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The Effects of Sugar Osmolytes on Reverse Micelle Systems

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Abstract

Reverse Micelles (RM) are nanoscopic pools of water encapsulated by an amphipathic surfactant molecule that allows the water pool to be suspended in a nonpolar solvent. We use RM systems because they allow for the study of water and dissolved osmolyte interactions in confinement. Gaining an understanding of how sugars interact with water in confinement has significant implications for biological systems. This project seeks to understand the effects of galactose as an osmolyte on the interactions, loading limits, and size of RMs when compared to RMs containing only water and RMs containing glucose. Galactose and glucose have slight structural differences, varying in the position of the hydroxyl group on the fourth carbon of each molecule allowing us to probe the structural nuances that often have large biochemical effects. RMs prepared using the surfactant Docusate Sodium (AOT) and the nonpolar solvent isooctane (2,2,4- trimethylpentane) were made in sizes of w_0=5, 10, and 20 where w_0 represents the ratio of the concentration of water to the concentration of surfactant ([water]/[surfactant]). The loading limit of galactose in RMs was determined to be less than that of glucose over a range of RM sizes with the highest loading limit found in w_0= 10 RMs. RM systems were also analyzed using Dynamic Light Scattering (DLS) to determine the impact of the osmolyte on RM size. We observed a reduction in the size of RMs when loaded with a sugar osmolyte, which we postulate happens because the interaction of the sugar with AOT headgroups disrupts the shape of RMs and causes a change in AOT surface area.

Key Molecules and RM Schematic

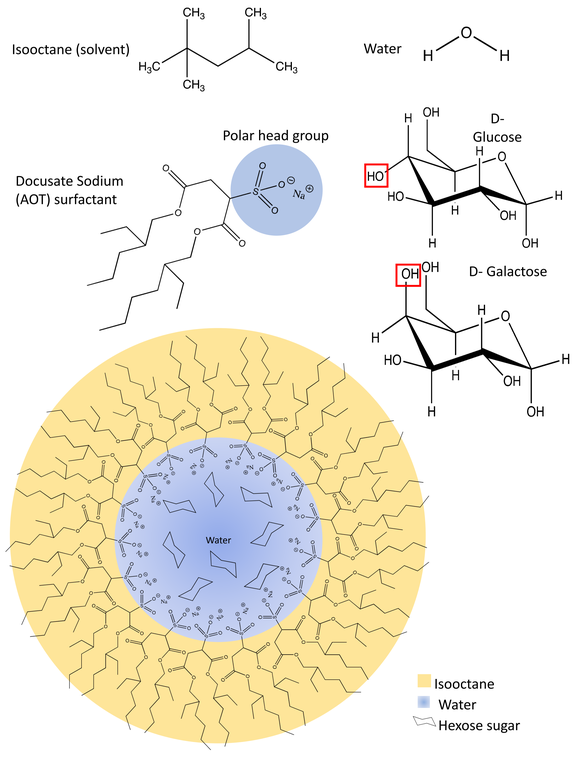


Figure 1. A diagram of a reverse micelle showing the surfactant AOT encapsulating a nanoscopic pool of water containing dissolved hexose sugars.

Methods

Making AOT Reverse Micelles

RM preparation
- Polar phase - pure water or galactose solution
- Non-polar phase - isooctane
- Surfactant - AOT

Sample sizes
- w_0=5, 10, and 20
- w_0=[water]/[surfactant]

Sample preparation
- Mix water, 0.1 M AOT in isooctane
- Sonicate 45 min
- Add galactose by mass
- Sonicate 45 min or until galactose is dissolved
- Samples equilibrate for 12 hours before use in data collection

Running DLS (Dynamic Light Scattering)
- DLS data collected using a Malvern Zetasizer SP at CSU
- Sample runs 25°C after 120 second equilibration
- 5 runs each sample with 10 measurements per run

Loading Limit experiments
- Using the sample preparation, RM mixtures were prepared with increasing quantities of galactose
- After 12 hour equilibration samples were check visually for homogeneity

Results

	Lowest ratio of water:galactose that can be dissolved into the RM
W_0 = 5	37:1
W_0 = 10	32:1
W_0 = 20	34:1

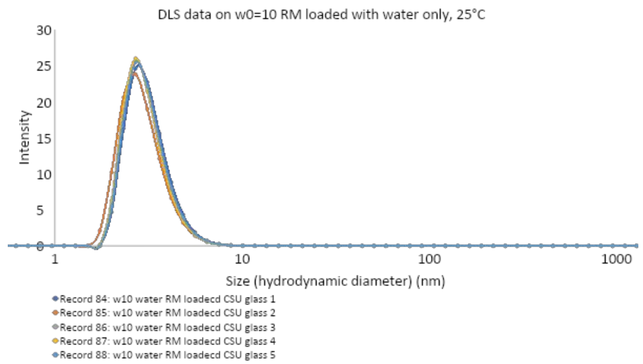
Table 1. Loading limits of galactose into AOT reverse micelles.

- The loading limit of galactose in RMs is less than that of glucose[1]. These results are preliminary and should be further investigated to determine statistical significance.
- The loading limit of galactose in RMs is greater in w_0= 10 RMs than in w_0=5 and w_0= 20 RMs.

	Diameter (nm) of RMs containing only water (polydispersity index)	Diameter (nm) of RMs containing 50:1 water:galactose (polydispersity index)	Percent size reduction (%)
W_0 = 5	4.15 (0.169)	3.08 (0.354)	25
W_0 = 10	5.71 (0.222)	4.82 (0.333)	15
W_0 = 20	7.20 (0.157)	6.47 (0.144)	10

Table 2. The measured hydrodynamic diameter of reverse micelles containing water and a 50:1 ratio of water to galactose with respective polydispersity. The percent reduction in size is also reported.

- RMs that are loaded with galactose are smaller than RMs containing only water.
- As the w_0 value increases, the percent reduction on size decreases.



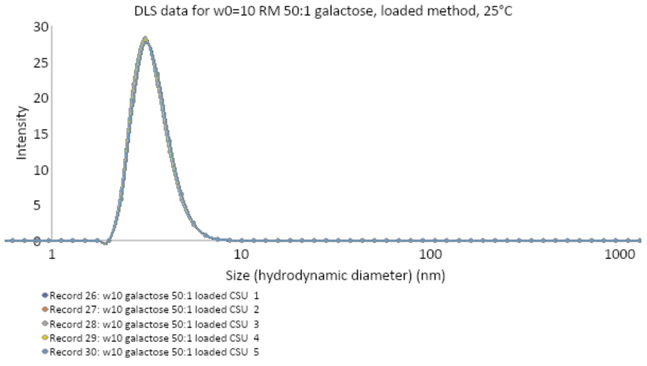


Figure 2. The distribution of size (hydrodynamic diameter) of reverse micelle particles in samples containing only water and containing 50:1 water to galactose. Record numbers indicate the five runs for each sample. Data shown is for samples made in Colorado at CSU using glass cuvettes. Note the logarithmic scale on the size axis.

- w_0= 5 and 10 RMs display an increase in polydispersity when loaded with galactose compared to loading with pure water.
- w_0= 20 RM samples do not display an increase in polydispersity when loaded with galactose.

Discussion

Loading Limits
- The loading limit refers to the maximum quantity of an osmolyte that can be dissolved into a RM sample while still forming stable RMs.
- The loading limits of galactose in RMs is less than that of glucose. This is unsurprising because galactose is less soluble in water than glucose.
- For smaller values of w_0, up to w_0=10, the loading limit increases as w_0 increases.
- We postulate that at different sizes of RMs the proportion of water located at the interface and interacting with the AOT headgroups decreases. Subsequently, the proportion of water that is free, or in the internal water pool, and available to solvate an osmolyte increases as w_0 increases.
- We propose that w_0= 20 RMs is large enough that the number and magnitude of interactions fundamentally change.

Size Data
- Because galactose is less soluble than glucose in water, the size data is for a 50:1 water to galactose ratio rather than the 30:1 ratio used by Wiebenga-Sanford et al.[1] in their glucose study.
- Galactose has a similar shrinking effect on RMs as has been previously observed with glucose[1]. At a 30:1 concentration of water to glucose, w_0=10 RMs display a 66% decrease in size[1]; at a 50:1 concentration of water to galactose, w_0=10 RMs display a 15% reduction in size.
- It is currently unknown if galactose causes the same magnitude of reduction in size as glucose because a direct comparison with the two sugars at the same concentration inside RMs has not yet been performed. If the two sugars were compared at the same concentration and a difference in size reduction were observed this could be attributed to the stereochemical differences and the impact of those differences on the intermolecular interactions between the two isomers.
- Since loading limits determined that galactose is not soluble in reverse micelles at a 30:1 ratio, new studies of glucose reverse micelles at a 50:1 concentration are needed to make this comparison.
- The reason that glucose (and galactose) create smaller RMs than those containing only water has not been conclusively determined. Wiebenga-Sanford et al.[1] proposed that the reduction in size is due to a combination of an increase in surface area per surfactant molecule and changes in RM shape. They argue that sugar osmolytes shield the sodium counterion from the polar headgroup of AOT causing an increase in surface area of the AOT molecule which effectively increases its concentration leading to a decrease in RM size. Furthermore, changes to particle eccentricity can cause an increase in the measured size of RMs because DLS instruments assume spherical particles.

Further Studies

- DLS data should be collected on fresh samples to ensure reproducibility of the size measurements.
- Loading limit experiments need to be repeated to confirm accuracy of the results.
- Complete DLS experiments on range of samples to compare the differences in RM size between RMs loaded with glucose, galactose, and mannose. Our research group has studied mannose in parallel with galactose because it is another isomer of glucose with distinct stereochemical differences. What is needed is DLS data on RMs containing the same concentrations of glucose, galactose, and mannose.
- Run COSY and NOSY NMR experiments to better understand the location and interactions of galactose within the RMs. This information would allow for a more conclusive explanation for the observed change in RM particle size when loaded with a sugar osmolyte.
- Run EXSY NMR experiments to determine the proton exchange rate between water and the hydroxyl groups of galactose and compare those rates to that of glucose. These experiments will help us better understand and characterize the interactions between water and different isomers of glucose.
- Repeat experiments using other isomers of glucose to determine the impacts of other stereochemical differences on RM systems.
- A disaccharide series could help us to understand the impacts of sugar osmolytes with added complexity which could be applied to biological systems where these osmolytes are present.

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