13 February 2021

Readme File

Data (Spreadsheets) for:

Incorporation of Membrane Proteins Into Bicontinuous Microemulsions Through Winsor-III System-Based Extraction

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Journal of Surfactants and Detergents (2021)

1. **Fig_2_CD of BR_AOT.xlsx** Data for circular dichroism (CD) spectroscopic analysis of bacteriorhodopsin (BR) in AOT/CK-2,13 bicontinuous microemulsions (BμEs). All data can be plotted and used directly by the user.

    - CD data used for **Fig 2** (plot = *CD_BR_AOT_gr* worksheet; data in *CD_data_BR* worksheet, *Col A-C*)
    - Fitting of CD data used for **Fig S1D**, via the CONTIN program) (plot = *CD_BR_AOT_fit_gr* worksheet; data in *CD_data_BR* worksheet, *Col E-G*)
    - Secondary structure content, as predicted by the CONTIN model; **Fig 2 inset** (plot = *BR_AOT_2nd_struct_gr* worksheet; data in *2nd_struct_data* worksheet)

2. **Fig_1AC_CD of asyn in AOT_CK2_13_syst.xlsx** Data for CD spectroscopic analysis of alpha-synuclein (ASYN) in AOT/CK-2,13 BμEs. All data can be plotted and used directly by the user.

    - CD) data used for **Fig 1A** (plot = *ASYN_AOT_gr* worksheet, data in *CD_data* worksheet, *Col A-G*)
    - Fitting of CD data used for **Fig S1A and B**, via the CONTIN program) (plots = *ASYN_AOT_fit_gr1* and *ASYN_AOT_fit_gr2* worksheets; data in *CD_data_BR* worksheet, *Col I-O*)
    - Secondary structure content, as predicted by the CONTIN model; **Fig 1C** (plot = *helix_gr* worksheet, data in *2nd_struct_data* worksheet)

3. **Fig_1BD_CD of asyn in SDS_pentOH syst.xlsx** Data for CD spectroscopic analysis of ASYN in SDS/pentanol BμEs. All data can be plotted and used directly by the user.

    - CD data used for **Fig 1B** (plot = *ASYN_SDS_gr* worksheet, data in *CD_data* worksheet, *Col A-E*)

- Fitting of CD data used for **Fig S1C**, via the CONTIN program) (plots = *ASYN_AOT_fit_gr1* and *ASYN_AOT_fit_gr2* worksheets; data in *CD_data_BR* worksheet, *Col G-K*)
- Secondary structure content, as predicted by the CONTIN model; **Fig 1C** (plot = *helix_gr* worksheet, data in *2nd_struct_data* worksheet)

4. **Fig_3_SANS_bulk_contrast.xlsx** Small-angle neutron scattering (SANS) data collected for BμEs in the presence or absence of ASYN and BR. All samples were bulk neutron contrast (deuteration present only in water or in oil)

   - All SANS data contained herein underwent background subtraction previously
   - The *info* worksheet provides information on where and when SANS analyses took place, and major instrumental settings.
   - Data for each sample are contained on separate worksheets
     - *Fig 3A AOT_blank* = AOT/CK-2,13 BμE system, no protein
     - *Fig 3A ASYN_AOT_1_gL* = AOT/CK-2,13 BμE system, ASYN present at 1 g/L
     - *Fig 3A ASYN_AOT_2_gL* = AOT/CK-2,13 BμE system, ASYN present at 2 g/L
     - *Fig 3A BR_AOT_1_gL* = AOT/CK-2,13 BμE system, BR present at 1 g/L
     - *Fig 3B SDS_blank* =SDS/pentanol BμE system, no protein
     - *Fig 3B ASYN_SDS_1_gL* =SDS/pentanol BμE system, ASYN present at 1 g/L
   - Each worksheet in arranged as follows:
     - *Col A* SANS data: momentum transfer, Q, $Å^{-2}$
     - *Col B* SANS data: scattered intensity, $I(Q)$, cm^{-1}
     - *Col C* SANS data: error bars for scattered intensity, $I(Q)$, cm^{-1}
     - *Col D* SANS data: error bars for momentum transfer, Q, $Å^{-1}$
     - NOTE: Error bars were not included in the figures since they were generally smaller than the data points
     - *Col F* Teubner-Strey (T-S) model fit to SANS data: momentum transfer, Q, $Å^{-1}$
     - *Col G* T-S model fit to SANS data: scattered intensity, $I(Q)$, cm^{-1}
     - The T-S model fit to the SANS data was obtained through form factor-structure factor modeling, as described in our paper
     - *Col I* Scaling factor multiplication of SANS data: scattered intensity, $I(Q)$, cm^{-1}
     - *Col J* Scaling factor multiplication of T-S model fit to SANS data: scattered intensity, $I(Q)$, cm^{-1}
     - *Block K2* Scaling factor for $I(Q)$ of SANS data and T-S model fit of SANS data
   - Graphs:
     - *ASYN+BR_AOT_offset_gr* : **Fig 3A** (SANS data for ASYN and BR in the AOT/CK-2,13 BμE system)
     - *ASYN+BR_AOT_gr* : data of Fig 3A plotted without an offset
     - *ASYN_SDS_offset_gr* : **Fig 3B** (SANS data for ASYN in the SDS/pentanol BμE system)
     - *ASYN+BR_AOT_gr* : data of Fig 3B plotted without an offset

5. **Fig_4_AOT_SANS_CMP_determination.xlsx** SANS data collected for BμEs in the absence of proteins (AOT/CK-2,13 system). All samples were film contrast (water and oil

possessing equivalent deuteration, hydrogenated surfactants). Each sample in the series differed in its (vol) % deuteration of water and oil. The purpose of this experiment was to determine the contrast match point (CMP) for BμEs: 17.2 (vol)% deuteration of water and of oil.

- All SANS data contained herein underwent background subtraction previously
- The *info* worksheet provides information on where and when SANS analyses took place, and major instrumental settings.
- *I_vs_Q_gr* worksheet: This graph is **Fig 4** [(*I(Q) vs Q*, log-log coordinates, for several different deuteration values]. Data plotted is contained in *I_vs_Q_*data worksheet
- *I_vs_Q_data* worksheet: SANS data used to create plot in *I_vs_Q_gr* [*I(Q) vs Q* for several different deuterations)]
    - All data share the same *Q* values (*Col A*)
    - "Err[*I(Q)*]" columns contain the error bars for *I(Q)*, determined through SANS data reduction software
- *0124_CMP_gr_fig* worksheet: This graph is the **inset for Fig 4** (SQRT[*I(Q)*] at *Q*=0.0124 $Å^{-1}$vs vol % deuteration). Plotted data is contained in *CMP_data* worksheet
    - *Blocks A173-179*: % deuteration
    - *Blocks C173-C179*: SQRT[*I(Q)*]
    - *Block E173-E179*: error bars for SQRT[*I(Q)*]
- *CMP_vs_Q_gr* worksheet: This graph is **Fig S2** (Apparent CMP vs. *Q* position). Data plotted is contained in *CMP_data worksheet*
    - *Col J, K, and L*: contain *Q*, Apparent CMP, and error for the Apparent CMP, respectively
    - The straight line indicates the CMP that was selected, 17.2 vol% deuteration
- *CMP_data* worksheet: Determination of the Apparent CMP at *Q* values within the range 0.0060-0.0351 $Å^{-1}$
    - *Col A, B*: Deuteration (vol % D_2O in water = % *d*-heptane in oil), *I(Q)*, organized for each of several *Q* values for a given range of 10 rows
    - *Col C*: SQRT[*I(Q)*], calculated via a simple formula
    - *Col D*: Error bars for *I(Q)*
    - *Col E*: Error bars for SQRT[*I(Q)*], calculated by uncertainty analysis, using *Col B* and *D* as inputs (Note: the uncertainty due to the background was determined to be negligible.)
    - *Col G, H*: Apparent CMP and its error bars were determined through the LINEST function: fitting of % deuteration vs SQRT[*I(Q)*]
    - *Col J-M*: % deuteration, Apparent CMP, error of Apparent CMP, and correlation coefficient (R^2) for % deuteration vs SQRT[*I(Q)*], respectively, via the LINEST function of Excel
    - *Col N*: Calculated *I(Q)* value at the CMP (17.2 vol % deuteration)
    - *Col O*: Calculated Error for *I(Q)* value at the CMP (17.2% deuteration) (Approach: we plotted Error for *I(Q)* as a percentage of *I(Q)* vs. % deuteration. Then, we interpolated the percent errors at 17.2% deuteration. The percentages, after dividing by 100%, appear in the formulae of *Col O*.)
    - The data in *Col J, N, and O* are plotted in Fig 5 (see below)

- *0060_CMP_gr* (and others) Plots to determine the CMP at Q=0.0060 Å^{-1} (and other Q values): % deuteration vs. SQRT[*I(Q)*].

6. **Fig_5_ASYN_AOT_SANS_CMP.xlsx** SANS data collected for BμEs (AOT/CK-2,13 system)in the presence of ASYN at the CMP (17.2 vol% deuteration). SANS data for ASYN-free BμEs at the CMP (estimated within the file *Fig_4_AOT_SANS_CMP_determination.xlsx*, as described above) was subtracted from the data for ASYN-encapsulated BμEs, and the resultant data was fit with a power law relationship.

- All SANS data contained herein underwent background subtraction previously
- The *info* worksheet provides information on where and when SANS analyses took place, and major instrumental settings.
- *ASYN_CMP_SANS_gr* worksheet: log-log plot of *I(Q) vs. Q* for ASYN-encapsulated and ASYN-free BμEs **(Fig. 5A)**
    - Data plotted are in the *CMP_data* worksheet: *Col J-K* and *A-B*, respectively, with error bars for *I(Q)* in *Col L* and *C*, respectively
    - Power law fit data are in the *CMP_data* worksheet: *Q and I(Q)* are in *Col A* and *H*, respectively
- *ASYN_CMP_subtd_SANS_gr* worksheet: log-log plot of *I(Q) vs. Q* for ASYN-CMP BμE data after subtraction of ASYN-free BμEs **(Fig 5B)**
    - Data plotted are in the *CMP_data* worksheet: *Col J and P*, with error bars for *I(Q)* in Col L
    - It is assumed that the error bars for ASYN-encapsulated BμEs are the same before and after subtraction of ASYN-free BμEs' scattering
    - Power law fit data are in the *CMP_data* worksheet: *Q and I(Q)* are in *Col J and U*, respectively
- *CMP_data* worksheet: Data used to prepare the two plots described above
    - *Col A-C*: SANS data for ASYN-free BμEs at the CMP: momentum transfer (*Q*), Å^{-1}, *I(Q)*, cm^{-1}, and error of *I(Q)* (cm^{-1}), respectively. This data was determined in *Fig_4_AOT_SANS_CMP_determination.xlsx*, Col J, N, and O therein, respectively.
    - *Col E-F*: Determination of the power law fit for the SANS data of *Col A and B* (ASYN-free BμEs), using the maroon-colored data.The slope, y-intercept, and correlation coefficient are in *Rows 22-24*, respectively (determined via linear regression).
    - *Col H, N*: Data generated for the power law relationship fitting the *Col A-B* data, using *Col A and Col J* as inputs (for *Q*), respectively
    - *Col J-L*: SANS data for ASYN encapsulated in BμEs at the CMP: momentum transfer (*Q*), Å^{-1}, *I(Q)*, cm^{-1}, and error of *I(Q)* (cm^{-1}), respectively. Data were measured at Bio-SANS, ORNL; reduced; and subsequently, background subtraction performed
    - *Col P*: Subtraction of I(Q) for ASYN-free BμEs (*Col N*) from *I(Q)* for ASY-encapsulated BμEs (*Col K*)
    - *Col R-S*: Determination of the power law fit for the SANS data of *Col J and P* (ASYN-encapsulated minus ASYN-free BμEs), using the green-colored data. The

LINEST function was employed (*Blocks R19-S23*). The slope, error for slope, y-intercept, error for y-int, and correlation coefficient are in *Rows 25-29*, respectively.

- *Col U*: Data generated for the power law relationship fitting the *Col J and P* data, using *Col J* as input (for *Q*)