Application of Contrast Variation to Structural Characterization of Rhamnolipid-Styrene Aggregates – A Small Angle Neutron Scattering (SANS) Study

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Morphologies of microemulsion from water-oil-surfactant mixtures have been extensively studied for decades. The general structures observed are spherical micelles (including oil or water "droplets"), cylindrical micelles (including cylindrical inter-connected network) and lamellar phase (including bicontinuous sponge phase) with the surfactant molecules residing at the water and oil interfaces [1] – [3]. Rhamnolipid is found to enhance dramatically the solubility of organic compounds (e.g., styrene) indicating that they self-assemble into stable structures in aqueous solutions. To understand the relationship between structure and solubility, small angle neutron scattering (SANS) is used to study the global morphologies of aggregates at various concentrations and contrast conditions. The analysis shows that rhamnolipid/styrene self-assemble into a mixture of cylindrical micelles and vesicles unlike the general structures observed in microemulsions.

The rhamnolipid with the trademark JBR-425 was from Jeneil Biosurfactant Co. For the purpose of contrast variation, deuterated styrene (*d*-styrene, from Cambridge Isotope Laboratories Inc.) and hydrogenated styrene (*h*-styrene, from Fisher Scientific Canada) were used for sample preparation individually. The rhamnolipid and styrene (either *h*- or *d*-) were then mixed and dissolved into D_2O to constitute the desired rhamnolipid (c_{rham}) and styrene (c_{styr}) concentrations. Neutron scattering experiments were conducted at N5, a triple-axis neutron spectrometer adapted for SANS measurements [4]. The employed wavelengths of the neutrons, λ were 5.23, 4 and 2.37 Å, chosen by a pyrolytic graphite monochromator. The raw scattering intensities were collected as a function of scattering angle, θ, by the 32-wire detector and then were corrected by sample and empty transmissions, empty cell scattering and background scattering/noise at the same range of θ and normalized to the absolute scale by a standard solution (1% polystyrene microbead solution with a diameter of 50 nm) with a known absolute scattering curve [4]. All the SANS data are presented as a function of the scattering vector, q, defined as $(4\pi/\lambda)\sin(\theta/2)$.

Figure 1 shows the SANS data of the c_{rham} = 2.0 wt.% and c_{styr} = 3600 mg/L (*h*-styrene) solution. Since the data cannot be fitted with any model of single morphology with reasonable physical parameters, a combinational SANS model of two form factors (a core-shell sphere, $P_{CSS}(q)$ and a cylinder, $P_{cyl}(q)$) is used to account for possible combinations of cylindrical, discoidal, spherical micelles and spherical vesicles. The $P_{cyl}(q)$ expressed in Equation 1 describes two possible scenarios: cylindrical or discoidal micelles, where hydrophilic group of the rhamnolipid shields the hydrophobic core and styrene molecules from water phase. Therefore the contrast factor, $\Delta\rho^2_{cyl}$, is calculated as the square of the scattering length density (SLD) differences of solvent (i.e., D_2O) and rhamnolipid/styrene.

$$P_{cyl}(q) = 4\Delta\rho^2_{cyl}\phi_{cyl}\left(\pi R^2_{cyl}L_{cyl}\right)\int_0^{\pi/2}\left(\frac{\sin\left(\frac{qL_{cyl}}{2}\cos(\alpha)\right)}{\frac{qL_{cyl}}{2}\cos(\alpha)}\frac{J_1(qr\sin(\alpha))}{qr\sin(\alpha)}\right)^2 d\alpha, \quad (1)$$

where ϕcyl, R_{cyl} and L_{cyl} are the volume fraction, radius and length of the cylindrical (or discoidal) micelles, respectively and $J_1(x)$ is the Bessel function of the first kind of the first order.

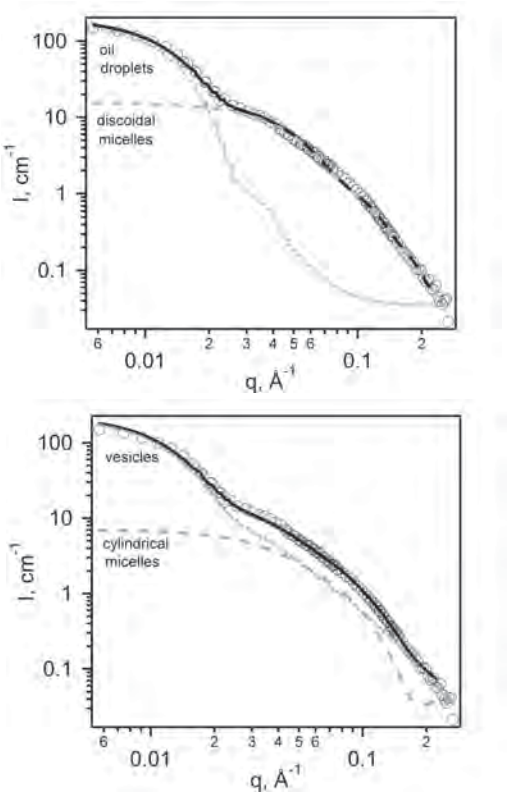

Fig 1. SANS data of the c_{rham} = 2.0 wt.% rhamnolipid solutions containing 3600 mg/L of *h*-styrene. (a) The solid, dotted and dashed lines are the best-fit using "discoidal micelles + oil droplets" combinational model, contributions from oil droplets and discoidal micelles, respectively; (b) The solid, dotted and dashed lines are the best-fit using "cylindrical micelles + vesicles" combinational model, contributions from vesicles and cylindrical micelles, respectively.

The $P_{CSS}(q)$ expressed in Equation 1 represents the other two possible morphologies: vesicles or microemulsion droplets. In the former, rhamnolipid/styrene is presumed to form a

spherical shell filled with D_2O, whereas the latter suggests rhamnolipid should form the interface of the styrene droplet and D_2O. In both cases, the outer radius, Ro of the spheres is described by a Schulz distribution function, *f(r)*, with a polydispersity of *p* defined as $\sigma/\langle R_o \rangle$, where $\langle R_o \rangle$ and σ^2 is the mean value and the variance of R_o. The scattering function is expressed in Equation 2 as follows.

$$P_{CSS}(q) = \frac{\phi_{CSS}}{V_s} \int_0^\infty f(r) \cdot [A(q,r)]^2 \, dr \,, \tag{2}$$

where ϕ_{CSS} is the volume fraction of the core-shell spheres in the solution, V_s is the volume of individual spheres and A*(q, r)* is the amplitude of the scattering intensity expressed as

$$A(q,r) = \frac{4\pi}{q^3}\left\{(\rho_i - \rho_o)\left[\sin\left(qr\frac{\langle R_i\rangle}{\langle R_o\rangle}\right) - qr\frac{\langle R_i\rangle}{\langle R_o\rangle}\cos\left(qr\frac{\langle R_i\rangle}{\langle R_o\rangle}\right)\right] + (\rho_o - \rho_{solv})[\sin(qr) - qr\cos(qr)]\right\}$$

$$\text{and } f(r) = \frac{p^{\frac{2}{p^2}}\left(\frac{r}{\langle R_o\rangle}\right)^{\frac{(1-p^2)}{p^2}} e^{-\frac{r}{p^2\langle R_o\rangle}}}{\langle R_o\rangle\Gamma\left(\frac{1}{p^2}\right)},$$

where $\Gamma(1/p^2)$ is the Gamma function to normalized the Schulz distribution, the SLDs of inner core, outer shell and solvent are ρ_i, ρ_o and ρ_{solv}, respectively and $\langle R_i \rangle$ is the core radius.

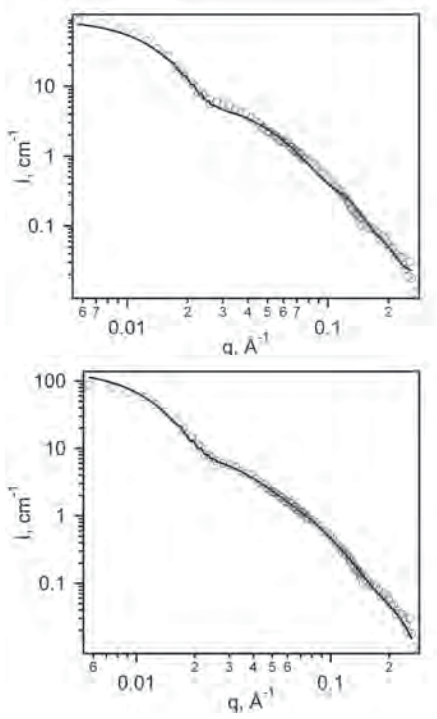

Fig 2. SANS data of the crham = 2.0 wt.% rhamnolipid solutions containing 3600 mg/L of d-styrene with the best-fit using (a) "discoidal micelles + oil droplets" and (b) "cylindrical micelles + vesicles" combinational models.

In the case of vesicles, $\rho_i = \rho_{solv} = \rho_{D2O}$ and $\rho_o = \rho_{rhamn\text{-}styr}$ (the average SLD of rhamnolipid and styrene mixture), whereas in the case of microemulsion, $\rho_i = \rho_{styr}$ (the SLD of styrene), $\rho_o = \rho_{rhamn}$ (the SLD of rhamnolipid) and $\rho_{solv} = \rho_{D2O}$. Therefore, we are able to differentiate the cases of microemulsion and vesicles through comparing the best fitting scattering curves of rhamnolipid/h-styrene and rhamnolipid/*d*-styrene in D_2O at the same conditions, assuming both result in the same morphologies (i.e., no isotope effect).

Fig 1 shows two best fitting curves using the combinational model: in one case (Figure 1a), a mixture of "oil droplet" (large spherical micelles) and discoidal micelles, where ρ_i, ρ_o and ρ_{solv} of spherical micelles are thus kept constant: $\rho_{styr} = 1.2 \times 10^{-6}$ Å^{-2}, $\rho_{rhamn} = 4.1 \times 10^{-7}$ Å^{-2} and $\rho_{D2O} = 6.4 \times 10^{-6}$ Å^{-2}. In the other case (Figure 1b), a mixture of vesicles and cylindrical micelles, where $\rho_i = \rho_{D2O}$ and $\rho_o = \rho_{rhamn\text{-}styr}$ (calculated to be ~ 5.7×10^{-7} Å^{-2}) for vesicular morphology. The intensity contribution from the individual morphology is also illustrated. Both models indicate reasonably good fits to the SANS data, although the value of χ^2 using the "cylindrical micelles + vesicles" model ($\chi^2 = 551$) seems slightly smaller than that from the "discoidal micelles + oil droplets" model ($\chi^2 = 1341$), leading to an inconclusive result.

A parallel SANS measurement is conducted on the sample under the same conditions except the h-styrene is substituted by the *d*-styrene as shown in Fig 2(a) and (b). Under the assumption of invariance in the structures, all the structural parameters are kept at the vicinity of the first best fitting values (only allowed ± 10% in variation) in either case but ρ_i and ρ_o vary freely while fitting through the "discoidal micelles + oil droplets" and "cylindrical micelles + vesicles" model, respectively. The best fits of both models seem to agree with the SANS data reasonably well in appearance. However χ^2 from the "discoidal micelles + oil droplets" model ($\chi^2 = 5008$) is nearly an order of magnitude larger than that from the "cylindrical micelles + vesicles" model ($\chi^2 = 551$). Moreover, the best fitting value of ρ_i, 2.8×10^{-6} Å^{-2}, in the "discoidal micelles + oil droplets" model is less than half of the calculated SLD value of *d*-styrene ($\rho_{d\text{-}styr} = 5.7 \times 10^{-6}$ Å^{-2}). On the contrary, the best fitting value of ρ_o, 1.16×10^{-6} Å^{-2}, in the "cylindrical micelles + vesicles" model reveals a volume fraction of 14% d-styrene in the vesicular shell, consistent with the calculated value of 15%, thus indicating that the "cylindrical micelles + vesicles" model a more valid model than the "discoidal micelles + oil droplets" model. This model also agrees with the previously reported structures of rhamnolipid aggregates at neutral and acidic conditions [5]. The SANS analysis illustrates that the application of contrast variation in neutron scattering is a powerful method to resolve the morphologies unambiguously.

References

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