Novel Eutectic Mixtures for Cryopreservation

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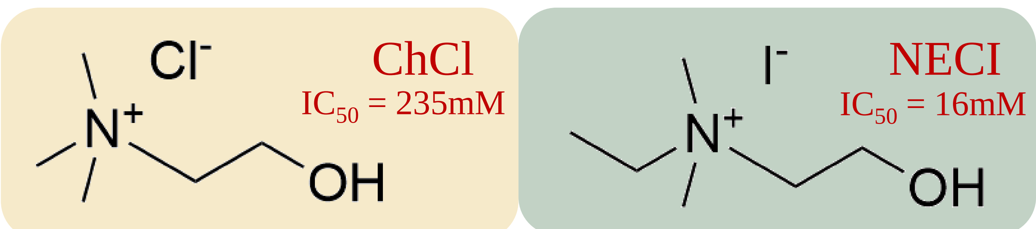
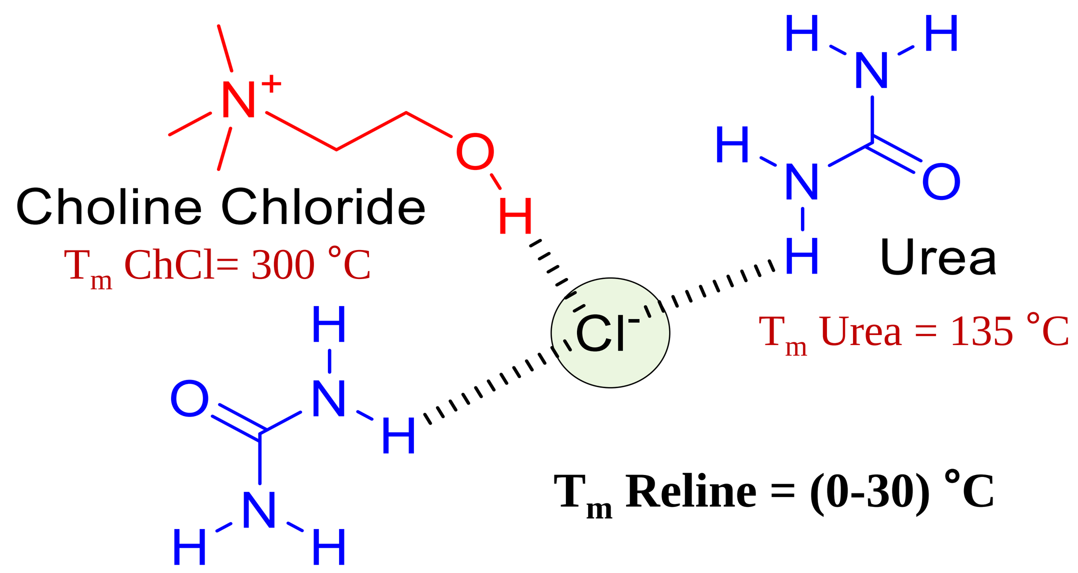
Introduction

Deep eutectic solvents (DES) form a mixture that exhibits lower melting and freezing points than its individual components. They are biocompatible, cost effective, and have low toxicity.[1]

This project aims to make a biocompatible cryoprotectant utilizing a ChCl derivative, NECI





DES show cryopreservation potential but lack ice recrystallization inhibition (IRI) activity. This project adds NECI, a ChCl-derived IRI, to address that gap.

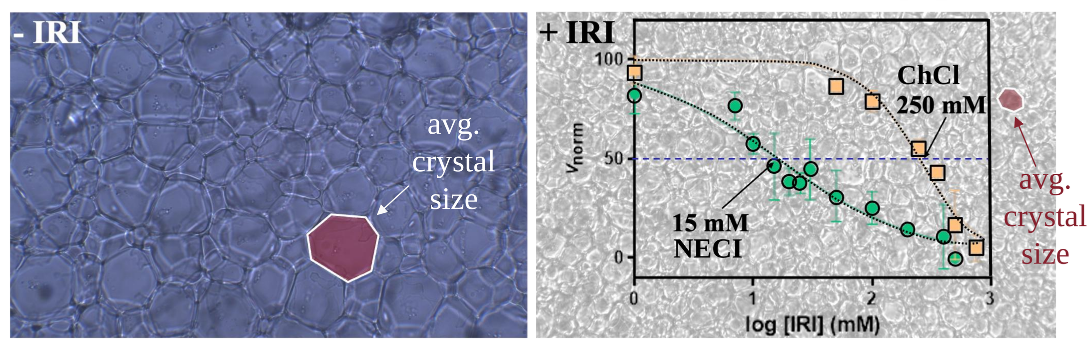


Figure 1: (left, blue) control IRI assay, (right, gray) 200mM NECI IRI assay, IC_{50} overlay showing NECI (green) vs ChCl (orange)

DES in cryomedia are made with 5-10% w/v[1], but mixtures over 51 wt% water create aqueous solutions.[3]

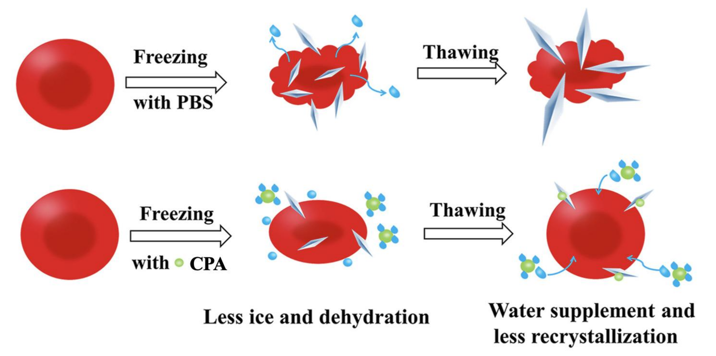


Figure 2: graphic showing how ice can impact red blood cell preservation: damaged (top), protected (bottom)[4]

Objectives

- Determine the eutectic point between NECI and Urea
- Determine the eutectic point between NECI and Glucose, and glucose derivatives
- Determine how water impacts the eutectic mixture

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Methods

Preparing eutectic mixture w/out H_2O:

- Add desired molar ratio of NECI and urea (or glucose) to mortar
- Grind with pestle for 10 mins
- Prepare ~2 mg sample for DSC

Preparing eutectic mixture w/H_2O:

- Add desired molar ratio of NECI and urea (or glucose) to mortar
- Add either 10% w/v water or 10 wt% water
- Grind with pestle for 10 mins
- Prepare ~2 mg sample for DSC

Differential Scanning Calorimetry (DSC) Methods:

- Linear: equilibrate at 90°C for 30 mins, cool at 5°C/min to -40°C, hold for 5 mins, heat at 5°C/min to 90°C
- Modulated: same as linear method except during cooling and heating, temp modulates ±2°C every 2 mins

Results (NECI:Urea)

By analyzing the melting peaks, the eutectic ratio between NECI:Urea was found to be 35:65 using the linear heating method

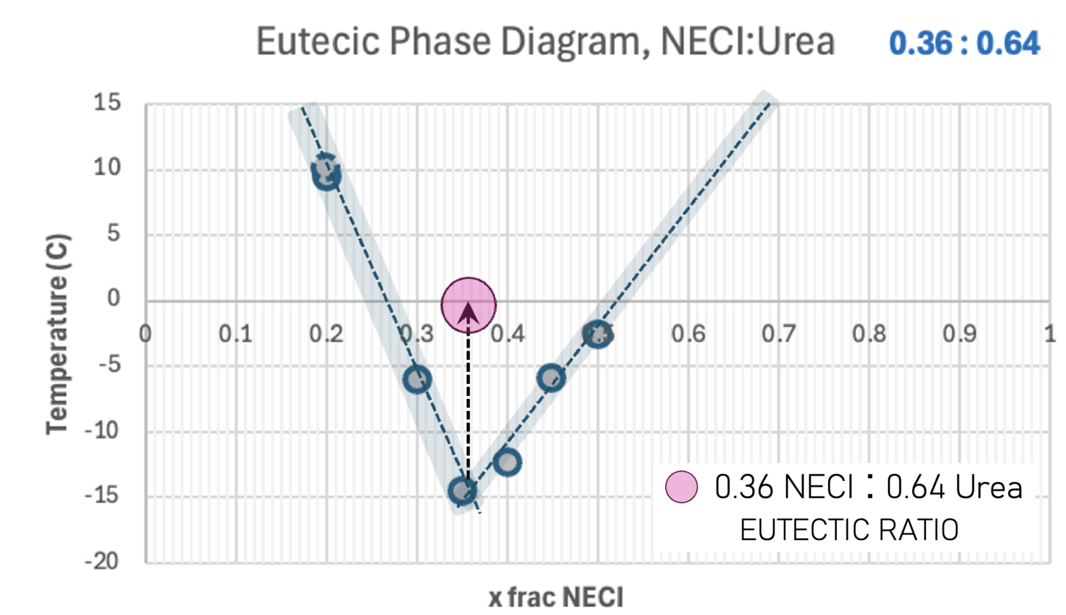

Figure 3: graph showing the correlation between molar fraction of NECI in DES and temperature

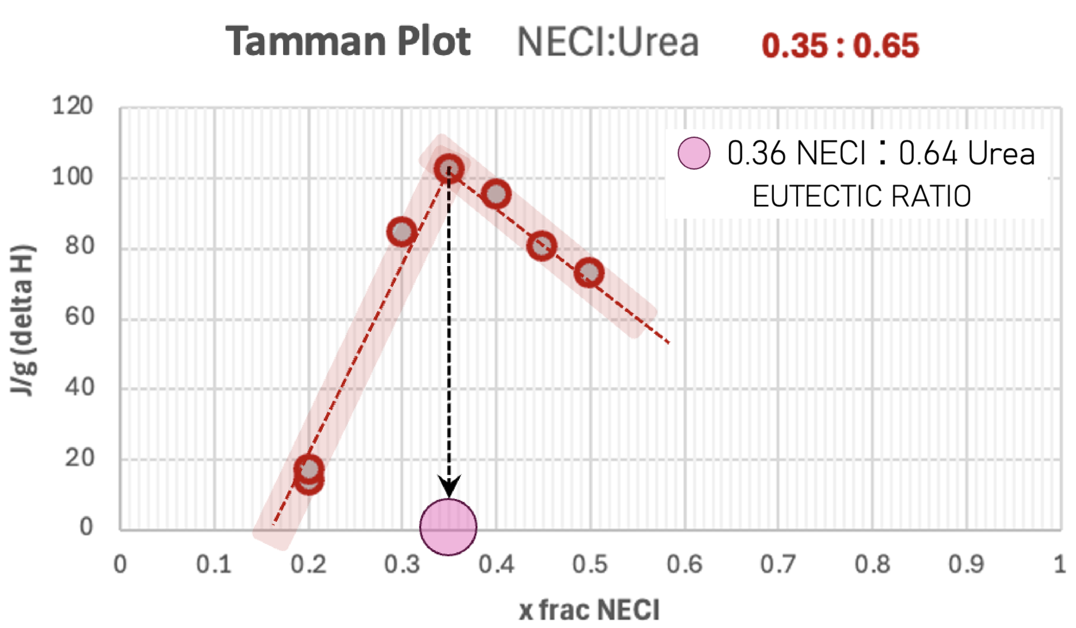

Figure 4: graph showing the correlation between molar fraction of NECI in DES and change in enthalpy

This is close to the tabulated eutectic ratio for ChCl:Urea, which is 33:67[5]

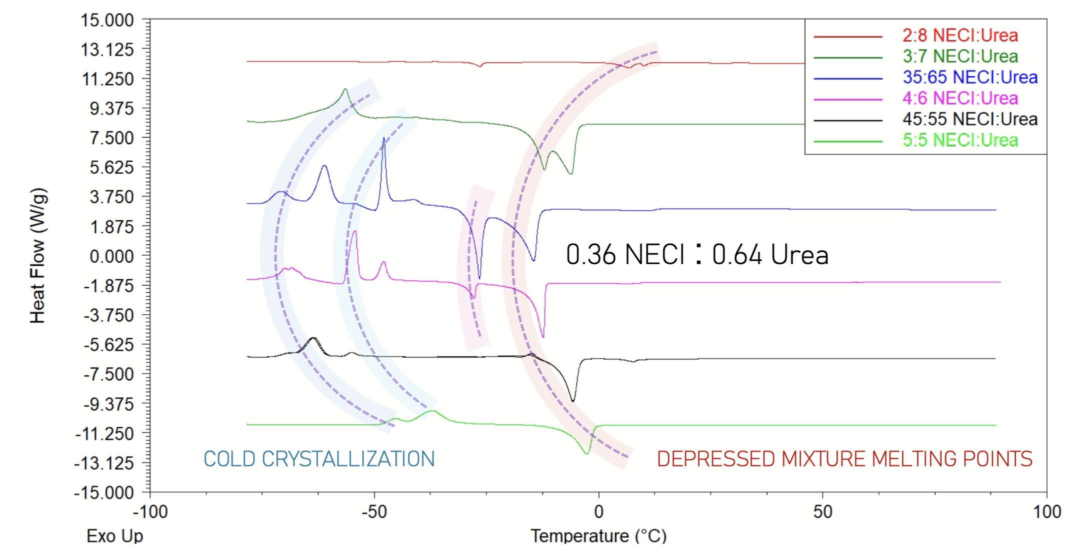

Figure 5: overlay of DSC runs for various NECI:Urea ratios

Results (NECI:Glucose)

Analyzing the melting peaks for NECI:Glucose proved to be inconclusive. However, analyzing the freezing peaks showed more promising results.

Using the modulated DSC temperature method and the data from the freezing peaks, the eutectic ratio for NECI:Glucose appears to be either 3:1 or 3:2 These values differ from the ChCl:Glucose eutectic ratio 1:1[6]

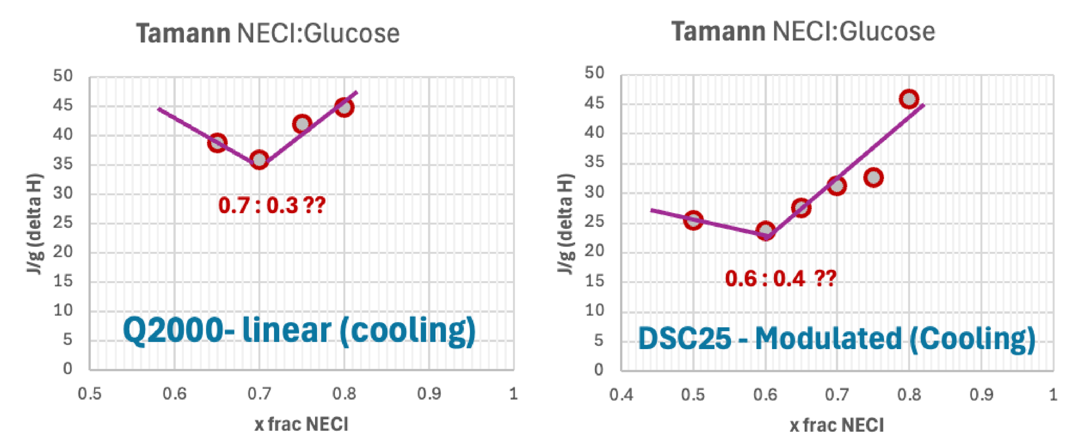

Figure 6: (left) correlation between molar fraction of NECI in DES and enthalpy using linear DSC method, (right) relationship between molar fraction of NECI in DES and enthalpy using modulated DSC method

There were difficulties in observing the eutectic point by DSC for NECI:Glucose using the linear temperature method on one of DSC instruments (DSC25). However, running with the modulated temperature method produced great results on both DSC instruments.

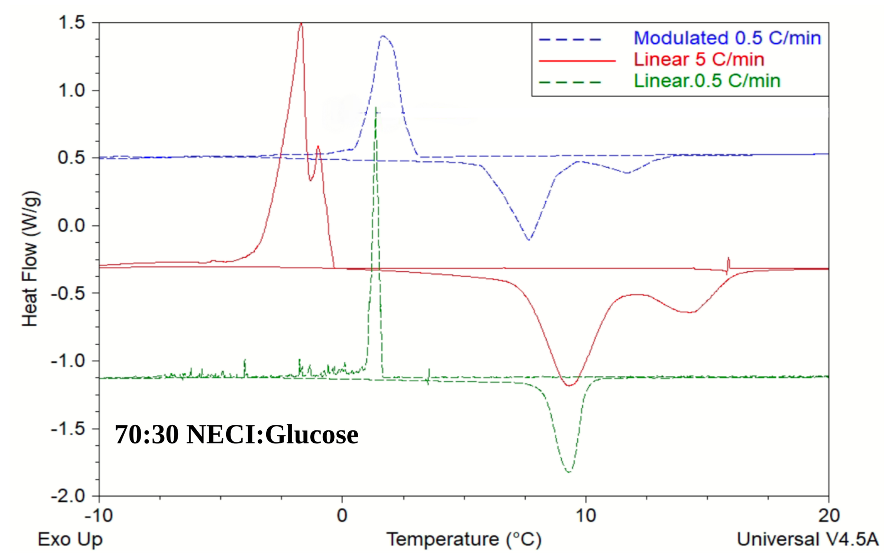

Figure 7: 70:30 NECI:Glucose overlay of different DSC methods

Water that is used to prep the cryoprotectant likely impacts H-bonding within the DES. Small amounts of water have been shown to incorporate into the DES lattice via H-bonding and improve the eutectic properties.

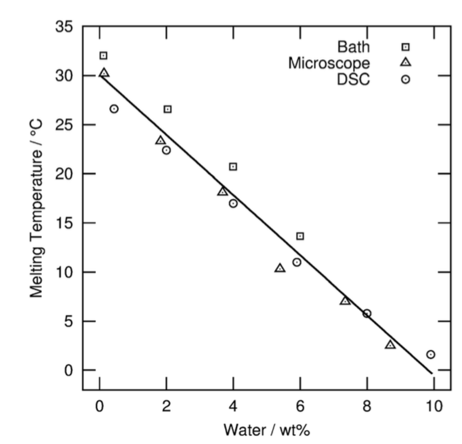

Figure 8: graph of DES ChCl:Urea melting temp changing due to wt% of water[5]

Results (Impact of H_2O on DES)

Too much water dilutes the DES, creating an aqueous solution. Some DES is maintained, but water dilutes the H-bonding in the DES. Small amounts of water conserved and enhanced eutectic properties.

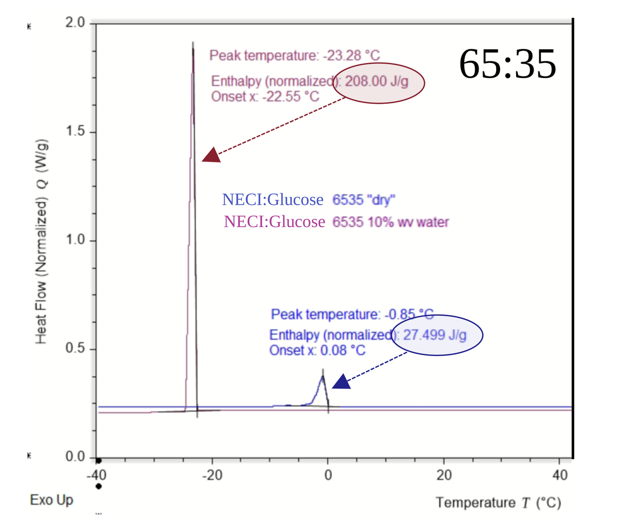

Figure 9: 65:35 dry NECI:Glucose compared to 65:35 NECI:Glucose in 10% w/v H_2O

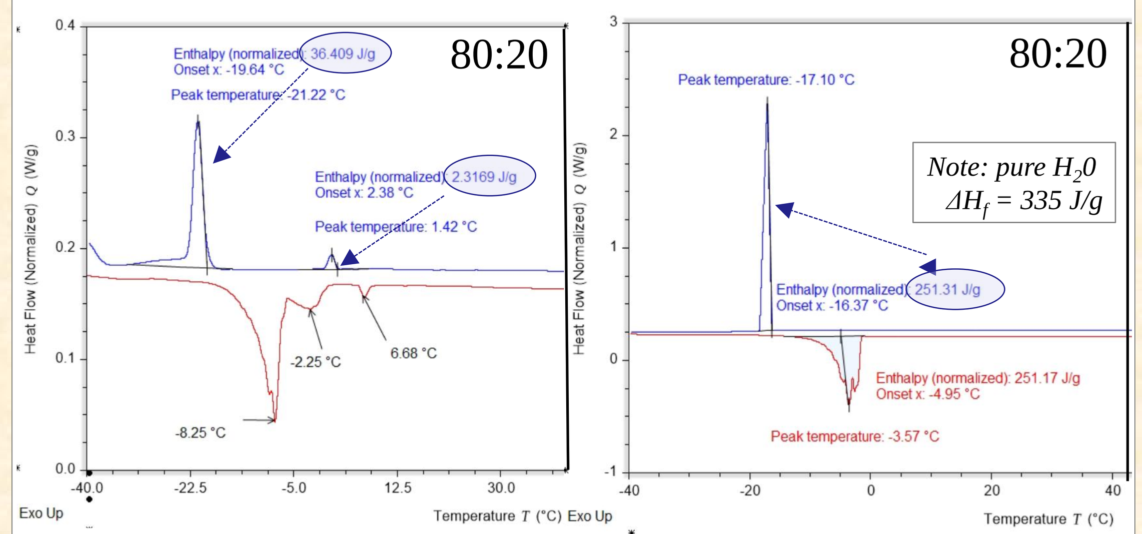

Figure 10: (left) 80:20 NECI:Glucose with 9 wt% H_2O, (right) 80:20 NECI:Glucose with 10% w/v (90 wt%) H_2O

Conclusions

- NECI:Urea eutectic molar ratio is 35:65 (i.e. 1:2)
- Modulated heating/cooling DSC method gives more useful results than linear heating/cooling
- NECI:Glucose eutectic molar ratio is either 3:1 or 3:2
- Mixing DES and water makes a tri-component DES
  - Too much water causes that tri-component DES to act like an aqueous (homogeneous) solution
  - Smaller amounts of water promote H-bonding in the DES and maintain eutectic with lower T_m

Next Steps

- Begin testing NECI with *glucose derivatives* to find their eutectic points relative to that with glucose
- Test other *choline derivatives* with urea to determine the impact of halogen type on the eutectic ratio
- Redo the NECI:Urea analysis using modulated DSC
- Explore range of different wt% water to see which ratio of DES:water is ideal (and suited to cryo apps)

References

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