Supplementary Information

Novel naturally-derived encapsulation agents in the ionic liquid form for sustainable emulsion-based products

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Material and methods

Materials

Materials used for ILs synthesis were diethanolamine (H_2EA, bis(2-hydroxyethyl)amine), stearic ($C_{18}OOH$) and oleic ($C_{18:1}OOH$) acids (Sigma-Aldrich, St. Louis, purity > 99% w/w) and choline (Ch) hydroxide (Sigma-Aldrich, St. Louis, aqueous solution, 46% w/w). The specific FAs were chosen to evaluate the effect of the unsaturation on the emulsion encapsulation, taking into account their significance in the FA profile of the main edible vegetable oils. Deionized water was used as emulsions' aqueous phase and tunisian rosemary (*Rosmarinus officinalis*) essential oil (Ferquima Ind. e Com. Ltda, Vargem Grande Paulista, Brazil) as core material. Aqueous choline hydroxide was chosen instead of the methanol-based one, considering the application potential of choline-based ILs in the food industry and the toxicity concerns associated to methanol.

Synthesis of the ILs and characterization

Diethanolammonium stearate ($[H_2EA][C_{18}OO]$) and diethanolammonium oleate ($[H_2EA][C_{18:1}OO]$) were synthesized through the Brønsted acid-base reaction between the FA and diethanolamine at x_1 = 0.5. The synthesis was performed in a nitrogen atmosphere at temperatures above the mesophase domain and cooled down to room temperature. Cholinium stearate ($[Ch][C_{18}OO]$) was synthesized through the acid-base reaction between stearic acid dissolved in ethanol solution and choline hydroxide at x_1 = 0.5. The synthesis was performed in a thermal fluid bath at room temperature and in a nitrogen atmosphere to avoid the oxidation of the compounds. The reaction resulted in IL aqueous-ethanolic solutions, which were dried under vacuum at 50 °C for 24 hours to obtain a white powder emulsifier. Their water content was determined by Karl Fisher titration, obtaining values lower than 1.5%. ILs were characterized by proton Nuclear

Magnetic Resonance (^{1}H NMR) by using a Bruker 300 Fourier 300 MHz and Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR ALPHA, Bruker Scientific, Banner Lane). The structures of ILs and the effectiveness of the synthesis were confirmed by the spectra.

Emulsion preparation

Emulsions were prepared by pre-mixing using Ultra Turrax (U) followed by high-intensity ultrasound-assisted encapsulation process (HIUS) using ILs ([H_2EA][C_{18}OO], [H_2EA][$C_{18:1}$OO] or [Ch][C_{18}OO]) at a fixed concentration (1.0%, w/w). Rosemary essential oil was used as oily phase at 1.0% (w/w). Emulsions were prepared at room temperature (± 25 °C) by pre-mixing the aqueous and oily phases using a U model T18 (IKA, Staufen, Germany). Previously, IL was dissolved into the water. The essential oil was added dropwise at 5.000 rpm for 5 min. Then, the pre-mixing condition was fixed at 10.000 rpm for 20 min for a 40 g sample, resulting in a specific energy of 6000 J/g obtained according to Eq. 1. After pre-mixing, the system was submitted to ultrasound treatment using an ultrasonic probe (13 mm of diameter) (Unique, Desruptor, 800 W, Indaiatuba, Brazil) at 720 W for 4.65 min for a 40 g sample, resulting in a specific energy of 5022 J/g (Eq. 1), totalizing 11022 J/g for both processes. A refrigerated bath at 5 °C was used to avoid excessive heating in all samples submitted to HIUS processing.

$$Specific\ energy\left(\frac{J}{g}\right) = \frac{Nominal\ power\ (W) \times Time\ (s)}{Weight\ (g)} \quad (1)$$

The acoustic power provided by the ultrasound probe for the nominal power of 720 W was determined by calorimetric assays according to a methodology previously described[1]. The HIUS intensity was calculated from the acoustic power according to Eq. 2:

$$HIUS\ intensity\left(\frac{W}{cm^2}\right) = \frac{4\ \times acoustic\ power}{\pi D^2} \quad (2)$$

where D (cm) is the probe diameter.

The HIUS intensity was 20 W/cm^2 for the nominal power of 720 W.

Trials were named according to the IL.

Kinetic stability

The visual aspect and kinetic stability of the emulsions were evaluated in cylindrical glass tubes (2.23 cm internal diameter and 12.81 cm height), properly sealed, and stored for 30 days at 25 °C. After preparation, 20 mL of each emulsion were immediately transferred to a glass tube. The oil phase separation was measured and may be expressed as the ratio between the height of the superior phase (H_S) and the total height of the emulsion (H_T), as shown in Eq. (3). The measurements were performed in duplicate at least.

$$Emulsion\ Stability\ (\%) = \frac{H_S}{H_T} \times 100 \quad (3)$$

The emulsion stability was also evaluated and confirmed using an optical scanning device Turbiscan LAB Expert (Formulaction, France), using the same sample that was placed in a cylindrical glass measurement cell (2.23 cm internal diameter and 12.81 cm height) and stored for 30 days. The backscattering (% BS) of monochromatic light (λ = 880 nm) was measured as a function of the emulsion height at 25 °C. The backscattering profile (BS versus emulsion height) of each emulsion was measured after 1, 2, 7, 14 and 30 days of storage time.

Optical microscopy

The microstructure of the emulsions was characterized after 1 and 7 days of storage using an optical microscope (Leica, Cambridge, UK). An emulsion droplet was put in slides with coverslips and observed with ×40 and ×100 objective lenses. The measurements were performed in triplicate at least.

Rheological assays

The rheological behavior and the viscosity profile of the emulsions were determined after 1 and 7 days of storage using an AR 1500ex stress controlled rotational rheometer (TA Instruments, New Castle, DE). Flow curves were obtained in an up-down-up step program at 25 °C, in which the shear rate ranged from 0 to 300 s^{-1}, using a stainless steel cone and plate (diameter = 40 mm, angle = 2° and cone truncation = 57 μm). In order to characterize the steady-state behavior of the shear stress (σ) – shear rate ($\dot{\gamma}$) profile of the emulsions, the results were obtained at the third step program. The measurements were performed in triplicate at least.

Flow curves were fitted to the Herschel-Bulkley (HB) model to obtain the rheological parameters according to Eq. (4).

$$\sigma = \sigma_0 + k\dot{\gamma}^n \quad (4)$$

where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}); σ_0 is the yield stress (Pa); k is the consistency index; n is the flow behavior index.

Encapsulation Efficiency

An aliquot of 0.5 mL of each sample was dissolved in 1.5 mL of ethyl acetate (Synth, lot 153016, Diadema, Brazil) and manually homogenized. Then, the mixture was centrifuged at 13,000 rpm for 10 min. Just after that, the samples were manually homogenized again and centrifuged at 13,000 rpm for 5 min. An aliquot of 1 mL of supernatant was filtered using nylon membrane (0.45 μm) and injected into the chromatograph. The sample split ration was 1:20. The carrier gas (Helium, 99.9% purity, White Martins, Campinas, Brazil) flowed at 1.1 mL/min. The injector and the detector temperatures were 220 °C and at 240 °C, respectively. The column was heated from 60 °C to 246 °C at 3 °C/min. A chromatograph GC-FID (Shimadzu, CG17A, Kyoto, Japan) equipped with a capillary column of fused silica DB-5 (J&W Scientific, 30 m × 0.25 mm × 0.25 μm, Folsom, USA) was used. The 1,8 cineole (Scheme S1) presented in emulsions was identified by comparing the retention indices of the samples and external standards. Quantification was performed using external standard calibration curves (Figure S1) and used to calculate the encapsulation efficiency of eucalyptol (1,8 cineole).

The encapsulation efficiency was measured and may be expressed as the ratio between the amount of bioactive effectively retained (Q_e) and the initial amount added to the emulsion (Q_i), according to Eq. (5).

$$Encapsulation\ Effciency\ (\%) = \frac{Q_e}{Q_i} \times 100 \quad (5)$$

Statistical analysis

The statistical analysis was performed using the software Statistica 7.0 (Statsoft Inc., Tulsa, USA). The significant differences ($p < 0.05$) between the treatments and the comparison between the mean values were evaluated by the Tukey test.

RESULTS

ILs characterization by Fourier Transform Infrared spectroscopy (FT-IR)

The list of main IR spectroscopy bands for all ILs obtained by FT-IR is presented below:

diethanolammonium stearate ([H_2EA][$C_{18}OO$])

IR (neat) ν = 2915, 2849, 1550, 1465, 1406, 1069, 952, 721 cm^{-1}.

diethanolammonium oleate ([H_2EA][$C_{18:1}OO$])

IR (neat) ν = 2923, 2851, 1556, 1456, 1402, 1071, 958, 722 cm^{-1}.

cholinium stearate ([Ch][$C_{18}OO$])

IR (neat) ν = 2914, 2848, 1569, 1467, 1388, 1092, 955, 718 cm^{-1}.

ILs characterization by Proton Nuclear Magnetic Resonance (^{1}H NMR)

The list of chemical shifts for all ILs obtained by ^{1}H NMR is presented below:

cholinium stearate ([Ch][$C_{18}OO$])

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.94 (m, 2H, -CH_2-O), 3.49 (t, 2H, -CH_2-), 3.17 (s, 9H, CH_3-N), 2.03 (t, 2H, -CH_2-COO^-), 1.45 (m, 2H, -CH_2-), 1.18 (m, 28H), 0.81 (t, 3H, CH_3-).

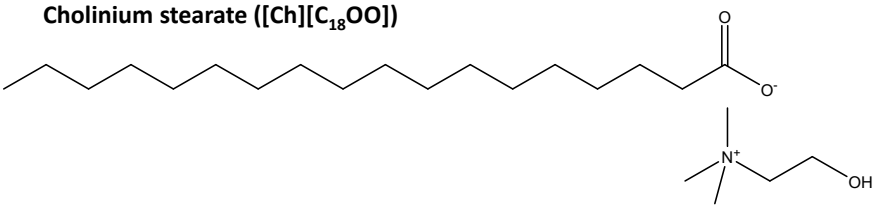
diethanolammonium stearate ([H_2EA][$C_{18}OO$])

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 5.79 (s, 2H, -NH_2^+), 3.77 (t, 2H, -CH_2-O), 3.01 (t, 2H, -CH_2-N), 2.09 (t, 2H, -CH_2-COO^-), 1.47 (m, 2H, -CH_2-), 1.18 (m, 28H), 0.81 (t, 3H, CH_3-).

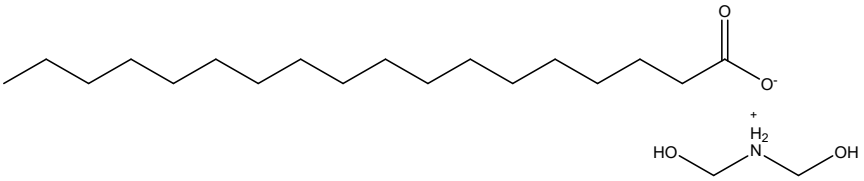
diethanolammonium oleate ([H_2EA][$C_{18:1}OO$])

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 5.29 (m, 3H, -NH_2^+), 3.53 (t, 2H, -CH_2-O), 2.75 (t, 2H, -CH_2-N), 2.04 (t, 2H, -CH_2-COO^-), 1.96 (m, 6H), 1.44 (m, 2H, -CH_2-), 1.22 (m, 20H), 0.84 (t, 3H, CH_3-).

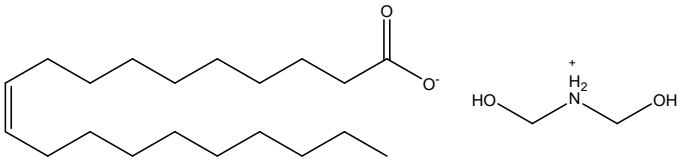
Cholinium stearate ([Ch][C_{18}OO])



Diethanolammonium stearate ([H_2EA][C_{18}OO])



Diethanolammonium oleate ([H_2EA][$C_{18:1}$OO])



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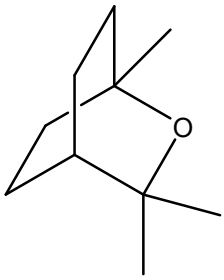
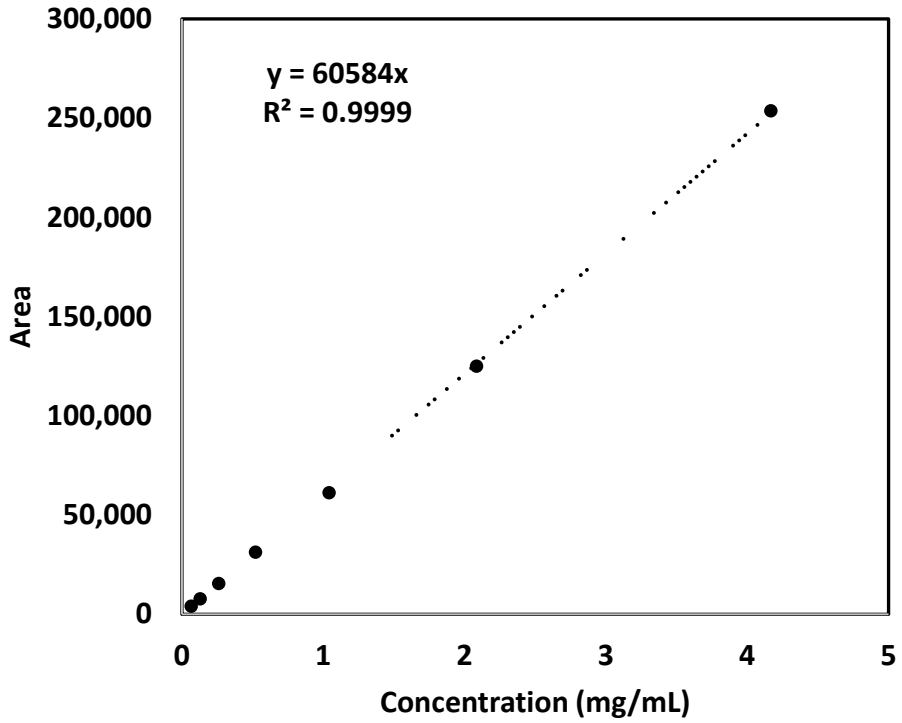


148 Eucalyptol

149 **Scheme S1.** Molecular structures of ILs and eucalyptol (1,8 cineole).



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151 **Figure S1.** Standard calibration curve for eucalyptol.

152 **Table S1.** Rheological model parameters related to shear stress/shear rate curves fitted to flow
153 experimental data and apparent viscosity for IL emulsions. Different letters in samples in the same
154 day of storage, using different ILs, indicate significant difference at $p < 0.05$. Capital letters:
155 difference between storage time using the same IL.

			Day 1		
IL	**σ_0/Pa**	***k*¥**	***n*ǂ**	**R^2**	**η/Pa·s at 100 s^{-1}**
$[H_2EA][C_{18}OO]$	0.0	0.0064	0.78	0.9981	0.0035 ± 0.00027 aA
$[H_2EA][C_{18:1}OO]$	0.0	0.0011	1.00	0.9990	0.0019 ± 0.00007 bA
$[Ch][C_{18}OO]$	0.0	0.0072	0.77	0.9987	0.0037 ± 0.00006 aA
			Day 7		
IL	**σ_0/Pa**	***k*¥**	***n*ǂ**	**R^2**	**η/Pa·s at 100 s^{-1}**
$[H_2EA][C_{18}OO]$	0.0	0.0075	0.73	0.9980	0.0032 ± 0.00002 aA
$[H_2EA][C_{18:1}OO]$	0.0	0.0011	1.00	0.9982	0.0019 ± 0.00001 bA
$[Ch][C_{18}OO]$	0.0	0.0065	0.77	0.9985	0.0033 ± 0.00002 aB

156 ¥*k* and ǂ*n* for Herschel-Bulkley model. η is the apparent viscosity.

References

1. T. J. Mason, J. P. Lorimer, D. M. Bates and Y. Zhao, *Ultrasonics Sonochemistry*, 1994, **1**, S91-S95.