Biobased Ionic Liquids as Cryo- and Lyoprotective Agents in TransfersomILs

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BACKGROUND

Transfersomes are flexible nanovesicular systems with potential for controlled delivery of bioactive.

Ionic liquids (ILs) offer tunable physicochemical properties, low volatility, and the ability to modulate interfacial interactions.

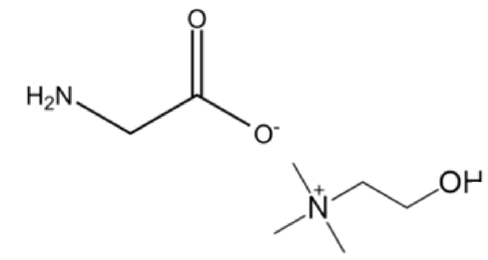
TransfersomILs combine the membrane flexibility of transfersomes and IL-enhanced colloidal stability.

Freeze-thawing and -drying are commonly used for stabilization but can cause membrane disruption, aggregation, and loss of encapsulated compounds.

BIOBASED IONIC LIQUIDS

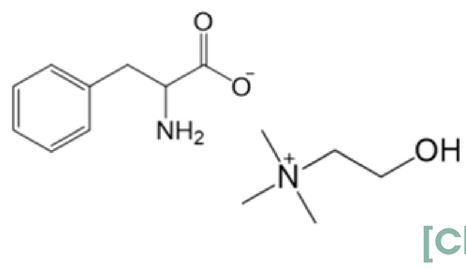
[Cho][Gly]

(2-hydroxyethyl)-trimethylammonium glycinate



[Cho][Phe]

(2-hydroxyethyl)-trimethylammonium-L-phenylalaninate)



HYPOTHESIS: Biobased ILs can act as cryo- and lyoprotective agents in TransfersomILs, maintaining physicochemical stability and antioxidant activity after freezing strategies

MATERIALS & METHODS

ILs Synthesis

[Cho][Gly] (yield = 68.7%)
[Cho][Phe] (yield = 65.2%)

TransfersomILs Preparation

Unloaded and Loaded with Caffeic Acid (CA) nanosystems with ILs at non-toxic concentrations

Freezing Strategies

Freeze-thawing
(4 hours)

Freeze-drying
(12 hours)

Characterisation of TransfersomILs

- Physicochemical properties
- Association Efficiency (AE)
- Ratio of Size
- Retention Efficiency
- Antioxidant Activity (Radical Scavenging Capacity (RSA) by DPPH Assay)

RESULTS

Table 1: Physicochemical properties, AE (%), and radical scavenging capacity (%) of unloaded and caffeic acid (CA) loaded TransfersomILs with and without [Cho][Phe] or [Cho][Gly], at 0.2%v/v. (n=3, mean ± SD)

Formulation	Size (nm)	Polidispersity Index	Zeta Potential (mV)	Association Efficiency (%)	Radical Scavenging Capacity (%)
Water	119 ± 3	0.25 ± 0.02	-26 ± 4	-	12 ± 2
[Cho][Phe]	75 ± 2	0.21 ± 0.01	-35 ± 2	-	10 ± 1
[Cho][Gly]	73 ± 2	0.24 ± 0.01	-31 ± 2	-	9 ± 3
CA	115 ± 2	0.21 ± 0.01	-20 ± 3	67.7 ± 1.3	61 ± 3
Ca + [Cho][Phe]	75 ± 2	0.21 ± 0.01	-44 ± 3	79.2 ± 0.8	65 ± 5
CA + [Cho][Gly]	71 ± 3	0.20 ± 0.01	-38 ± 3	87.5 ± 1.5	66 ± 2

FREEZE-THAWING

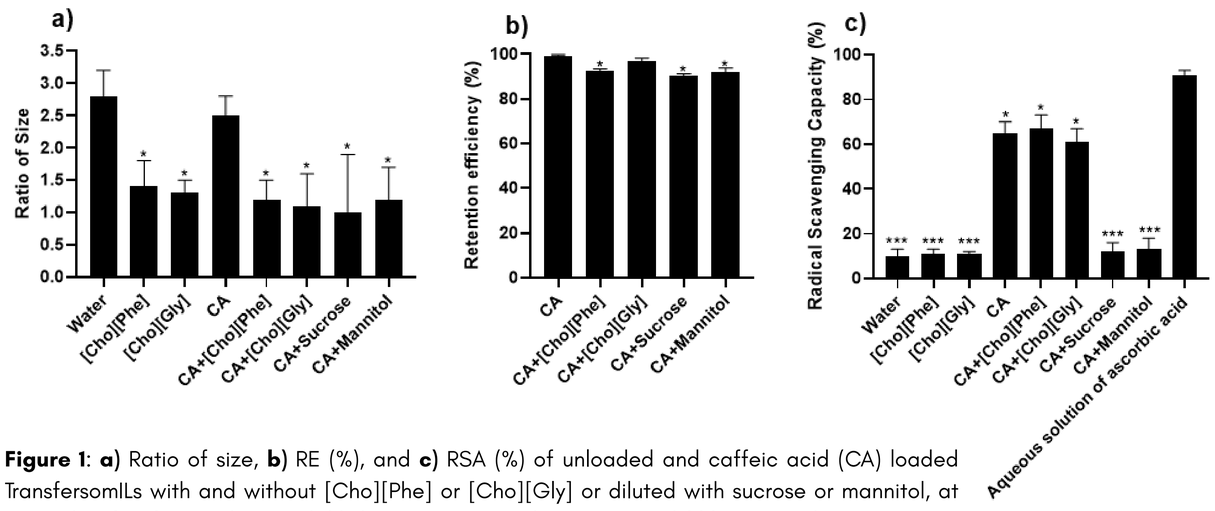


Figure 1: **a)** Ratio of size, **b)** RE (%), and **c)** RSA (%) of unloaded and caffeic acid (CA) loaded TransfersomILs with and without [Cho][Phe] or [Cho][Gly] or diluted with sucrose or mannitol, at 0.2%v/v, after freeze-thawing (4h). (n=3, mean ± SD, * $p < 0.05$, and *** $p < 0.001$)

FREEZE-DRYING

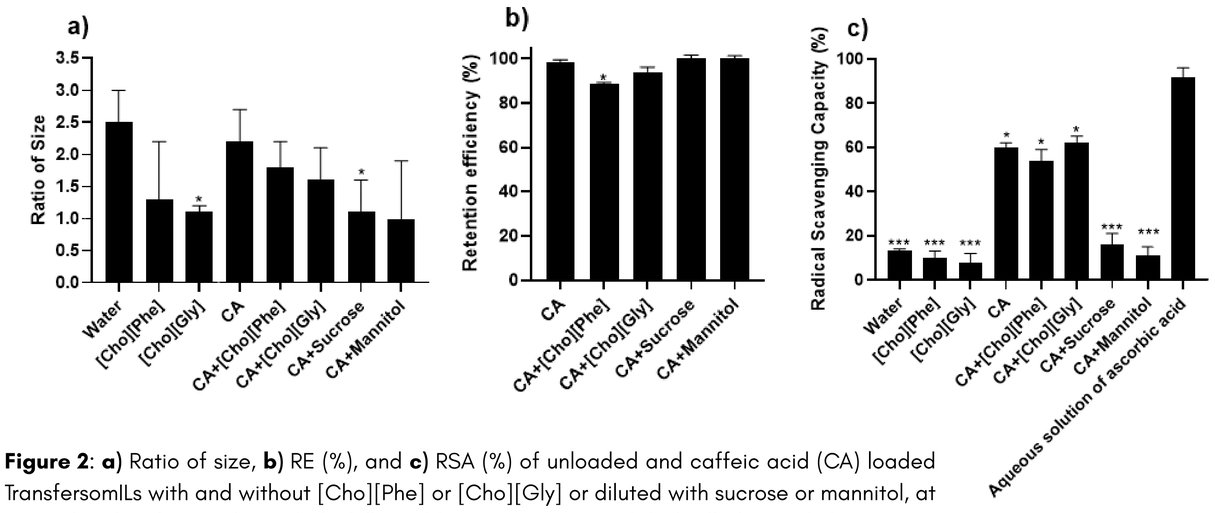


Figure 2: **a)** Ratio of size, **b)** RE (%), and **c)** RSA (%) of unloaded and caffeic acid (CA) loaded TransfersomILs with and without [Cho][Phe] or [Cho][Gly] or diluted with sucrose or mannitol, at 0.2%v/v, after freeze-drying (12h, followed by resuspension with bidistilled water). (n=3, mean ± SD, * $p < 0.05$, and *** $p < 0.001$)

DISCUSSION & CONCLUSION

- Biobased ILs [Cho][Phe] and [Cho][Gly] at 0.2% (v/v) seem to protect TransfersomILs during freeze-thawing, preserving particle size and colloidal stability.
- In freeze-drying, these ILs partially retained their retention efficiency while maintaining physicochemical properties and antioxidant activity.
- Biobased ILs show promise as sustainable, multifunctional cryoprotective agents for nanovesicular systems.
- Further optimization is needed to enhance their lyoprotective capacity and achieve higher retention efficiencies.

REFERENCES

[1] Júlio, A., Costa, J. G., Pereira-Leite, C., & Santos de Almeida, T. (2021). TransfersomILs: From Ionic Liquids to a New Class of Nanovesicular Systems. Nanomaterials (Basel, Switzerland), 12(1), 7. https://doi.org/10.3390/nano12010007

[2] Khayrani, A. C., Fahmi, M., Nurhayati, R. W., Manas, N. H. A., & Suhaeri, M. (2024). Effect of freeze-thaw cycles method on transfersome characteristics for growth protein encapsulation. International Journal of Technology, 15(2), 267-278. https://doi.org/10.14716/ijtech.v15i2.6670

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