Effects of Additives on the Structure of Rhamnolipid (Biosurfactant): A Small-Angle Neutron Scattering (SANS) Study

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Biosurfactants are surface active agents produced by microorganisms. The rhamnolipids used in this study, R1 and R2, are biosurfactants from the glycolipid group produced by the bacterium Pseudomonas aeruginosa [1, 2]. They are capable of effectively removing heavy metals (such as copper and zinc) from sediments [3, 4] and enhancing the removal of oil grease and metal ions from contaminated soil [5-7]. Addition of 1% NaOH showed significant enhancement of the removal of copper from sediments and mining residues [8]. The changes in heavy metal removal efficiency under different conditions presumably pertain to the change in the structure of rhamnolipid [6, 9, 10]. Small angle neutron scattering (SANS) has been widely used in resolving the structures of surfactants, phospholipids and microemulsions with the length scale ranged from 10 to 1000 Å. We have used the following two SANS instruments to investigate the morphology of the rhamnolipid in the absence and the presence of different additives including NaOH, KOH and NaCl to obtain more insight in to the structural transformations: NG3 at the National Institute of Standards and Technology (NIST) and E3 at the Canadian Neutron Beam Centre (CNBC).

The scattering data of the 2% rhamnolipid/D2O solutions containing 100mg/L of various ions (i.e., Cu^{2+}, Ni^{2+}, Zn^{2+} and all three) obtained from E3 diffractometer (Figure 1) indicate that the scattering pattern strongly depends on the pH values of the systems instead of ions. All the curves of the samples in the basic condition collapse onto one curve with a low-q plateau followed by a high-q decay, indicative of small particles. This is different from all the scattering curves of the acidic samples, which have a common pattern that two monotonic decays with different slopes at low- and high-q regimes are observed. Due to a strong smearing effect from vertical divergence, a detailed analysis is not performed on these data. We conducted a detailed neutron scattering study using higher resolution NG3-SANS (at NIST) on representative samples, which were prepared including 100 mg/L of Cu^{2+}, Ni^{2+} and Zn^{2+} ions in 2% rhamnolipid/D_2O solutions. The pH of the samples were adjusted using 10% HNO_3 and (1) NaOH (1M), (2) 1% NaOH, (3) 1% NaCl, and (4) 1% KOH, respectively to yield a value of 6.5 (S#1), 13.2 (S#2), 5.5 (S#3) and 13.2 (S#4), respectively.

The SANS data in Figure 2 shows that S#1 and S#4 have a similar pattern, while S#2 and S#3 are almost identical to each other. This result confirms the E3 neutron diffraction data, indicating that pH value is one of the most influential parameters on morphology. The scattering intensity of S#1 at the low q regime (from 0.003 Å-1 to 0.05 Å-1) follows a q^{-2} decay, a characteristic of scattering from two-dimensional objects, presumably, unilamellar vesicles. Moreover, there are weak oscillations along the curve indicating the vesicular size distribution is somewhat narrow. Therefore, a simple model could be used, a polydisperse spherical shell [11], to fit the experimental data. The shell, presumably, is composed of the rhamnolipid bilayer and the best fitting result indicates a bilayer thickness of 15 ± 2 Å, an average diameter of 550 ± 50 Å and polydispersity of 0.28 ± 0.05.

The best fitting curve does not agree with the SANS data very well at low q, presumably due to the strong influence of interparticle interaction (known as the "structure factor") or the existence of another population of smaller aggregates (e.g. micelles). However, the feature of oscillation and the position of the broad peak are captured, indicative of reasonably reliable size and polydispersity from the best fitting result. In the case of S#4, whose pH value is lower than that of S#1, the scattering pattern also shows a q^{-2} dependence at low q. However, the absolute intensity is slightly higher than that of S#1 at the same q-range and the intensity oscillation is almost absent with the broad peak seemingly shifting to a lower q value, indicative of a higher polydispersity and slightly larger particles in the system. After fitting the data using the same model, the bilayer thickness, diameter and polydispersity of the S#4 vesicles are obtained to be 14 ± 1, 580 ± 50 Å and 0.38 ± 0.10, respectively.

For both S#2 and S#3 (at strong basic condition), the intensity decays as a function of q^{-4} (corresponding to Porod's law [12] of scattering from the interface) at the low-q regime ($q < 0.007$ Å^{-1}), indicative of the existence of large aggregates (> 200 nm). Then, the intensity remains practically constant over the q range between 0.012 and 0.06 Å^{-1} followed by another q^{-4} decay at $q > 0.1$ Å^{-1}, indicative of another population of smaller aggregates, possibly micelles (Figure 2). The scattering intensity contributed from the micelles can be approximated as the following where $(R_G^2 \cdot q^2/3) \leq 1$,

1). $$I(q) \approx I(0)e^{-R_G^2 q^2/3}$$

where I(0) and R_G is the zero-angle intensity and radius of gyration of the micelles [12].

A Guinier plot, where ln[I(q)] is plotted against q^2, can therefore be constructed to obtain the dimension of the micelles (inset of Figure 2). This approach is based on the following two assumptions: the inter-micellar interaction is minimal, and the contribution of SANS intensity from large aggregates at the q region in interest (in this case, q > 0.03 Å-1) is negligible. The obtained slope of the line, ln[I(q)] vs. q^2, is $-R_G^2/3$ according to Equation 1. Applying the Guinier plot on the SANS data over a q range between 0.04 and 0.1 $Å^{-1}$ results in a value of R_G = 17.2 ± 1.0 Å. The same data analysis can be applied to the scattering curve of S#3 as well. The obtained R_G is 17.9 ± 1.0 Å, which is practically the same dimension as that of S#2 within the error. Therefore, it can be concluded that they presumably have the same micellar structure. The data are also fitted by a spherical model (the solid curves) [13] yielding a radius of 17.5 Å for both cases confirming the result from Guinier analysis. Since the larger aggregates (causing the uprising at low q) are outside the scale of the SANS probing range, we cannot conclude the structure based on current SANS data. However, they are possibly not of unilamellar structure, since the scattering decay follows q^{-4} instead of q^{-2}.

In conclusion, we are able to successfully obtain the global structures of R1 and R2 aggregates in solutions using SANS. Based on the SANS data, it can also be concluded that pH is the determining factor for the transition. In fact, the pH-sensitive vesicles have the potential for the use of controlled release nanoparticles to deliver drugs. From an environmental standing point, the pH in the media to which the metal techniques applied is a major controlling parameter in the efficiency of the process. This is due to change in the morphological transition of the rhamnolipid structure.

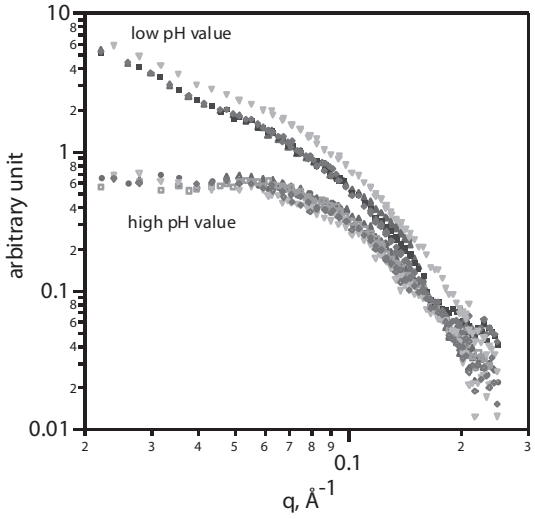

Fig. 1 CNBC E3 neutron diffraction data for samples of various ion dopants at acidic (solid symbols) and basic (open symbols) conditions: no dopant: (diamonds), Cu^{2+} (tip-up triangles), Ni^{2+} (squares), Zn^{2+} (tip-down triangles), all ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, circles).

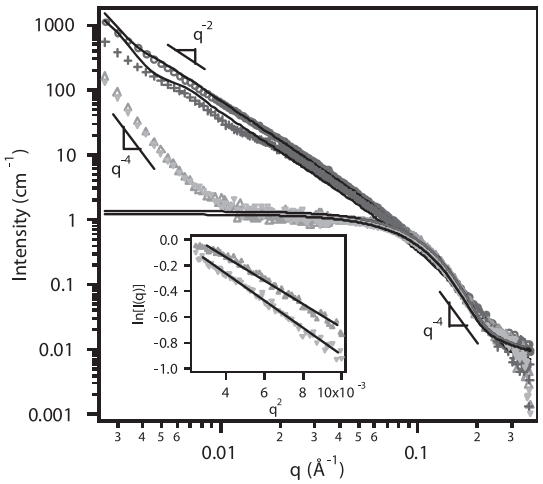

Fig. 2 NIST SANS data of S#1 (crosses), S#2 (tip-down triangles), S#3 (tip-down triangles) and S#4 (circles). The solid curves are the best fitting results for S#1 and S#4 using the polydisperse spherical shell model and S#2 and S#3 using spherical model. Inset: Giunier plots [ln(I) vs. q^2] of S#2 (tip-down triangles) and S#3 (tip-up triangles). The slopes of the solid regression lines, $-R_G^2/3$, reveal the size of micelles.

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