Natural Deep Eutectic Solvents: a promising green approach to increase the bioavailability of BCS class II drugs

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1. Introduction

Most orally administered drugs, especially those belonging to BCS classes II and IV, are characterized by low water solubility and this requires a complex formulation design and adequate technologies to improve their limited solubility and/or the dissolution rate [1]. The pursued strategies are many but a lot of them display pros and cons. In recent years, a great deal of attention has been paid to more sustainable pharmaceutical manufacturing processes and **Natural Deep Eutectic Solvents (NaDES)** have emerged as potential solvents for drugs bioavailability enhancement [2]. NaDES are natural eutectic mixture obtained by the complexation of a Hydrogen Bond Acceptor (HBA) with a Hydrogen Bond Donor (HBD). NaDES meet the principles of Green Chemistry in fact they are produced with simple methods, using inexpensive, natural and non-toxic substances and their chemical-physical characteristics can be adapted as needed [3].

2. Aim

The aim of this project was to increase the solubility of four BCS class II drugs (domperidone - **DOM**, cinnarizine - **CIN**, nimesulide - **NIM**, tolbutamide - **TBM**) with different physico-chemical properties such as pKa, log P and melting point (**Table 1**) and to investigate the molecular interactions between NaDES and low soluble APIs. Then, we have investigated the biopharmaceutical properties of loaded NaDES using in vitro dissolution tests in buffer solutions and in biorelevant media. For this reason, we have tested and characterized NaDES based on different combinations of HBA and HBD (**Table 2**).

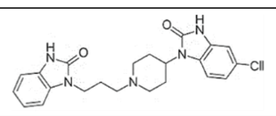
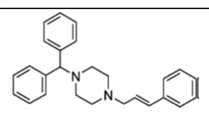
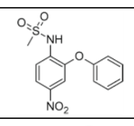
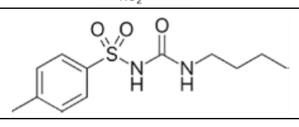
APIs	Chemical structure	MW (g/mol)	pKa	logP	MP (°C)
DOM		425.91	7.9	9.90	242.5
CIN		368.52	8.1	5.77	117–121
NIM		308.31	6.7	2.56	143-144.5
TBM		270.35	5.16	2.34	128.5

Table 1. Structure and characteristics of the APIs under investigation

3. NaDES preparation

NaDES	HBA	HBD	Molar ratio	H_2O (%w/w)
1	Choline chloride	Glucose	5:2	2.5
2		Xylitol	5:2	5
3		Proline	2:1	27.5
4		Citric acid	3:1	7.5
5			2:1	7.5
6		Tartaric acid	2:1	7.5
7		Malic acid	1:1	5
8	Proline	Glucose	1:1	22.5
9		Xylitol	1:1	20
10	Citric acid	Glucose	1:1	20

Table 2. NaDES composition

NaDES 1 and 2 were unstable during storage, suggesting that the selected molar ratio and/or the amount of added water was not appropriate. For this reason, these two NaDES were excluded from the next steps of this project.

Ternary NaDES with a specific molar ratio were prepared in glass scintillation vials with caps by stirring the mixture of solids slowly setting the temperature at 70°C until a clear and homogeneous solution was observed (**Figure 1**).

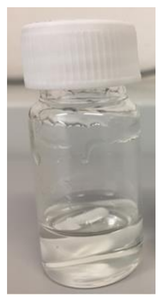


Figure 1. NaDES

A certain amount of water was included in all NaDES composition (**Table 2**) to allow the formation and/or to reduce the preparation time and viscosity. Finally, the mixture was allowed to cool down to room temperature.

4. Characterization of unloaded NaDES

NaDES	Calculated water amount (%) ± SD	pH	Viscosity ± SD (mPa*s)
3	31.36 ± 1.33	7.0	40
4	8.56 ± 0.10	2.0-2.5	2070 ± 170
5	8.51 ± 0.08	2.0-2.5	3327 ± 340
6	8.11 ± 0.08	1.5-2.0	2693 ± 101
7	6.00 ± 0.20	1.5-2.0	2115 ± 304
8	24.61 ± 0.17	6.0-6.5	2070 ± 210
9	20.67 ± 0.36	7.0	1008 ± 238
10	20.50 ± 0.45	1.0-1.5	3980 ± 57

Table 3. Characterization of stable NaDES

The stable unloaded NaDES were characterized in terms of water content, pH and viscosity.
The results (**Table 3)** show that the type of HBA/HBD, their molar ratio and water amount strongly affect the pH, the viscosity and thus the extent of H-bonding interactions. In fact, NaDES 10 showed the highest viscosity despite the 20% of water.

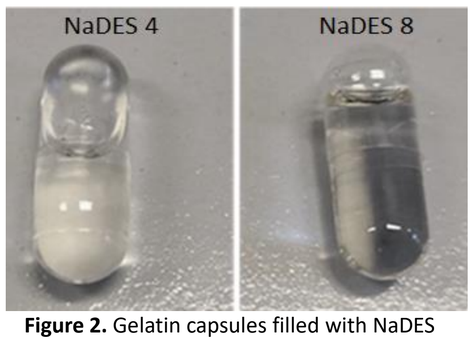


Figure 2. Gelatin capsules filled with NaDES

Hard gelatin capsules filled with NaDES containing up to about 25% by weight of water **(Figure 2) maintained their integrity until 6 months, proving that all water participate in H-bonds network.**

5. APIs solubility increase

The greatest solubility enhance of APIs was obtained with acidic NaDES. The solubility increase was significant in all NaDES compared to buffer solution at pH 1.2 (**Figure 3**). In particular, NaDES 7 resulted the best solvent for DOM, CIN and NIM; while TBM reached the highest solubility in NaDES 4.

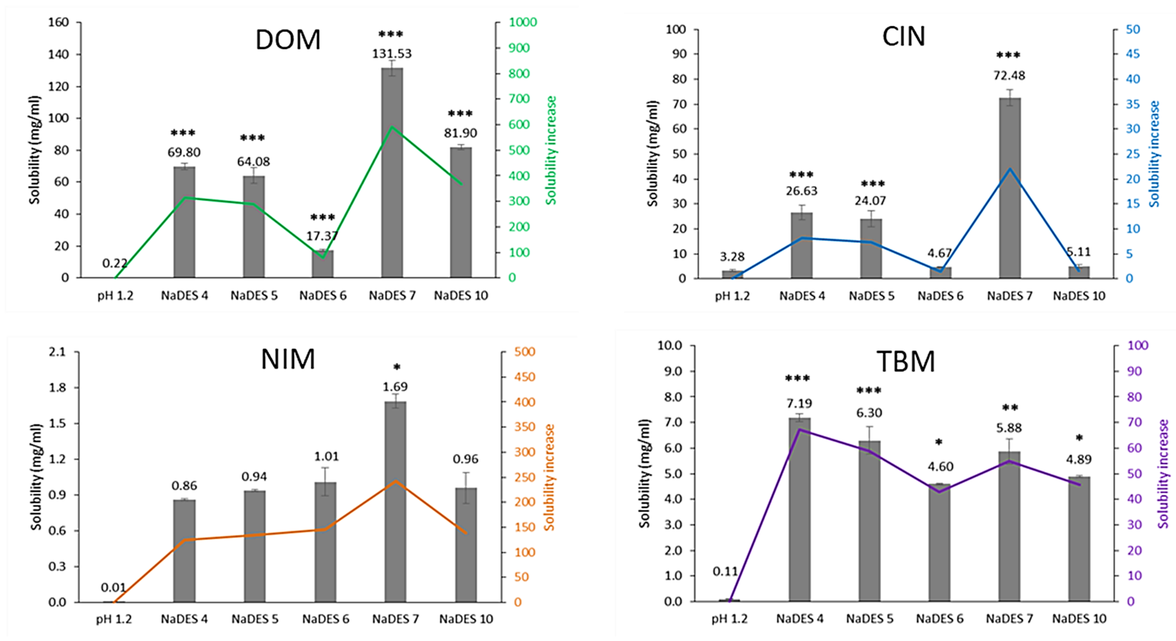


Figure 3. Solubility values of the APIs in acidic NaDES compared to aqueous solution pH 1.2. Values are expressed as mean (n = 3) ± S.D. * p < 0.5, **p < 0.01 and ***p < 0.001, significant difference compared to the solubility in aqueous solution.

6. NMR spectroscopy

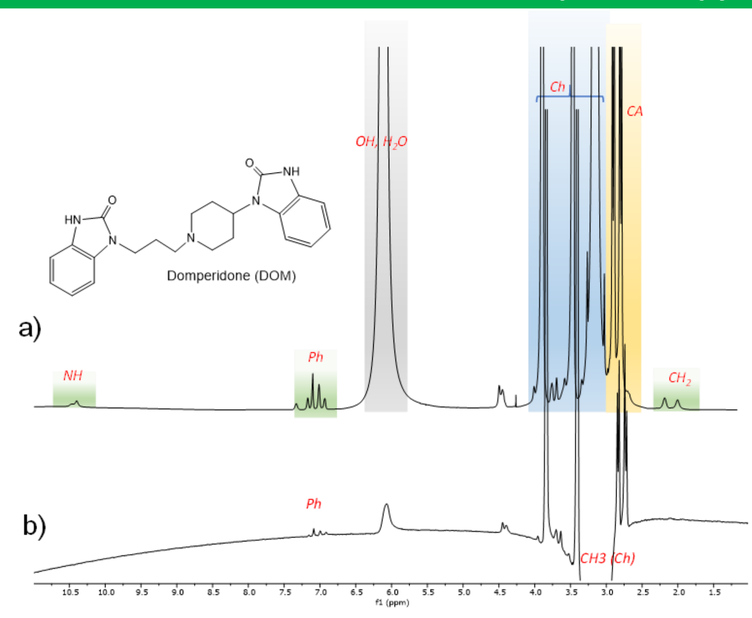


Figure 4.
a) ^{1}H NMR spectrum at 80°C of DOM in NaDES 4 (grey box, peaks related to water/mobile protons; blue box, peaks related to ChCl; yellow box, peaks related to citric acid and green box, peaks related to DOM);

b) 1D ROESY partial spectrum at 80°C of DOM in NaDES 4 obtained selectively irradiating choline methyl protons.

NOE experiments demonstrated the formation of a robust supramolecular structure among the protons of choline, those of organic acid and water. Moreover, **1D-ROESY studies revealed the presence of hydrophobic interactions between the methyl substituents of ammonium cation of choline chloride and the butyl group of TBM, the aromatic portion of DOM (Figure 4), and the aliphatic protons of CIN, respectively.** Unfortunately, NMR studies could not be performed for NIM because the signals of the drug fall under those of the evaluated eutectic mixtures.

7. In vitro dissolution test

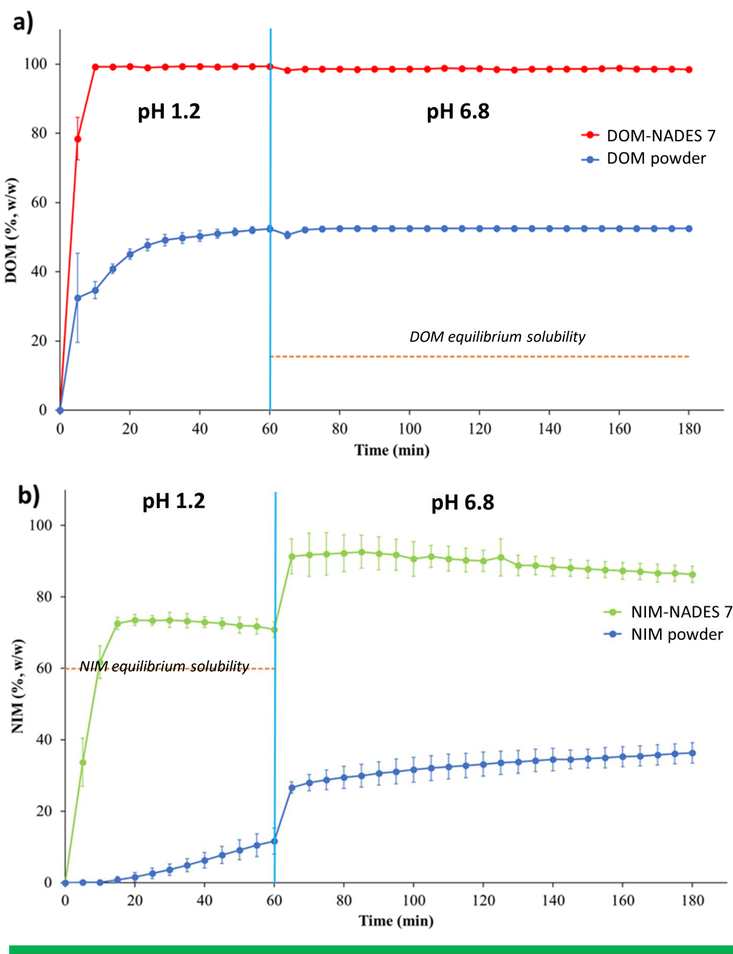


Figure 5. Dissolution profiles of
a) DOM-NaDES 7 compared with DOM powder in buffer solution at pH 1.2 and 6.8
b) NIM-NaDES 7 compared with NIM powder in buffer solution at pH 1.2 and 6.8.
The dotted orange line indicates the equilibrium solubility of the APIs (8.15 µg/mL for DOM and 8.98 µg/mL for NIM) at the respective critical pH, expressed as % of the total concentration.

The results show that **NaDES containing DOM and NIM** are able to improve the biopharmaceutical characteristics of these APIs, **allowing the formation and maintenance of an oversaturated solution in the respective critical pH**, despite the NaDES dilution in the dissolution medium and the disruption of the H-bonds network.
The results obtained using biorelevant media confirm the behavior of DOM-NaDES observed in buffers (figure not reported), while the dissolution profile of raw drug increased significantly due to the micellar solubilization process by the surfactants of the FaSSGF and FaSSIF.

8. Conclusion

In conclusion, some formulations of NaDES were able to increase the solubility of these APIs compared to water solubility. NMR spectroscopy studies demonstrate, for the first time, that the solubilization mechanism of NaDES towards the poorly soluble drugs is a complex process and depends not only to the extent of intra/intermolecular H-bonds, but even to hydrophobic interactions between drug and choline chloride. These results are important for better understanding the role of choline-based natural eutectic mixture as solubilization enhancers.
Finally, in vitro dissolution tests performed in buffer solutions (pH 1.2 and 6.8) and in biorelevant media (fasted state) proved that the eutectic mixture formed a supersaturated solution of the APIs at the critical pH. For this reason, NaDES are green solvents potentially able to increase the bioavailability of APIs BCS class II.

REFERENCES

1. *Göke, K. et al. Novel strategies for the formulation and processing of poorly water-soluble drugs. Eur. J. Pharm. Biopharm, 126:40-56 (2018).*
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