Experimental report

26/08/2021

Proposal: 9-13-927 **Council:** 4/2020

Title: Interactions of the Choline And GEranate (CAGE) Deep Eutectic Solventwith Lipid Bilayers

Research area: Chemistry

This proposal is a new proposal

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Experimental team: Samantha MICCIULLA

Local contacts: Samantha MICCIULLA

Samples: DMPC
d54-DMPC
choline geranate (C5ONH14.2C10H15O2)
choline octanoate (C5ONH14.2C8H15O2)
choline d15-octanoate (C5ONH14.2C8D15O2)
choline d6-geranate (C5ONH14.2C10D6H9O2)

Instrument	Requested days	Allocated days	From	To
D17	3	0		
FIGARO User-supplied	3	2	28/03/2021	30/03/2021

Abstract:

Deep eutectic solvents (DES) are a form of ionic liquid, composed of mixtures of a salt with a neutral molecule, which are stable liquids at room temperature. They can be prepared using non-toxic components from natural sources and are of increasing interest in pharmaceutical formulations due to their low irritancy. In particular the choline and geranate (CAGE) DES has shown a remarkable ability to enhance transdermal delivery of pharmaceuticals, from small molecules to large proteins. The mechanism by which it enhances skin penetration however is not well understood, and so far few studies have focused on this. We propose to use contrast variation neutron reflectivity to undertake an initial study on how CAGE in dilute solutions interacts with a model lipid bilayer, and to compare this with a closely related but inactive species, choline octanoate. Neutron reflectivity and selective deuteration will provide the ability to locate the choline and geranate or octanoate species in the bilayers and so help to determine how CAGE can facilitate the passage of other species through the skin.

1.0 Objectives & Experimental

CAGE has previously been shown to insert and disrupt lipid bilayers, useful for transdermal delivery of larger pharmaceuticals [1]. However, little is known about the mechanism of activity. By comparing the interactions between CAGE and lipid bilayers with those of a non-active, yet closely-related analogue, choline octanoate (CAOT), a chemical basis for this effect may be investigated.

Overall, six systems were studied by neutron reflectivity (NR). Initially, d_{54}-DMPC bilayers were formed on SiO_2 surfaces before exposure to a deep eutectic solvent (DES). By exploiting contrast variation and selectively deuterating components of the DES, it was hoped that these could be isolated and located within the lipid bilayer. This included hCA-hGE, dCA-hGE, hCA-hOT, dCA-hOT, hCA-dOT, dCA-dOT measured under three solvent contrasts, 100% and 0% D_2O and Si-matched water (SiMW).

Data will be processed using the *Motofit* package within *Igor Pro*. Unfinished, tentative fits are shown here to help explain an initial understanding of the results.

2.0 Report

2.1 Lipid Bilayer Formation

After initial characterisation of the bare Si blocks in D_2O, blocks were exposed to small unilamellar vesicle (SUV) solutions. Here, $CaCl_2$ (aq, 1 mM) and the small size of vesicles encourages their collapse to a bilayer. Although each bilayer was characterised individually, collectively, the six bilayers were highly consistent, attaining high coverage without evidence of multilamellar formation. A good fit was achieved for these bilayers using previously found parameters [2] for a dDMPC bilayer on SiO_2 (Figure 1).

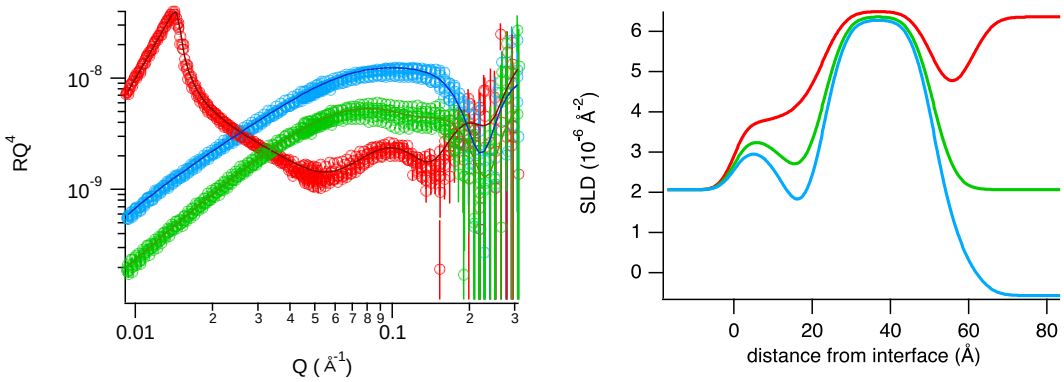

Figure 1: Data, fits and model for x6 dDMPC bilayers in (red) D2O, (blue) H2O and (green) Si-matched.

2.2 Interactions of CAGE

Post incubation with CAGE, both the hCA-hGE and dCA-hGE systems appeared to have induced distortions to the bilayer (Figure 2). Tentatively, these changes appeared to relate to the SLD of the lipid head and tail groups, as well as their hydration.

For dCA-hGE, SLD changes to the head and tail groups indicated an approximate 10% exchange of the lipid head groups with choline, and 10% exchange of the lipid tails with geranate, without dimension changes to the bilayer. There was also an associated 10% increase in hydration of the tails as well as 10% decrease in hydration of the heads. This is reasonable, as increased hydration of tail groups would be necessary for transdermal delivery, and a loss of hydration in the heads may be expected as CA replaces water molecules in this layer during insertion.

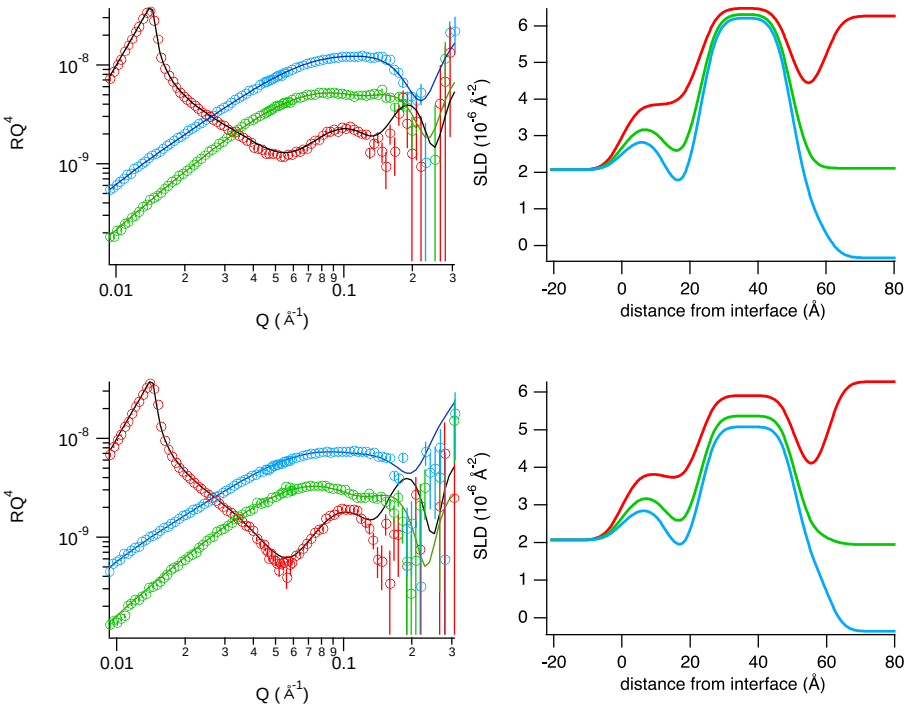

Figure 2: Bilayer fit and model (top) before and (bottom) after dCA-hGE incubation in (red) D2O, (blue) H2O and (green) Si-matched.

Changes in hCA-hGE were less apparent, however this may be expected given the lesser contrast between hydrogenated lipid head groups and hydrogenated choline. Here, SLD changes appeared to relate to a 3.5% exchange with choline and gernanate, located as before, again with the corresponding 3.5% changes to hydration.

Hence, whilst showing differences to the extent of interaction, the CAGE in both systems has been isolated to the same location within the bilayer, verified through contrast variation to choline. In both cases, there was also an associated increase in the hydration of the tails, suggesting the induced disruption of lipid packing required to facilitate transdermal delivery.

2.3 Interactions of CAOT

Incubation with CAOT also appeared to distort the bilayer, however it was apparent that this occurred by a different mechanism compared to CAGE.

To begin to explain this, attention is drawn to the systems with deuterated octanoate (dOT) (Figure 3). Although contrast variation to CA has again confirmed its location within the lipid head group region like CAGE, this is not true for the OT unit of the DES. If the insertion of the DES within the bilayer occurred in the same manner as CAGE, dOT would be expected to lower the SLD of the lipid tails only very slightly, from 6.5 to 6.3 at 100% exchange. However, a much larger decrease in the SLD of the tails was observed (5.7 and 6.1, respectively). It was believed this indicated that OT, unlike GE, was unable to penetrate the tail region, and instead only cation insertion was observed, as has been seen in other systems.

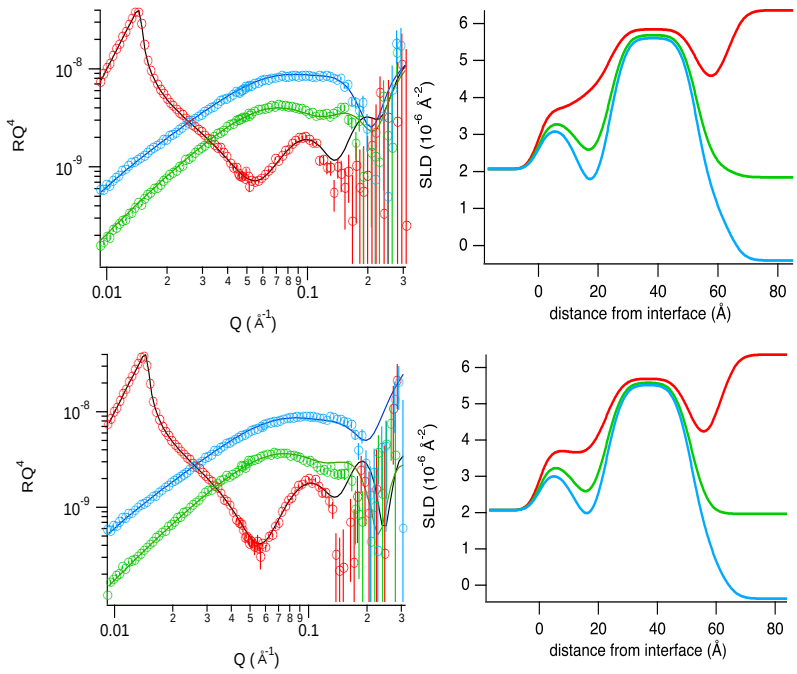

Figure 3: Bilayer fit after (Top) dCA-dOT and (Bottom) hCA-dOT incubation in (red) D2O, (blue) H2O and (green) Si-matched.

Fitting for the hCA-hOT and dCA-hOT systems is ongoing, but already indicate similar trends. Interestingly, no increase of hydration was observed for the lipid tail groups. Hence, although the bilayer has clearly been disrupted, this would not be expected to allow polar molecules to cross the bilayer. A distinct mechanistic has been identified between CAGE and CAOT, whereby anion insertion appears critical for delivery capabilities.

3.0 Concluding remarks

Tentatively, it appears that CAGE may facilitate transdermal delivery via the insertion of GE into the lipid tails, subsequently disrupting packing and causing an increase in hydration, whilst CA moieties remain tethered in the head group region. A key feature is that the rigidity of the GE molecules prevents rearrangement of the DES, as was seen by the separation of cation and anion in the mechanism of activity of CAOT.

This work will contribute towards the PhD thesis of George Neville, and we expect to publish the work within the next few months. We would like to express strong thanks to the beamline scientist, Samantha Micciulla who ran the samples for us at FIGARO due to our inability to travel during the COVID pandemic and was very helpful with advice and assistance during the remote experiment.

4.0 References

1. Qi, Q.M.; Mitragotri, S. Mechanistic study of transdermal delivery of macromolecules assisted by ionic liquids. *Journal of Controlled Release* **2019**, *311-312*, 162-169, doi:https://doi.org/10.1016/j.jconrel.2019.08.029.

2. Hall, S.C.L.; Clifton, L.A.; Tognoloni, C.; Morrison, K.A.; Knowles, T.J.; Kinane, C.J.; Dafforn, T.R.; Edler, K.J.; Arnold, T. Adsorption of a styrene maleic acid (SMA) copolymer-stabilized phospholipid nanodisc on a solid-supported planar lipid bilayer. *Journal of Colloid and Interface Science* **2020**, *574*, 272-284, doi:https://doi.org/10.1016/j.jcis.2020.04.013.