Ionic liquids to upgrade the performance of O/W emulsions containing sparling soluble phenolic compounds

A. Júlio[1,2], R. Caparica[1,2], F. Fernandes[3], J. G. Costa[1], T. Santos de Almeida[1,*]
[1]CBIOS-Research Center for Biosciences & Health Technologies, Lusófona University, Lisboa, Portugal.
[2]Department of Biomedical Sciences, University of Alcalá, Ctra. Madrid-Barcelona Km. 33.600, Alcalá de Henares, 28871 Madrid, Spain.
[3]Escola de Ciências e Tecnologias da Saúde, Lusófona University, Lisboa, Portugal.
*tania.almeida@ulusofona.pt

INTRODUCTION

The poor drug solubility of many drugs and the low stability of some delivery systems can be quite defiant for the pharmaceutical industry[1]. In this scope, ionic liquids (ILs) can be a useful tool, due to their multifunctionality[1-3].

Phenolic compounds have presented huge potential in terms of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer. However, many are sparling soluble, which limits their applicability[1]. This work aimed to study the influence of different ILs (choline amino acid ILs, imidazole halogenated ILs and imidazole amino acid ILs), used at non-toxic concentrations, on the stability of oil-in-water (O/W) emulsions containing ferulic, caffeic, *p*-coumaric acids, or rutin.[4]

RESULTS AND DISCUSSION

Eight ILs, (2-hydroxyethyl)trimethylammonium phenylalaninate [Cho][Phe], (2-hydroxyethyl)trimethylammonium glycinate [Cho][Gly], 1-ethyl-3-methylimidazolium bromide [Emim][Br], 1-butyl-3-methylimidazolium bromide [Bmim][Br], 1-ethyl-3-methylimidazolium phenylalaninate [Emim][Phe], 1-ethyl-3-methylimidazolium glycinate [Emim][Gly], 1-butyl-3-methylimidazolium phenylalaninate [Bmim][Phe], and 1-butyl-3-methylimidazolium glycinate [Bmim][Gly], were studied.

The ILs improved the performance of the O/W emulsions in multiple ways. The concentration of each IL incorporated was the upper concentration where the human keratinocytes (HaCaT cells) viability was maintained (MTT Assay; 24h).

The formulations containing ILs were easier to formulate. Additionally, the ILs led to more viscous formulations, with the amino acid ILs conveying higher viscosity than the halogenated salts (**Table 1**).

The stability studies uncovered that without ILs the systems are unstable, presenting phase separation, while in their presence no instability phenomena were detected.

Table 1: Results from the stability studies of the O/W emulsions prepared in the presence and absence of 0.2% (v/v) [Cho][Br] or of each of the ILs (n = 3). Viscosity values were measured after formulation and following six temperature cycles (at −5 °C and 45 °C).

IL	% IL	After Formulation		Stability Studies		
		Visual Analysis	Viscosity (mPas)	After Centrifugation	After Gradual Heating	Viscosity (mPas) after 6 Temperature Cycles
Control 1	-	Stable	5170 ± 90	Unstable	Unstable	-
[Cho][Br]	0.2	Stable	9000 ± 100	Stable	Stable	10320 ± 80
[Cho][Phe]	0.2	Stable	12700 ± 102			15400 ± 100
[Cho][Gly]	0.2	Stable	11800 ± 52			13100 ± 105
[Emim][Br]	0.2	Stable	9100 ± 97			11000 ± 101
[Emim][Phe]	0.2	Stable	10000 ± 132			12400 ± 129
[Emim][Gly]	0.2	Stable	10400 ± 188			13100 ± 77
[Bmim][Br]	0.2	Stable	8750 ± 65			9500 ± 90
[Bmim][Phe]	0.2	Stable	9100 ± 80			11000 ± 85
[Bmim][Gly]	0.2	Stable	9200 ± 120			11400 ± 112

Table 2: Results from the stability studies of the O/W emulsions prepared in the presence of each drug individually and in the presence or absence of the glycinate derived ILs (n = 3). Viscosity values were measured after formulation and following six temperature cycles (at −5 °C and 45 °C).

Drug	IL	% IL	After Formulation		Stability studies		
			Visual Analysis	Viscosity (mPas)	After Centrifuge	After Gradual Heating	Viscosity (mPas) after 6 Temperature Cycles
Ferulic Acid	Control 2a	-	Stable	8000 ± 80	Unstable	Unstable	-
	[Cho][Gly]	0.2	Stable	12000 ± 75	Stable	Stable	12500 ± 100
		0.5	Stable	13700 ± 110			15000 ± 90
	[Emim][Gly]	0.2	Stable	11300 ± 100			12000 ± 100
	[Bmim][Gly]	0.2	Stable	10000 ± 130			12600 ± 100
Caffeic Acid	Control 2b	-	Stable	8500 ± 100	Unstable	Unstable	-
	[Cho][Gly]	0.2	Stable	11000 ± 95	Stable	Stable	12000 ± 90
		0.5	Stable	12000 ± 100			15500 ± 95
	[Emim][Gly]	0.2	Stable	11200 ± 90			14100 ± 80
	[Bmim][Gly]	0.2	Stable	11000 ± 80			14500 ± 90
p-Coumaric Acid	Control 2c	-	Stable	8200 ± 100	Unstable	Unstable	-
	[Cho][Gly]	0.2	Stable	12000 ± 100	Stable	Stable	15500 ± 100
		0.5	Stable	13500 ± 100			17000 ± 90
	[Emim][Gly]	0.2	Stable	10300 ± 90			14600 ± 100
	[Bmim][Gly]	0.2	Stable	10200 ± 100			14000 ± 100
Rutin	Control 2d	-	Stable	7500 ± 150	Unstable	Unstable	-
	[Cho][Gly]	0.2	Stable	12800 ± 100	Stable	Stable	13100 ± 105
		0.5	Stable	13400 ± 90			16000 ± 100
	[Emim][Gly]	0.2	Stable	10400 ± 188			13100 ± 77
	[Bmim][Gly]	0.2	Stable	9220 ± 50			11140 ± 52

Table 3: Results from the accelerated and shelf-life stability studies of the O/W emulsions prepared in the presence of 0.2% (v/v) of [Cho][Br] or of each of the ILs and without the drug (n = 3). Viscosity values were measured after 90 days in an oven (40 ± 2 °C), in a refrigerator (5 ± 2 °C), or at room temperature.

IL	% IL	Accelerated Stability		Shelf Test
		Heating at Oven	Cooling at Refrigerator	Viscosity (mPas)
[Cho][Br]	0.2	12100 ± 100	12215 ± 120	13400 ± 50
[Cho][Phe]	0.2	15500 ± 85	14950 ± 80	16200 ± 65
[Cho][Gly]	0.2	15220 ± 50	15000 ± 80	16050 ± 100
[Emim][Br]	0.2	11900 ± 100	11500 ± 50	13100 ± 90
[Emim][Phe]	0.2	12200 ± 100	11950 ± 50	13450 ± 70
[Emim][Gly]	0.2	12450 ± 100	12320 ± 100	14120 ± 90
[Bmim][Br]	0.2	9500 ± 100	9900 ± 100	9900 ± 50
[Bmim][Phe]	0.2	10300 ± 50	10220 ± 100	11000 ± 100
[Bmim][Gly]	0.2	10175 ± 50	10300 ± 70	11110 ± 50

Since the ILs derived from glycinate allow a higher enhancement in drug solubility, the [Cho][Gly], [Emim][Gly], and [Bmim][Gly] were further studied as a mean to incorporate the four phenolic compounds at their maximum solubility in each water:IL mixture studied. Once again, the formulations without ILs were unstable, after the stability studies, while the formulations containing the ILs were all stable (**Table 2**).

The stable emulsions were stored at room temperature, in the oven and in the refrigerator. These formulations were macroscopically evaluated over time. After 90 days, no signs of instability were observed in all the batches (**Table 3** and **4**).

Table 4: Results from the accelerated and shelf-life stability studies of the O/W emulsions prepared in the presence of each drug individually and in the presence of the glycinate derived ILs (n = 3). Viscosity values were measured after 90 days in an oven (40 ± 2 °C), in a refrigerator (5 ± 2 °C), or at room temperature.

Drug	IL	% IL	Accelerated Stability		Shelf Test
			Heating at Oven	Cooling at Refrigerator	Viscosity (mPas)
Ferulic Acid	[Cho][Gly]	0.2	15500 ± 100	15410 ± 50	16250 ± 100
		0.5	16300 ± 50	16570 ± 110	17120 ± 120
	[Emim][Gly]	0.2	12320 ± 80	12200 ± 50	14200 ± 110
	[Bmim][Gly]	0.2	11000 ± 50	10900 ± 50	12100 ± 100
Caffeic Acid	[Cho][Gly]	0.2	13100 ± 50	13310 ± 80	14850 ± 50
		0.5	13300 ± 60	13140 ± 100	15680 ± 50
	[Emim][Gly]	0.2	12520 ± 100	12600 ± 100	14200 ± 150
	[Bmim][Gly]	0.2	12100 ± 100	11990 ± 100	14620 ± 80
p-Coumaric Acid	[Cho][Gly]	0.2	13250 ± 50	13500 ± 100	16000 ± 100
		0.5	13900 ± 50	14540 ± 100	17120 ± 150
	[Emim][Gly]	0.2	11950 ± 50	12000 ± 100	14850 ± 100
	[Bmim][Gly]	0.2	11400 ± 50	11355 ± 100	14700 ± 100
Rutin	[Cho][Gly]	0.2	15800 ± 50	16000 ± 100	16550 ± 120
		0.5	16750 ± 60	17010 ± 100	18225 ± 115
	[Emim][Gly]	0.2	12450 ± 50	12800 ± 100	14780 ± 100
	[Bmim][Gly]	0.2	11500 ± 50	11650 ± 100	12380 ± 100

CONCLUSION

Overall, the incorporation of ILs led to an upgrade to multiple features of O/W emulsions, namely by improving the topical delivery of various poorly soluble drugs, the preparation procedure and the stability of the formulations.

Acknowledgments

This work was financially supported by Fundação para a Ciência e Tecnologia, through funding UID/DTP/04567/2019 and UIDB/0456/2020 (both general funding to CBIOS), and by funding from Universidade Lusófona/ILIND (Grant Programme FIPID 2019/2020). A.J. and R.C. would like to thank ALIES for the grant PADDIC 2018-2019 and PADDIC 2019-20.

References

1. T. Santos de Almeida, *et al., Drug Development and Industrial Pharmacy*, 43 *(2017), 1858-1865.*
2. A. Júlio *et al., Nanomaterials*, 9 (2019), 1148-1165.
3. R. Caparica, et al., *Biomolecules*, 10 (2020), 233-248.
4. R. Caparica, *et al.*, *International Journal of Molecular Sciences*, 22 (2021), 4338-4360.