Science and Technology.

28   ²Helmholtz-ZentrumGeesthacht.

29   [†]These authors contributed equally to this work.

Abstract: The interaction between biosurfactant Surfactin and cationic Gemini surfactant ethanediyl-1,3-bis(dodecyldimethylammoniumbromide) (abbreviated as 12-3-12) was investigated using turbidity, surface tension, dynamic light scattering (DLS) and small angle neutron scattering (SANS). Analysis of critical micelle concentration (CMC) values in Surfactin/12-3-12 mixture indicates that there is synergism in formation of mixed Surfactin/12-3-12 micelles. Although Surfactin and 12-3-12 are oppositely charged in phosphate buffer solution (PBS, pH 7.4), there are no precipitates observed at the concentrations below the CMC of Surfactin/12-3-12 system. However, at the concentration above CMC value, the Surfactin/12-3-12 mixture is severely turbid with high 12-3-12 content. DLS and SANS measurements follow the size and shape changes of mixed Surfactin/12-3-12 aggregates from small spherical micelles via elongated aggregates to large bulk complexes with increasing fraction of Gemini surfactant.

Keywords: Surfactin, Gemini surfactant, micelle, surface tension, SANS

1. Introduction

The mixtures of different types of surfactants have attracted significant interest since mixtures provide a synergistic enhancement of performance and functionality which cannot take place in single surfactant systems¹⁻³. Synergism increases with the degree of charge difference⁴⁻⁶ meaning that the higher level of synergism is obtained by mixing anionic and cationic surfactants. There have been plenty of studies of anionic/cationic surfactant mixtures. The behavior and physicochemical properties of mixtures of a highly branched cationic and sodium alkyl sulfate have been widely studied by Yu and co-workers⁷⁻⁹. And, it has been reported that the asymmetrically double-tailed cationic surfactants fail to fit well into a crystal lattice structure and hence minimize precipitation¹⁰. Bergström and co-workers have studied the structures in cationic/anionic surfactant including Gemini systems using the small angle neutron scattering technique¹¹⁻¹³. However, precipitation is a common phenomenon that can occur in all of the anionic/cationic surfactants mixture mentioned above and in most cases precipitation is undesirable because it renders the surfactant ineffective in solution. Therefore, a better understanding of such system is necessary to broaden the horizon for their application.

Surfactin, a biosurfactant produced by various *Bacillus subtilis* strains, is cyclic lipopeptide built from a heptapeptide (Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu) and a β -hydroxy fatty acid with variable chain lengths of 13-15 carbon atoms¹⁴⁻¹⁶ (Figure 1(a)). Surfactin has been receiving attention for industrial, biotechnological, and therapeutical applications^{17,18} because of its high surface activity, which could reduce the surface tension of water to 27 mN/m¹⁷ by that concentration as low as 10^{-5} M. It can be seen that Surfactin has two $-\text{COOH}$ groups in L-Glu1 and L-Asp5. The pK_a values¹⁹ of Asp and Glu are around 4.3 and 4.5, respectively, which means it exists as an anionic molecule in neutral solution.

Gemini surfactants contain two single-chain surfactant moieties joined by a spacer group.²⁰⁻²² The two amphiphilic moieties are close to each other, especially when the spacer group is short. Owing to this, on the one hand, the interaction between the hydrophobic chains is enhanced; on the other hand, repulsion between the hydrophilic

90 groups (particularly the electrostatic between the ionic head groups) will be greatly
91 reduced due to the chemical bonds connection. This novel class of surfactants
92 has many unique properties that are superior to those of conventional single-chain
93 surfactants, such as remarkably low critical micelle concentration (CMC), much
94 higher surface activity, and better wetting properties. Moreover, it has
95 unusual aggregation morphologies. Since the surfactant micellization is driven by
96 hydrophobic interactions but opposed by the repulsion of charged head groups (for
97 ionic surfactants) and hydration (for nonionic surfactants), Gemini surfactant is more
98 readily to form aggregates. The quaternary ammonium Gemini surfactants are widely
99 investigated about their properties and applications^{23,24}. For the 12-s-12 (12 carbon
100 atoms on the hydrophobic alkyl chain and 's' represents the number of carbon atoms
101 of the spacer) series, at the concentration close to CMC value, spherical micelles are
102 formed. Increasing the surfactant concentration, for the short spacer, such as $s = 2, 3$,
103 elongated rod-like micelles are formed; for the medium length like $s = 4, 6, 8, 10, 12$,
104 spherical micelles are formed, and for the longer spacer, $s = 16, 20$, vesicles are
105 readily to form²⁴.

106 There are a lot of studies on Surfactin¹⁴⁻¹⁹ and Gemini²⁰⁻²⁴ from various aspects.
107 However, to the best of our knowledge, there is no research about their mixtures. The
108 dynamic interfacial tension between the Surfactin/12-3-12 binary aqueous system and
109 crude oil has been carried out in our group, which revealed that Surfactin/12-3-12
110 mixtures can reduce the interfacial tension to an ultralow level in alkaline
111 environment (see details in Supporting Information) which can be used in the
112 application in oil recovery. The better understanding of the fundamental
113 physicochemical property of the mixture of Surfactin/Gemini surfactant system is the
114 key point for their practical application. In our previous work, we have investigated
115 the micelle formation and interfacial behavior of Surfactin²⁵ in PBS buffer. A further
116 research²⁶ has also been carried out on the interaction of Surfactin with betaines which
117 revealed that there was a synergistic effect between them, and the configuration of
118 mixed micelles changed with the head-groups of betaines. Then, the interactions
119 among neutral polymer PAM, Surfactin and four betaines in PBS buffer were

120 further studied. Transition from spherical to rodlike aggregates (micelles) has been
121 observed in solutions of Surfactin and
122 Surfactin/SDDAB(N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate) with
123 addition of 0.8 wt % of PAM²⁷. Wang et al²⁸ studied the interaction between surfactin
124 and cationic surfactant Cetyltrimethylammonium Bromide (CTAB) in mixed micelle,
125 There existed synergism between anionic Surfactin and cationic surfactant CTAB, and the
126 mixed system might be able to form vesicle spontaneously at high molar fraction
127 of surfactin. However they did not study the precipitation condition and the aggregates
128 properties (shape and size) of the anionic/cationic mixed surfactant aqueous system. A
129 continuation of the aggregation behavior of Surfactin/12-3-12 mixtures were
130 investigated in present paper by surface tension, small angle neutron scattering and
131 dynamic light scattering, which will broaden the potential application for both
132 Surfactin and Gemini surfactants.

133 **2. Materials and Experiments**

134 **2.1 Materials.**

135 **2.1.1 Surfactin.** Surfactin was produced by *Bacillus subtilis* TD7 cultured in a
136 laboratory of East China University of Science and Technology^{29,30}. Surfactin isoform
137 (Figure 1(a)) was separated by extraction with anhydrous ether, isolated with normal
138 pressure ODS C18 column and purified by the RP-HPLC (Jasco, Japan). The structure
139 of the isolated lipopeptide was determined by the electrospray
140 ionization-time-of-flight mass spectrometer (ESI-TOF MS/MS) and GC/MS^{29,30}.

141

b

142 **Figure 1.** The chemical structure of Surfactin(a) and 12-3-12(b).

143 **2.1.2 GeminiSurfactant.** Crude ethanediyl-1,3-bis(dodecyldimethylammonium)
 144 bromide (abbreviated as 12-3-12) was obtained from Daochun Chemical Technology
 145 Co. Ltd. It was recrystallized at least four times with ethanol and ethyl acetate³¹ then
 146 dried under vacuum with low heat at 40°C for 1 day. The purity of 12-3-12 was
 147 checked by ¹H NMR. The ¹H NMR Spectra of 12-3-12 and the
 148 related analyzing data are presented in Supporting Information, and the impurity content
 149 was estimated to be below 2%. The chemical structure of 12-3-12 is presented in
 150 Figure 1(b).

151 **2.2 Sample Preparation.** First, 10 mM phosphate buffer solution (PBS,
 152 pH=7.4, this pH value was chosen to achieve better water solubility for Surfactin) were
 153 prepared as the solvent, then high concentration of pure surfactant solutions were
 154 prepared by weighing the desired amounts of surfactants and dissolved in the solvent.
 155 The mixtures of the desired composition of surfactants were made by mixing high
 156 concentration of two pure surfactant solutions with a certain amount and added

157 solvent to a settled volume. The composition of the Surfactin/12-3-12 binary system
158 is described with $\alpha_{\text{Surfactin}}$, the mole fraction of Surfactin.

$$159 \quad \alpha_{\text{surfactin}} = \frac{c_{\text{surfactin}}}{c_{\text{surfactin}} + c_{12-3-12}} \quad (1)$$

160 For SANS experiments, the samples were prepared under the same condition except
161 doubly distilled water was replaced by D₂O (99.9% D, from Sigma-Aldrich). The pD
162 value was mediated by eq2, with proper corrections by Krezel et al^{32,33}.

$$\text{pD} = 0.929 \text{ pH meter trading} + 0.42 \quad (2)$$

163 **2.3 Precipitation Determination.** For the series of mixed solutions of Surfactin and
164 12-3-12, since surfactant solutions can remain supersaturated for long periods of time
165 before precipitation started^{34,35}, therefore, to determine whether there is
166 precipitation formed, all solutions were cooled at 0°C to force precipitation to
167 occur³⁵. The solutions were then placed in a water bath at 25.0 ± 0.1 °C, shaken
168 periodically, and allowed to equilibrate for at least 4 days before measurements. If a
169 solution is outside of the precipitation region, crystals would dissolve so that the
170 solution became isotropic. If crystals remained in solution after equilibration, the initial
171 solution composition was considered to be inside the precipitation region. The presence
172 of precipitate in solution was determined by visual inspection using a high intensity
173 light.

174 **2.4 Surface Tension Measurements.** For CMC measurements, surface tension was
175 measured after the samples were properly mixed by ultra-sonic and equilibrated for 1 day.
176 Measurements were carried out at 25°C by surface tension meter DCAT21
177 (Dataphysics, Germany) using a Wilhelmy small platinum plate of ca. 4 cm perimeter.
178 The plate was first rinsed with doubly distilled water and then burned to red to make
179 sure there are no surfactants left on the plate. Prior to the measurements, the equipment
180 was tested by determination of a surface tension of double distilled water (72 ± 0.2
181 mN/m) at 25.0 °C.

182 **2.5 DLS Measurements.** DLS measurements were carried out at 25°C with Nano-ZS
183 (Malvern Instruments Ltd. UK), equipped with 4 mW He-Ne laser at $\lambda_0 = 633 \text{ nm}$ as a
184 light source. PAL (Phase Analysis Light Scattering) and LDV (Laser Doppler

185 Velocimetry) were used to measure the size at a scattering angle of 173° . Samples
186 were kept in quartz cuvette. DLS measurements were made at 0.5 mM surfactant
187 concentration with $\alpha_{\text{Surfactin}}$ ranges from 0 to 1, and each sample was measured for
188 three times. Experimental data are given by the Malvern Zetasizer software.

189 **2.6 Small angle neutron scattering (SANS) Measurements.** SANS measurements
190 were performed at SANS1 instrument operated by HZG and TUM at the
191 Meier-Leibniz-Zentrum (FRM2, Garching, Germany)³⁶. The wavelength of the neutron
192 beam was $6 \text{ \AA}$ with $\Delta\lambda/\lambda=10\%$. A position-sensitive detector with 128×128 pixels and a
193 pixel size of 8 mm was used. These settings allowed us to cover a q range from 0.008 to
194 $0.4 \text{ \AA}^{-1}$. Samples were placed in 1 mm thick quartz cell and measured at $T = 25 \pm 1^\circ \text{C}$.
195 Data have been normalized by water measurements at same geometry and corrected for
196 detector response and electronic noise. The scattering from PBS buffer prepared in D_2O
197 was subtracted as the background. Data reduction has been done by the QtiKWS³⁷ data
198 analysis software. The dispersion of resolution function has been calculated and taken
199 into account for further data analysis.

200 **3. Results and Discussion**

201 **3.1 Precipitation Phase Boundary.** Since Surfactin and 12-3-12 are oppositely
202 charged in PBS (pH 7.4), it was expected that precipitates will form in their mixtures. In
203 order to evaluate the precipitation potential of these mixtures as well as to ensure that
204 the surface tension studies were conducted in regions without precipitates, turbidity
205 studies were conducted. At a constant $\alpha_{\text{Surfactin}}$ value, the measurements were made at
206 series concentrations to confirm the phase boundaries.

207 In Figure 2(a), the red line represents the precipitation phase boundary which was
208 determined by visual observation using a high intensity light. The precipitation region
209 was further divided into two parts as dark gray and light grey in Figure 2(a), where
210 the precipitation potential is different between these two parts. In order to better
211 explain the meaning of “dark and light grey” in Figure 2(a), the photos of 0.5
212 mM Surfactin/12-3-12 solutions at different $\alpha_{\text{Surfactin}}$ values is shown in Figure 2(b). The
213 dark gray represents high turbid solutions ($\alpha_{\text{Surfactin}} = 0.1, 0.2, \text{ and } 0.3$ in Figure 2(b))
214 which are entirely opaque, while the light gray corresponds to turbid solutions

243 ($\alpha_{\text{Surfactin}} = 0.4, 0.5$, and pure Gemini in Figure 2(b)) which can still transfer light.
 240 Besides, the white region means visually clear and transparent solutions ($\alpha_{\text{Surfactin}}$
 247 $= 0.6-1$ in Figure 2(b)).

229
 269 **Figure 2.a:** The precipitation phase boundary of Surfactin/12-3-12 mixture over a wide
 270 range of concentration, b: The photos of 0.5 mM Surfactin/12-3-12 solutions with $\alpha_{\text{Surfactin}}$
 271 ranges from 0 to 1, c: The peptide ring of Surfactin can result in reduced precipitation,
 272 c(1) is the precipitation schematic diagram of conventional anionic/cationic mixtures
 273 (Adapted from Panswad³⁵).

334 It was reported that pure 12-3-12 aqueous solution of concentration close to 1 wt%
 337 (16 mM) was still clear by visual observation at 25 °C³⁸. However, it can be seen from
 338 Figure 2(a) that the 0.4 mM 12-3-12 solution is turbid when it was dissolved in 10 mM
 339 PBS buffer. This result means that there are big aggregates formed in 12-3-12 solution.
 340 Therefore, PBS buffer should have some effect on the aggregation behavior of 12-3-12.
 341 Then negatively charged hydrogen phosphates and dihydrogen phosphates will screen
 350 the positive charges on the head groups of 12-3-12 and weaken the electrostatic
 351 repulsion between 12-3-12 molecules, thus large aggregates of 12-3-12 formed in PBS
 352 buffer. Figure 2(a) also shows that the solution in the high 12-3-12 content region is
 353 more turbid and the region of dark grey is much larger than that in the high Surfactin
 354 content region. In aqueous solution the peptide ring of Surfactin shows a
 357 “horse-saddle” topology³⁹, the two negatively charged amino acid residues Glu and

Asp constitute a minor polar domain. On the opposite side, Val residue extends down, facing the fatty acid chain, making up a major hydrophobic domain. When mixed with 12-3-12, the hydrophobic domain of Surfactin sterically hindered the interaction between oppositely charged head groups and tail–tail interaction (see in Figure 2(c)). In Panswad's work³⁵, extended anionic surfactants (a novel class of surfactants that have groups of intermediate polarity such as polypropyleneoxides (PO) or ethylene oxides (EO) inserted between the hydrocarbon tail and the hydrophilic head group) have the similar property when mixed with cationic surfactants.

So there are no precipitates formed in Surfactin/12-3-12 mixture at low surfactant concentration. The CMC values of Surfactin/12-3-12 mixture presented in Table 1 with different $\alpha_{\text{Surfactin}}$ values, which were determined by surface tension measurements as shown in Figure 3. It can be found that all CMC values are smaller than the surfactant concentrations suggested by the precipitation phase boundary in Figure 2(a). And it is interesting that the precipitates region reduced with the increase of $\alpha_{\text{Surfactin}}$ value. This excellent property can mitigate the main disadvantage of mixed anionic and cationic surfactant systems which is their tendency to form precipitates⁴⁰. The precipitation happening in the anionic/cationic surfactant system is unfavorable for their applications, such as detergency performance and subsurface remediation of oil contamination^{40,41}.

3.2 Interaction between Surfactin and Gemini (12-3-12). Since the precipitation phase boundary of Surfactin/12-3-12 mixture has been studied, the synergism between them was expected and further studied. The surface tension (γ) as a function of total surfactant concentration with different molar fractions of Surfactin is presented in Figure 3. The relevant results obtained from the analysis of surface tension data are summarized in Table 1.

261
 262 **Figure 3.**Surface tension isotherm of Surfactin/12-3-12 system in 10 mM PBS
 263 medium at 25°C.
 264

265 **Table 1.CMC and γ_{CMC} Values of Surfactin/12-3-12 system**

$\alpha_{\text{Surfactin}}$	$\text{CMC}_{\text{exp}} \times 10^2$ (mM)	$\text{CMC}_{\text{ideal}} \times 10^2$ (mM)	$\gamma_{\text{CMC}}(\text{mN/m})$ (± 0.03)
0	18.9 $\pm$ 0.3	18.9 $\pm$ 0.3	30.43
0.1	3.75 $\pm$ 0.01	6.91 $\pm$ 0.02	30.39
0.2	1.21 $\pm$ 0.02	4.23 $\pm$ 0.03	30.15
0.4	1.14 $\pm$ 0.01	2.38 $\pm$ 0.02	29.77
0.6	1.05 $\pm$ 0.03	1.66 $\pm$ 0.05	28.91
0.8	1.06 $\pm$ 0.01	1.27 $\pm$ 0.03	30.32
1	1.03 $\pm$ 0.03	1.03 $\pm$ 0.03	30.97

266 Figure 3 presents the surface tension results of pure Surfactin, 12-3-12, and their
 267 mixtures at $\alpha_{\text{Surfactin}} = 0.1, 0.2, 0.4, 0.6$ and 0.8 . Compared with that of pure
 268 Surfactin and 12-3-12, the γ_{CMC} values of Surfactin/12-3-12 mixtures are smaller, all
 269 also in the surface tension decreasing region before CMC, a lower surfactants
 270 concentration is needed to reach the same surface tension.

271 Table 1 shows that the CMC of 12-3-12 is $1.89 \times 10^{-1} \text{ mM}$ in 10 mM PBS
 272 ($\text{pH}=7.4$). Shanget al⁴² has reported that the CMC of 12-3-12 in aqueous solution is 9.21
 273 $\times 10^{-1} \text{ mM}$, while its CMC decreased to $1.16 \times 10^{-1} \text{ mM}$ when 5 mM NaBr was added in
 274 the system, which is close to our result. The surface activity of 12-3-12 changes
 275 remarkably when Surfactin is added to pure 12-3-12 solution. When $\alpha_{\text{Surfactin}}$ value is 0.1 ,
 276 The CMC value is reduced to $3.75 \times 10^{-2} \text{ mM}$ (Table 1) by one order of magnitude
 277 compared with that of pure 12-3-12. With $\alpha_{\text{Surfactin}}$ increases to 0.8 , the CMC value
 278 gradually decreases and approaches to that of pure Surfactin. The lower γ_{CMC} and CMC
 279 values show that the mixture of Surfactin and 12-3-12 has better ability and efficiency
 280 in reducing surface tension than the individual surfactant.

281 The regular solution theory (RST) is applied to obtain $\text{CMC}_{\text{ideal}}$ values for the mixed
 282 Surfactin/12-3-12 systems, and the $\text{CMC}_{\text{ideal}}$ values were calculated according to Eq.
 283 3⁴³. Here 1 and 2 refers to Surfactin and 12-3-12, respectively. Parameters α_1 is the
 284 molar fraction of Surfactin, f_1 and f_2 are the activity coefficients of Surfactin and

12-3-12 in mixed micelles (for ideal mixing $f_1=f_2=1$), respectively.

$$\frac{1}{\text{CMC}_{\text{ideal}}} = \frac{\alpha_1}{f_1 \text{CMC}_1} + \frac{(1 - \alpha_1)}{f_2 \text{CMC}_2} \quad (3)$$

The experimental CMC values (Table 2) are always lower than the values expected for ideal mixing, indicating that there is synergism pointing to the formation of mixed micelles in Surfactin/12-3-12 solution.

The strong synergistic behavior is also supported by negative values of Molecular Interaction Parameter β^m calculated with Eq. 4 and 5 according to the Regular Solution Theory⁴⁴.

$$\frac{(X_1^m)^2 \ln [(\alpha_1 C_{12}^m / X_1^m C_1^m)]}{(1 - X_1^m)^2 \ln [(1 - \alpha_1) C_{12}^m / (1 - X_1^m) C_2^m]} = 1 \quad (4)$$

$$\beta^m = \frac{\ln(\alpha_1 C_{12}^m / X_1^m C_1^m)}{(1 - X_1^m)^2} \quad (5)$$

Where α_1 is the mole fraction of Surfactin in solution, X_1^m is the mole fraction of Surfactin in the mixed micelle. C_1^m , C_2^m and C_{12}^m is the CMC values of pure Surfactin, pure 12-3-12 and mixture respectively. The conditions for synergism to exist in the mixture are as follows: (a) β^m is negative; (b) $|\beta^m| > |\ln(C_1^m / C_2^m)|$. In Table 2, the values of β^m , and $|\ln(C_1^m / C_2^m)|$ obtained for different compositions of Surfactin/12-3-12 system are summarized. It can be seen that the average negative values of the interaction parameter β_{ave} for Surfactin/12-3-12 mixtures support the synergism between Surfactin and 12-3-12.

For conventional anionic/cationic mixtures, the β^m values are reported to be in the range of -10 to -20 by Rosen⁴⁵, while the values report here are in the range of -2 to -6. Although Surfactin and Gemini mixtures show lower synergism than conventional surfactant mixtures, this unique combination actually has better ability in practical application due to the less precipitation potential (see Fig. 2(a) and discussion). Wang²⁸ and co-workers have investigated the synergism of Surfactin/CTAB mixtures using the surface tension method over a wide range of mole fraction which means the precipitation is not heavy enough to be obstacle to CMC measurement, the β^m values of the mixtures are in the range of -1 to -3 which also show much lower

309 synergism than conventional anionic/cationic mixtures. Therefore, we can speculate
 310 that Surfactin can reduce precipitation when mixed with not only 12-3-12 but also
 311 other kinds of cationic surfactants due to its special structure.

312 **Table 2. Molecular Interaction and Synergism Parameters for Surfactin/12-3-12**
 313 **mixtures.**

α_1	β^m	X_1^m	$ \ln(C_1^m / C_2^m) $
0.1	-2.68	0.5795	
0.2	-5.77	0.5982	
0.4	-4.54	0.6885	2.91
0.6	-4.04	0.7654	
0.8	-3.28	0.8695	
$\beta_{ave} = -4.06$			

314 Prof. Maeda⁴⁶ suggested a new approach which is based on the phase separation
 315 model and describes the thermodynamic stability of mixed micelle using Gibbs
 316 energy of micellization. The values of ΔG_M , B_0 , B_1 , and B_2 were also calculated and
 317 discussed (presented in Support Information). Negative B_1 values indicate that with an
 318 increasing molar fraction of Surfactin, the chain-chain interaction initially gets
 319 stronger then becomes weak. The ΔG_M values also reveal that mixed micelles are
 320 more stable than pure surfactant micelles.

321 **3.3 Structure Study of the Aggregates in Bulk Phase**

322 **3.3.1 Dynamic Light Scattering (DLS).** DLS was performed to determine the size
 323 distribution of the aggregates, as shown in Figure 4 by the volume size
 324 distribution. Measurements were made at a constant total surfactant concentration of
 325 0.5 mM. For pure 12-3-12, it can be seen that large aggregates around 200 nm are
 326 mainly formed in PBS, which is consistent with the turbidity shown in figure 2(a). For
 327 Surfactin/12-3-12 mixtures, at higher 12-3-12 content region ($\alpha_{Surfactin} = 0.1, 0.2$, and
 328 0.3), large size aggregates with hydrodynamic r around 400 nm are mainly formed in
 329 Surfactin/12-3-12 solution. That is why the Surfactin/12-3-12 solutions at this
 330 composition are obviously turbid. With the increasing amount of Surfactin ($\alpha_{Surfactin} =$

0.4 and 0.5), the size distribution of aggregates also appears the bimodal distribution, most aggregates are around 30 nm, and there remains some amount of large aggregates around 400 nm. Thus, the Surfactin/12-3-12 solution ($\alpha_{\text{Surfactin}} = 0.4$ and 0.5) is clear and transparent. When $\alpha_{\text{Surfactin}}$ is 0.6, there are only small micelles around 6 nm in Surfactin/12-3-12 solution. At higher Surfactin content region ($\alpha_{\text{Surfactin}}$ value between 0.7 and 0.9), the size of Surfactin/12-3-12 aggregate is reduced to about 3 nm. For pure Surfactin solution, micelles around 4 nm are mainly formed, which is similar to the result reported by Zou²⁵.

Figure 4. Volume Size Distribution of Surfactin/12-3-12 Aggregates at different $\alpha_{\text{Surfactin}}$ values.

The DLS results indicate that large aggregates are formed in Surfactin/12-3-12 solution at high 12-3-12 content, and the smaller micelles start to form at high Surfactin content. This result agrees well with the phenomenon observed in Figure 2(a), where the severely turbid solution shade into clear and transparent ones with the increasing molar fraction of Surfactin.

3.3.2 Small Angle Neutron Scattering (SANS) measurements. DLS measures the hydrated size of particles (particles plus water), whereas SANS points to the “dry size”.⁴⁷ So SANS measurements were performed to further confirm the size and shape changes of Surfactin/12-3-12 aggregates with $\alpha_{\text{Surfactin}}$ values. Here the measurements were made at a constant total surfactant concentration of 0.5 mM. As shown in Figure 2, the Surfactin/12-3-12 solution is turbid when $\alpha_{\text{Surfactin}}$ is in the range of 0 to 0.5. Therefore, the SANS measurements were only performed for pure Surfactin and

354 Surfactin/12-3-12 mixtures with $\alpha_{\text{Surfactin}}$ between 0.6 to 0.8 . The SANS results are
 355 presented in Figure 5.

356
 357 **Figure 5.** The SANS results of Surfactin/12-3-12 mixtures at different $\alpha_{\text{Surfactin}}$
 358 values. Solid lines are fits according to the models described in the text below.

359 At constant concentration the scattering intensities $I(q)$ are proportional to
 360 the volume square of aggregates and the contrast between neutron scattering length
 361 densities of the aggregates and the solvent⁴⁷. Figure 5 reveals that the scattering
 362 intensity decreases with the increase of $\alpha_{\text{Surfactin}}$ value. This result is consistent with the
 363 above results in Section 3.2 that there is synergetic interaction between Surfactin and
 364 Gemini, where the interaction parameters between Surfactin and 12-3-12 decrease in
 365 the same $\alpha_{\text{Surfactin}}$ range. We have performed the data analysis by employing the Indirect
 366 Fourier Transformation (IFT) method developed by Glatter⁴⁸ and using the version
 367 reported by J. S. Pedersen⁴⁹.

368

369 **Figure 6.** $P(r)$ function obtained from the corresponding scattering curves in Figure 5.

370 The $p(r)$ functions for $\alpha_{\text{Surfactin}}=0.6-1$ were presented in Figure 6. The estimation of
 371 the micelle diameter D_{max} was obtained from the maximum distance of $P(r)$, and all
 372 the fitted data are listed in Table 3. $p(r)$ function for $\alpha_{\text{Surfactin}} = 0.6$ in Figure 6 reveals
 373 that large aggregates form in the system. Table 3 shows that radius of gyration R_g for
 374 $\alpha_{\text{Surfactin}} = 0.6$ is $87 \text{ \AA}$, which is highest among these systems and with the DLS
 375 results. The slope of scattering curve around -1 and shape of $p(r)$ function of $\alpha_{\text{Surfactin}}$
 376 $=0.6$ indicates that unspherical (elongated) aggregates form in the solution. The overall
 377 size of aggregates is too large for measured q range and it is possible to estimate that
 378 length of aggregates is larger than $300 \text{ \AA}$. The data have been analysed by IFT in
 379 approximation of infinitely long cylinder. In this case the information about cross
 380 section properties as pair distance distribution function of cross section $p_{\text{CS}}(r)$ and
 381 diameter of cross section approximately $50 \text{ \AA}$ have been obtained. When $\alpha_{\text{Surfactin}} =$
 382 0.7 , $p(r)$ function shows that both spherical micelles (symmetric part) with $D_{\text{max}} = 50$
 383 $\text{\AA}$ and rod like micelles with length of $80 \text{ \AA}$ are formed ($p(r)$ with larger D_{max} of $100 \text{ \AA}$
 384 for $\alpha_{\text{Surfactin}} = 0.7$ was also calculated and shown in supporting info). Besides, the shape

of $P(r)$ function of $\alpha_{\text{Surfactin}}=0.8$ and 1 change to symmetric shows that the aggregates shape is close to spherical.

To confirm assumption from shape of $p(r)$ function the direct modelling has been performed^[50]. At high Surfactin content the model fitting has been done with model of elliptical object with semi axis of a , b , b (model fits are shown as lines in Fig. 5). The parameters $a = b = 20 \pm 1 \text{ \AA}$ for $\alpha_{\text{Surfactin}}=1$, $a = 20 \pm 1 \text{ \AA}$ and $b = 26 \pm 1 \text{ \AA}$ for $\alpha_{\text{Surfactin}}=0.8$ were obtained. At $\alpha_{\text{Surfactin}} = 0.6$ the fit with model of cylinders with length of $280 \pm 10 \text{ \AA}$ and radius of cross section of $20 \pm 1 \text{ \AA}$ shows some deviation in intermediate q range which can point on presence of small amount of small spherical aggregates. For the most complicated case of $\alpha_{\text{Surfactin}} = 0.7$, it is possible to fit data with mixture of volume fraction 0.33 of cylinders of length $80 \pm 5 \text{ \AA}$ and cross section radius of $20 \pm 1 \text{ \AA}$ and volume fraction 0.67 of spherical objects of radius $20 \pm 1 \text{ \AA}$. Due to limited q range and SANS data measured only at one scattering contrast the fit parameters should be consider with caution.

The shape of the surfactant aggregates in solution could be predicted by the critical packing parameter (cpp) which is defined as $\text{cpp} = V_c / Al_c$, where V_c is the alkyl chain molecular volume, l_c is the extended chain length, and A is the areas per molecule. The value A was measured to be $0.78 \pm 0.04 \text{ nm}^2$ for 12-3-12⁵¹ and $1.47 \pm 0.05 \text{ nm}^2$ for Surfactin⁵² by Neutron reflectometry. The variables l_c and V_c can be calculated by Tanford's formula, eqs 6 and 7.⁵³

$$l_c = (0.154 + 0.127n_c)\text{nm} \quad (6)$$

$$V_c \approx (0.0274 + 0.0269n_c)\text{nm}^3 \quad (7)$$

where n_c is the number of methylene groups on the hydrocarbon chain of the surfactant. For Gemini surfactants, the hydrocarbon tail chains cannot extend beyond a certain length l_c , and each tail must occupy a certain volume V_c .⁵⁴ Surfactants with the cpp value of less than 1/3 are supposed to form spherical micelles. When the cpp value is between 1/3 and 1/2, it indicates that the surfactant is to form rod-shaped micelles, whereas when the cpp value is between 1/2 and 1, it generally corresponds to a monolayer or multilayer vesicles. The cpp value is 0.142 ± 0.05 for surfactin which is below 1/3, suggesting that they form spherical micelles in solution. While the value

is 0.514 ± 0.027 for 12-3-12 which is close to $1/2$, it indicates that they form rodlike micelles or vesicles in solution. In a word, The $\alpha_{\text{Surfactin}}$ value for 12-3-12 is much bigger than Surfactin. If the surfactant composition at interface is nonuniform, with each dominating in regions of preferred curvature, then the spherical Surfactin micelles can be deformed into rod-like micelles as 12-3-12 is increasingly added to the system (see in figure 7).

Table 3. The fitted results of SANS Data for the Surfactin and 12-3-12 Mixtures

$\alpha_{\text{Surfactin}}$	$D_{\text{max}} (\text{\AA})$	$I(0), (\text{cm}^{-1})$	$R_g, (\text{\AA})$
0.6	300	0.28 ± 0.01	87 ± 4
0.7	85	0.060 ± 0.001	26 ± 1
0.8	50	0.038 ± 0.001	17 ± 1
1	44	0.027 ± 0.001	16 ± 1

Thus it is summarized that at low Gemini ($\alpha_{\text{Surfactin}} = 0.8-1$) content there are spherical aggregates around 44 Å in diameter. With increase of Gemini these aggregates expand and transform to short elongated aggregates ($\alpha_{\text{Surfactin}} = 0.7$). With further increase of Gemini part of aggregates in elongated form ($\alpha_{\text{Surfactin}} = 0.6$).

434

Figure 7. Sketch of the mixed surfactants micelles in the bulk solution.

Conclusions

Surfactin, a biosurfactant has large potential applications in industrial, biotechnological, and therapeutical fields^{17,18} due to its high surface activity and less toxic. To minimize the cost of using biosurfactant Surfactin, there are a number of

430 studies on Surfactin/synthetic surfactants mixtures in recent years. These synthetic
431 surfactants include anionic Sodium Dodecylbenzenesulphonate (SDBS)⁵⁵,
432 ampholytic Betaines²⁶ and cationic CTAB²⁸. However, the precipitation condition and
433 the aggregates properties (shape and size) of such Surfactin/cationic surfactants system
434 were still unclear.

435 In the present work, we study the precipitation phase boundary and
436 micellar properties of binary systems formed by Surfactin and Cationic Gemini
437 surfactant 12-3-12. Precipitation phase boundary of mixed Surfactin and 12-3-12 were
438 evaluated to ensure that the surface tension measurements are in regions without
439 precipitate. At high concentrations, the solutions are turbid, but with the increasing
440 molar fraction of Surfactin ($\alpha_{\text{Surfactin}}$) turbidity weakened. The CMC analysis shows
441 that the mixtures exhibit synergism in the formation of Surfactin/12-3-12 mixed
442 micelles. DLS and SANS results indicated that in the high 12-3-12 content region
443 larger aggregates were formed, and with the increasing of $\alpha_{\text{Surfactin}}$, micelles of smaller
444 size were formed, so the system became more stable. Besides elongated micelles are
445 formed at $\alpha_{\text{Surfactin}}=0.6$, mixture of elongated and spherical micelles at $\alpha_{\text{Surfactin}}=0.7$ and
446 only spherical micelles at $\alpha_{\text{Surfactin}}=0.8, 1$.

447 It is interesting to note that Surfactin may have the capability to reduce
448 precipitation when mixed with cationic surfactants due to the peptide ring. The results
449 of Wang⁴⁵ also support the conjecture which has been discussed in section 3.2. In
450 addition, the asymmetrically double-tailed cationic surfactants¹⁰ and extended anionic
451 surfactants³⁵ have been reported to have the similar property to minimize precipitation
452 when mixed with opposite-charged surfactants. It is worth mentioning
453 that Surfactin/12-3-12 mixtures can reduce the interfacial tension to an ultralow level
454 in alkaline environment (see details in Supporting Information). These fundamental
455 physicochemical properties are favorable to the use of Surfactin/Gemini mixture in
456 daily chemical and oil chemistry field.

457 **Supporting Information**

458 The experimental details on the dynamic interfacial tension (IFT) between
459 surfactin/12-3-12 and crude oil in alkaline solution. The ¹H NMR Spectra and the

related analyzing date of 12-3-12. Details on the new approach to describe the thermodynamic stability of mixed micelle using Gibbs energy of micellization. $p(r)$ with larger $D_{\max}$ of 100 Å for $\alpha_{\text{Surfactin}} = 0.7$.

Acknowledgements

We gratefully acknowledge the support of this work by Shanghai Natural Science Foundation (Grant No. 15ZR1409900), the National Natural Science Foundation of China (No. 21573070), and Fundamental Research Funds for the Central Universities.

This work benefited from the use of the SasView application, originally developed under NSF award DMR-0520547.

References:

- (1) Y.Konodo, H.Uchiyama, N.Yoshino, K.Nishiyama, M.Abe, Spontaneous Vesicle Formation from Aqueous Solutions of Didodecyldimethylammonium Bromide and Sodium Dodecyl sulfate Mixtures, *Langmuir*, 11 (1995)2380-2384.
- (2) A.Khan, E.F.Marques, Synergism and Polymorphism in Mixed Surfactant Systems, *Curr.Opin.ColloidInterf. Sci.* 4 (1999)402-410.
- (3) C.Tondre, C.Caillet, Properties of the Amphiphilic Films in Mixed Cationic/Anionic Vesicles: A Comprehensive View from a Literature Analysis, *Adv. Colloid Interf. Sci.* 93 (2001)115-134.
- (4) J.M.Peacock, E.Matijevic, Precipitation of alkylbenzenesulfonates with metal ions, *J Colloid Interface Sci.* 77 (1980)548-554.
- (5) N.Kallay, M.Pastuovic, E.Matijevic, Solubility and enthalpy of precipitation of magnesium, calcium, strontium, and barium dodecyl sulfates, *J Colloids Interface Sci.* 106 (1985)452-458.
- (6) A. Mehreteab, F.J.Loprest, Formation of pseudo-nonionic complexes of anionic and cationic surfactants, *J Colloid Interface Sci.* 125(1988)602-609.
- (7) Z.J. Yu, G.Xu, Physicochemical properties of aqueous mixtures of tetrabutylammonium bromide and anionic surfactants. 1. Temperature-induced micellar growth and cloud point phenomenon, *J PhysChem.* 93(1989)7441-7445.
- (8) Z.J. Yu, Z. Zhou, G.Xu, Physicochemical properties of aqueous mixtures of tetrabutylammonium bromide and anionic surfactants. 2. Micellar growth patterns and

- the effect of intermicellar interactions on light-scattering data, *J Phys. Chem.* 93 (1989) 7446-7451.
- (9) Z.J. Yu, X. Zhang, G. Xu, G.X. Zhao, Physicochemical properties of aqueous mixtures of tetrabutylammonium bromide and anionic surfactants. 3. Effects of surfactant chain length and salinity, *J Phys Chem.* 94 (1990) 3675-3681.
- (10) A. Upadhyaya, E.J. Acosta, J.F. Scamehorn, D.A. Sabatini, Microemulsion Phase Behavior of Anionic-Cationic Surfactant Mixtures: Effect of Tail Branching, *J Surfact Deterg.* 9(2006), 169–179.
- (11) M. Bergstrom, J.S. Pedersen, Formation of tablet-shaped and ribbonlike micelles in mixtures of an anionic and a cationic surfactant, *Langmuir.* 15 (1999) 2250-2253.
- (12) M. Bergstrom, J.S. Pedersen, P. Schurtenberger, S.U. Egelhaaf, Small-angle neutron scattering (SANS) study of vesicles and lamellar sheets formed from mixtures of an anionic and a cationic surfactant, *J Phys Chem B.* 103 (1999) 9888-9897.
- (13) M. Bergstrom, J.S. Pedersen, A small-angle neutron scattering study of surfactant aggregates formed in aqueous mixtures of sodium dodecyl sulfate and didodecyldimethylammonium bromide, *J Phys Chem B.* 104 (2000) 4155-4163.
- (14) F. Baumgart, B. Kluge, C. Ullrich, J. Vater, D. Ziessow, Identification of amino acid substitutions in the lipopeptide Surfactin using 2D NMR spectroscopy. *Biochem. Biophys. Res. Commun.* 177(1991)998-1005.
- (15) K. Oka, T. Hirano, M. Homma, H. Ishii, K. Murakami, S. Mogami, A. Motizuki, H. Morita, K. Takeya, H. Itokawa, Satisfactory separation and MS-MS spectrometry of six Surfactins isolated from *Bacillus subtilis* natto, *Chem. Pharm. Bull.* 41(1993) 1000-1002.
- (16) N. Hue, L. Serani, O. Laprevote, Structural investigation of cyclic peptidolipids from *Bacillus subtilis* by high-energy tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 15 (2001) 203-209.
- (17) A.H. Zou, J. Liu, V.M. Garamus, K. Zheng, R. Willumeit, B.Z. Mu, Interaction between the Natural Lipopeptide [Glu1, Asp5] Surfactin-C15 and Hemoglobin in Aqueous Solution, *Biomacromolecules.* 11(2010)593–599.
- (18) C.N. Mulligan, Environmental applications for biosurfactants, *Env. Pol.* 133 (2005)

183-198.

(19) K.Ma, E.L.Clancy, Y.Zhang, D.G.Ray, K.Wollenberg, M.G.Zagorski, Residue-Specific pKa Measurements of the β -Peptide and Mechanism of pH-Induced Amyloid Formation, *J. Am. Chem. Soc.* 121 (1999) 8698–8706.

(20) F.M.Menger, C.A.Littau, Activation of hydrocarbons by a ruthenium(II) (fluoroalkyl)phosphine hydride complex, *J. Am. Chem. Soc.* 113 (1991) 1450-1451.

(21) F.M.Menger, C.A.Littau, Gemini surfactants: a new class of self-assembling molecules, *J. Am. Chem. Soc.* 115(1993)10083-10090.

(22) R.Zana, Dimeric and oligomeric surfactants. Behavior at interfaces and in aqueous solution: a review, *Adv Colloid and Interface Sci.* 97(2002) 205-253.

(23) J. Zhao, D.C. Sherril, B.M. Fung, Mixtures of Monomeric and Dimeric Cationic Surfactants, *J. Phys. Chem. B.* 102 (1998) 7613–7618.

(24) D.Danino, Y.Talmon, R.Zana, Alkanediyl- α,ω -Bis(Dimethylalkylammonium Bromide) Surfactants (Dimeric Surfactants). 5. Aggregation and Microstructure in Aqueous Solutions, *Langmuir.* 11 (199) 1448-1456.

(25) A.H.Zou, J.Liu, V.M.Garamus, Y.Yang, R. Willumeit, B.Z.Mu, Micellization Activity of the Natural Lipopeptide [Glu1, Asp5] Surfactin-C15 in Aqueous Solution, *J. Phys. Chem. B.* 114 (2010) 2712-2718.

(26) F.Liu, J.W.Xiao, V.M.Garamus, L.Almasy, R.Willumeit, B.Z.Mu, A.H. Zou, Interaction of the Biosurfactant, Surfactin with Betaines in Aqueous Solution, *Langmuir.* 29(2013)10648-10657.

(27) J.W.Xiao, F.Liu, V. M.Garamus, L.Almásy, U.A.Handge, R.Willumeit, B. Z.Mu, A.H.Zou, Insights into the Interactions among Surfactin, Betaines, and PAM: Surface Tension, Small-Angle Neutron Scattering, and Small-Angle X-ray Scattering Study, *Langmuir.* 30 (2014) 3363-3372.

(28) H. Wang, H.Z. Gang, R.Q. Ye, B.Z. Mu, Interaction between biosurfactant surfactin and cationic surfactant cetyltrimethyl ammonium bromide in mixed micelle, *Colloid Polym Sci.* 292 (2014) 3169–3176.

(29) Y.M.Li, N.A.Haddad, S.Z.Yang, B.Z.Mu, Variants of Lipopeptides Produced by *Bacillus licheniformis* HSN221 in Different Medium Components Evaluated by a

550 Rapid Method ESI-MS, *Int. J. Pept. Res. Ther.* 14 (2008) 229-235.

551 (30) C.S.Song, R.Q.Ye, B.Z.Mu, Molecular Behavior of a Microbial Lipopeptide
552 Monolayer at the Air-Water Interface, *Colloid Surf. A.* 302 (2007), 82-87.

553 (31) R.Zana, M.Benraou, R.Rueff, Alkanediyl- α,ω -bis(dimethylalkylammonium
554 bromide) Surfactants. 1. Effect of the spacer chain length on the critical micelle
555 concentration and micelle ionization degree, *Langmuir*. 7(1991) 1072-1075.

556 (32) L.A.Bastardo, V.M.Garamus, M.Bergström, P.M.Claesson, The Structures of
557 Complexes between Polyethylene Imine and Sodium Dodecyl Sulfate in D₂O: A
558 Scattering Study, *J. Phys. Chem. B.* 109 (2005) 167-174.

559 (33) A.Krezel, W. Bal, A Formula for Correlating pK_a Values Determined in D₂O and
560 H₂O, *J. Inorg. Biochem.* 98 (2004) 161-166.

561 (34) X.J.Fan, P.Stenius, N.Kallay, E.Matijevic, Precipitation of Surfactant Salts II. The
562 Effect of Nonionic Surfactants on Precipitation of Calcium Dodecyl Sulfate, *J*
563 *COLLOID INTERF SCI.*, 121 (1988) 571-578.

564 (35) D.Panswad, D.A.Sabatini, S.Khaodhiar, Precipitation and Micellar Properties of
565 Novel Mixed Anionic Extended Surfactants and a Cationic Surfactant, *J*
566 *Surfact Deterg.* 14 (2011) 577-583.

567 (36) A. Heinemann, S.Mühlbauer, SANS-1: Small angle neutron scattering, *Journal of*
568 *large-scale research facilities.* <http://dx.doi.org/10.17815/jlsrf-1-32>, 2015 (accessed
569 16.03.22).

570 (37) Pipich, V. QtiKWS: user-friendly program for reduction, visualization, analysis
571 and fit of SA(N)S data. URL <http://www.qtikws.de>, 2007 (accessed 16.03.22).

572 (38) R.Zana, Alkanediyl- α,ω -bis(dimethylalkylammonium bromide) Surfactants: II.
573 Krafft Temperature and Melting Temperature, *Journal of Colloid and Interface*
574 *Science.* 252 (2002) 259-261.

575 (39) J.M.Bonmatin, M.Genest, H.Labbe, M.Ptak, Solution three-dimensional structure
576 of surfactin: A cyclic lipopeptide studied by ¹H-nmr, distance geometry, and
577 molecular dynamics, *Biopolymers.* 34 (1994) 975-986.

578 (40) J.F.Scamehorn, J.H.Harwell, Surfactant-Based Separations. Science and
579 Technology, American Chemical Society, New York, 2005, pp.601.

- (41) J.H.Harwell, Factors Affecting Surfactant Performance in Groundwater Remediation Applications, in Transport and Remediations of Subsurface Contaminants,ACS, Washington DC, 1992, pp. 124.
- (42) Y.Z.Shang,T.F.Wang, X.Han,C.J.Peng,H.L.Liu, Effect of Ionic Liquids $C_n\text{mimBr}$ on Properties of Gemini Surfactant 12-3-12 Aqueous Solution,Ind. Eng. Chem. Res. 49 (2010) 8852–8857.
- (43)S. Chavda,P. Bahadur,V.K.Aswal,Interaction Between Nonionic and Gemini (Cationic) Surfactants: Effect of Spacer Chain Length,JSurfactDeterg. 14 (2011) 353–362.
- (44) D.N.Rubingh, Mixed Micelle Solutions. In Solution Chemistry of Surfactants. Springer, New York, 1979,pp.337-354.
- (45)Rosen, M.J.Surfactants and interfacial phenomena,3rd edn.Wiley, New Jersey, 2004,pp. 386-396.
- (46) H.Maeda, Simple thermodynamic analysis of stability of ionic/nonionic mixed micelles,J Colloid Interface Sci.172(1995) 98–105.
- (47)X.M. Yang,L. Zhao,L. Almasy,V.M.Garamus,A.HZou, R. Willumeit, S.J. Fan,International Journal of Pharmaceutics.450 (2013) 225– 234.
- (48)O.Glatter, A New Method for the Evaluation of Small-AngleScattering Data,J. Appl. Crystallogr. 10 (1977) 415–421.
- (49)J.S.Pedersen, Analysis of Small-Angle Scattering Data from Colloids and Polymer Solutions: Modeling and Least-Squares Fitting,Adv.ColloidInterf. Sci.70 (1997) 171-210.
- (50) I.Breßler, J.Kohlbrecher, A.F.Thünemann. SASfit : a tool for small-angle scattering data analysis using a library of analytical expressions. Journal of Applied Crystallography, 48(2015):1587–1598.
- (51) H.H Shen, R.K. Thomas, C.Y.Chen, Aggregation of the Naturally Occurring Lipopeptide, Surfactin, at Interfaces and in Solution: An Unusual Type of Surfactant, Langmuir, 25(2009)4211-4218.
- (52) X.L.Pei, C.D. Chu, R.K. Thomas, Adsorption of Gemini Surfactants with Dodecyl Side Chains and Different Spacers, Including Partially Fluorinated Spacers,

610 on Different Surfaces: Neutron Reflectometry Results, Langmuir,
 611 27(2011):1844-1852.

612 (53)C.Tanford, The Hydrophobic Effect: Formation of Micelles and Biological
 613 Membranes, Wiley, New York, 1980, pp. 51–53.

614 (54)R. Zana, GEMINI SURFACTANTSSynthesis, Interfacial and Solution-Phase
 615 Behavior, and Applications,MARCEL DEKKER, INC: New York,2004,pp. 41-42.

616 (55) S.A Onaizi, MS Nasser, F.A Twaiq,Micellization and interfacial behavior of a
 617 synthetic surfactant–biosurfactant mixture, Colloids & Surfaces A,
 618 415(2012):388–393.