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Menthol-based Eutectic Mixtures: Hydrophobic Low Viscosity Solvents

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S Supporting Information

ABSTRACT: Inspired by one of the major problems in the pharmaceutical industry, we advantageously used the formation of eutectic mixtures to synthesize new solvents. The aim of this work is to identify low viscosity, cheap, biodegradable and hydrophobic eutectic solvents from natural resources. Consequently, novel eutectic mixtures based on DL-menthol and naturally occurring acids, namely pyruvic acid, acetic acid, L-lactic acid, and lauric acid, were synthesized and are here reported for the first time. The obtained DL-menthol-based eutectic mixtures were analyzed using NMR and FTIR spectroscopy in order to check their structures and purities and to confirm the interaction of the two compounds leading to the eutectic formation. Important solvent thermophysical properties, such as density and viscosity, of the prepared eutectic solvents with different water contents (dried and water-saturated) were measured. Finally, taking advantage of their hydrophobic character, namely the formation of two phases with water at room temperature, four different biomolecules, caffeine, tryptophan, isophthalic acid, and vanillin, were extracted and the extraction efficiencies of the prepared eutectic solvents compared.

KEYWORDS: *Eutectic mixtures, Menthol, Green solvents, Hydrophobicity, Thermophysical and aqueous phase equilibria properties*

INTRODUCTION

One of the major challenges in modern chemistry and chemical engineering is the formulation of new solvents that simultaneously meet the Green Chemistry criteria and have the ability to dissolve as large as possible spectrum of solutes. Currently, two major classes of solvents, ionic liquids (ILs) and eutectic mixtures,[1,2] are being developed and used by academia and industry. Regarding ILs, their commercial availability promoted the production of a large body of information concerning their thermophysical and transport properties, toxicity, and phase equilibria, and consequently, new applications can now be properly evaluated. Conversely, eutectic mixtures, and in particular deep eutectic solvents, prepared from the combination of a salt or an ionic liquid with a neutral compound, are still in their infancy, lacking organization and properties systematization. Most of the eutectic mixtures proposed so far are based on materials from renewable resources, such as carboxylic acids, sugars, amines, polyols, etc., and although they are cheap and usually considered nontoxic and biodegradable, their inherent toxicity and biodegradability needs to be evaluated case by case. For example, it has been shown that choline chloride:glucose and choline chloride:glycerol deep eutectic solvents display low cytotoxicity, whereas choline chloride:oxalic acid possesses moderate cytotoxicity. Nevertheless, these three deep eutectic solvents were classified as "readily biodegradable" based on their high levels of mineralization.[3] It has been shown that some deep eutectic solvents can be a good replacement for analogous ILs, due to the similarity between their properties and the fact that they can be advantageously prepared in a cheaper, quicker and easier way.[4] The preparation of deep eutectic solvents depends usually on the establishment of hydrogen bonds between the two compounds, one acting as hydrogen bond donor and the other as hydrogen bond acceptor. However, as in other fluids where hydrogen bonds are the dominant interactions, one of the major drawbacks of this class of solvents is their general high viscosity when compared to organic solvents and even sometimes to ILs or even their solid/pasty/gel-like state.

The main purpose of this work is to identify cheap and biodegradable solvents based on eutectic mixtures that can be prepared from natural resources. A quick look into the eutectic solvents from natural resources that have been proposed in the literature tells us that the vast majority, if not all, are hydrophilic or very hydrophilic. Consequently, the eutectic mixtures from renewable resources with a certain hydrophobic character, which displayed liquid−liquid equilibria with water, are in need. Because of its very small solubility in water (0.46 mg/mL at 25 °C[5]), menthol is a good candidate to prepare sustainable, cheap and hydrophobic solvents using the formation of eutectic mixtures approach. Menthol can be extracted from *Mentha* species, is abundant, and cheap (∼€60/kg). Eutectic mixtures

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containing menthol have been reported in the pharmaceutical field. For example, menthol and borneol[6,7] and camphor[8] and *N*-ethyl-5-methyl-2-(1-methylethyl)cyclohexanecarboxamide[9] eutectic mixtures have been prepared and used as vehicles for transdermal drug delivery, whereas eutectic mixtures of menthol and testosterone,[10] ibuprofen,[11,12] lidocaine,[13] ubiquinone,[14] captopril,[8] daidzein,[6] and fluconazole[7] have been used as analgesic, antimicrobial, antipruritic, anti-inflammatory, and antitussive[15,16] compounds. Thermal properties of some of these eutectic mixtures have been researched: for example, although L-menthol/testosterone 80:20 molar ratio and L-menthol/ubiquinone 70:30 molar ratio display a melting temperature (T_m) of 39.3[10] and 38.5 °C,[14] respectively, the L-menthol/lidocaine 70:30 molar ratio has a lower T_m value of 26 °C,[13] and the L-menthol/ibuprofen 70:30 molar ratio even lower, 19 °C.[11] These examples clearly illustrate the role of the second component on the melting temperature of these eutectics.

In this work, four eutectic mixtures composed of DL-menthol and different acids were prepared just by heating and mixing the pure compounds and then studied, as listed in Table 1. The chemical structures of DL-menthol and the used organic acids are depicted in Figure 1. To evaluate the real technological value of the prepared eutectic mixtures as solvents, relevant thermophysical properties, such as density and viscosity, were measured, within the temperature range indicated by the DSC and TGA studies and at different water contents, dried, and water-saturated. Finally, and taking advantage of the hydrophobic character of these eutectics, and thus their two liquid phase formation in the presence of water at room temperature, the extraction efficiency of four different biomolecules, caffeine, tryptophan, isophthalic acid, and vanillin from the water phase to the eutectic mixture phase, was evaluated in terms of their partition coefficients.

Table 1. Summary of Different Eutectic Mixtures Studied in This Work Using DL-Menthol as Hydrogen Bond Acceptor and Different Hydrogen Bond Donors

hydrogen bond donor	molar ratio	aspect
acetic acid	1:1	transparent liquid
pyruvic acid	1:2	transparent yellow liquid
lactic acid	1:2	transparent liquid
lauric acid	2:1	transparent liquid

EXPERIMENTAL SECTION

Materials. DL-Menthol (≥95% mass fraction purity), pyruvic acid (purity > 98%), acetic acid (purity ≥ 99.7%), lauric acid (purity > 98%), caffeine (purity 99%), vanillic acid (purity ≥ 97%, tetracycline (purity ≥ 98%), and tryptophan (purity ≥ 98%) were purchased from Sigma-Aldrich and used as received. L-Lactic acid solution (81 wt % in water) was supplied by Fluka. The water used was ultrapure, double distilled, passed through a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification apparatus.

Preparation Methodology. DL-Menthol-based eutectic mixtures were prepared by adding different hydrogen bond donors (polyethylene glycol, monoethylene glycol, cholesterol, pyruvic acid, acetic acid, L-lactic acid, butyric acid, hexanoic acid, and lauric acid) to DL-menthol in different molar ratios. The mixture of the two compounds was then heated up to 50 °C for 15 min and then cooled down slowly until it reached room temperature. Some of these eutectic mixtures yielded either pasty compounds or liquids with melting points higher than that of DL-menthol. Others also did not yield an eutectic mixture (such as in the case when glycols were used) and thus other ratios were attempted without success. Consequently, these eutectic mixtures were discarded. Only four eutectic mixtures of DL-menthol and different acids were studied in this work, as listed in Table 1.

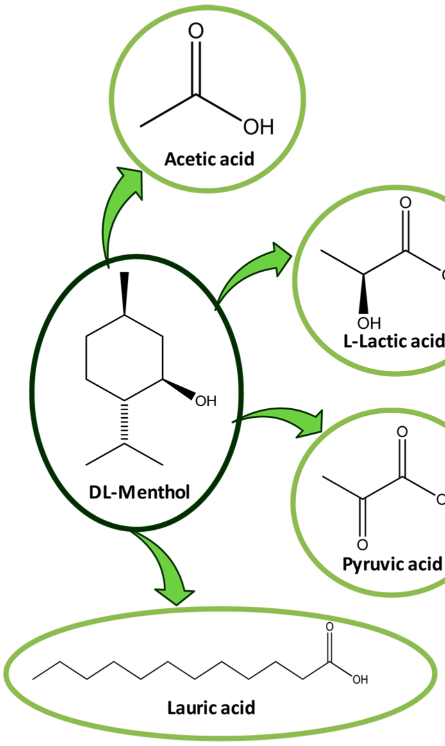

Figure 1. Chemical structure of the compounds used for the DL-menthol-based eutectic mixtures studied in this work.

For the preparation of the dried samples, the DL-menthol-based eutectic mixtures were maintained for at least 4 days in a Schlenk under high vacuum (ca. 10^{-1} Pa) at room temperature. For the water saturated samples, binary mixtures containing water and the eutectic mixture were prepared, vigorously shaken, and left to equilibrate for 1 day. Samples of the eutectic mixture phase were carefully taken with a syringe. The amount of water present in each dried and saturated sample was measured, in order to infer their relative hygroscopicity, and thus hydrophobicity. The water content was determined by Karl Fisher titration (model Metrohm 831 Karl Fisher coulometer). After preparation of eutectic mixtures, proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) and Fourier transform infrared spectroscopy (FTIR) were used to check the eutectic mixtures structures and purities and to confirm the interaction between two compounds leading to the eutectic formation.

NMR Measurements. All the experiments were carried out on a Bruker AVANCE 400 spectrometer operated at room temperature with 16 and 500 scans for the ^{1}H NMR and ^{13}C NMR spectra, respectively. The eutectic mixtures samples were prepared on 5 mm NMR tubes by weight approximately 30 mg of eutectic mixture and then adding 0.5 mL of deuterated chloroform ($CDCl_3$). The homogeneity of the sample was assured by vortex mixing.

FTIR Measurements. FTIR measurements were carried out using a Brüker IFS66/S FTIR spectrometer (Brüker Daltonics, MA, USA) with a single reflection ATR cell (DuraDisk, equipped with a diamond crystal). The data relative to spectral region were recorded between 4000 and 600 cm^{-1} at room temperature. For each sample, 290 scans were recorded at a spectral resolution of 4 cm^{-1} and five replica spectra were collected in order to evaluate reproducibility (OPUS v5.0).

Thermophysical Properties. DSC (differential scanning calorimetry, TA Instrument Model DSC Q200) and TGA (TA Instruments Model TGA Q50) were used to measure the thermal properties of the prepared menthol-based eutectic mixtures. All solid−liquid phase transitions were determined in a range of temperatures from 193.15 at 373.15 K, with a heating and cooling rate of 5 °C·min^{-1}. Condensation in the DSC furnace was prevented by using dry nitrogen as a purge gas with a flow rate of 50 mL/min. Indium (T_{melting} = 429.76 K) was used as the standard compound for calibration. Samples of each mixture, typically 3 to 15 mg, were transferred to the aluminum DSC pan, which was then hermetically sealed in order to prevent vaporization. The uncertainty in the melting point temperature obtained by calculation of the standard deviation of several consecutive measurements for the same sample is better than ±1 K.

The TGA was used for measuring the decomposition temperature of the eutectic mixtures, using also nitrogen at a flow rate of 60 mL·min^{-1}. Samples were placed inside of aluminum pans and heated to 600 °C at a rate of 10 °C·min^{-1} until complete thermal degradation was achieved.

Measurements of viscosity and density of the eutectic mixtures were carried out in the temperature range from 293.15 up to 353.15 K and at atmospheric pressure, using an Anton Paar (model SVM 3000) automated rotational Stabinger viscometer−densimeter. The temperature uncertainty is ±0.02 K. The relative uncertainty of the dynamic viscosity is ±0.35%, and the absolute uncertainty of the density is ±0.0005 g·cm^{-3}. To evaluate the effect of water on the density and viscosity of the prepared eutectic mixtures, two different scenarios were studied: dried and water-saturated. The eutectic mixtures were simultaneously sampled for the water content determination and the density and viscosity measurements.

Biomolecules Extraction. Aqueous solutions of different biomolecules, namely caffeine, tryptophan, and vanillin, with concentrations of 0.2 g dm^{-3}, and isophthalic acid with a concentration 0.1 g dm^{-3}, were prepared. Then, 1 mL of these solutions was put into contact with equal amount of each one of the eutectic mixtures and stirred at 3000 rpm, at room temperature and pressure conditions, to ensure that the equilibrium of the biomolecules between the two phases was reached. Afterward, the flasks were let to separate the phases for 24 h, so that complete separation of the two phases and full equilibration of biomolecules between the two phases was attained. Samples of the aqueous phase were taken and the quantification of the biomolecules was carried out by UV−vis spectroscopy using a SHIMADZU UV-1700, Pharma-Spec spectrophotometer, at wavelengths of 278 nm for tryptophan and 273 nm for other compounds, by means of a calibration curve previously established for each compound. Evaluation of the biomolecules concentration during this 24 h period indicates that this is the minimum time period required for equilibrium to be reached. At least three samples of each individual aqueous phase were quantified and the deviations evaluated.

■ RESULTS AND DISCUSSION

Eutectic mixtures containing menthol have already been reported in the literature,[6−14] but in a pharmaceutical context. In this work, eutectic mixtures of DL-menthol and organic acids were prepared and important solvent properties, such as density and viscosity, were measured in order to evaluate their feasibility as extraction solvents. Their performance as extraction solvents for several model biomolecules was also evaluated. For this purpose, different proportions of several natural organic acids were mixed with DL-menthol and those which are liquid at room temperature are listed in Table 1 and their chemical structures depicted in Figure 1.

Eutectic mixtures of DL-menthol and other compounds, such as polyethylene glycol, monoethylene glycol, and cholesterol, were also prepared by yielded either pasty compounds or liquids with melting points higher than that of menthol. Consequently, these eutectic mixtures were discarded. Figure 2 shows an illustration of the formation of an eutectic mixture, in this case DL-menthol and lauric acid, which are both solids and when they are heated become liquid mixture. After, when water is added, two phases are formed.

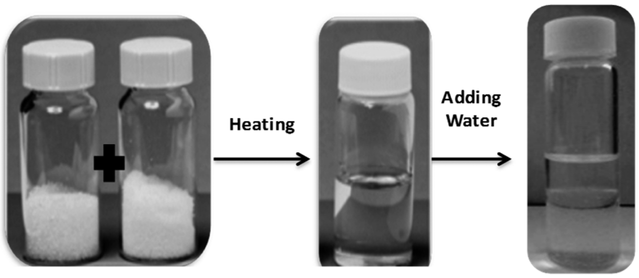

Figure 2. Illustration of the formation of an eutectic mixture and its phase splitting with water. From left to right: DL-menthol + lauric acid (both solids), DL-menthol:lauric acid eutectic mixture (1:2), and eutectic mixture after mixing with water.

Thermal Properties. The thermal behavior of the eutectic mixtures was studied using TGA and DSC. TGA was used to acquire the eutectic mixtures decomposition temperature (T_{dec}), which is one of the most important properties of these compounds, especially for their application as new, alternative solvents so that optimization of the working conditions below this temperature can be achieved. These temperatures determined for the studied eutectic mixtures are summarized in Table S1, present in the Supporting Information. It can be observed that the decomposition temperature of all the prepared eutectic mixtures is above 200 °C. Note that in the case of the DL-menthol:pyruvic acid, the eutectic mixture is more stable than the separate compounds.

Differential scanning calorimetry is another one of the most widely used methods of thermal analysis, measuring the temperature and energy associated with a range of thermal events including melting, crystallization, and glass transitions.[17] Morrison et al.[18] have used this technique to characterize urea−choline chloride and malonic acid−choline chloride eutectic systems. The melting temperatures of the pure compounds, DL-menthol and acids, and their eutectic mixtures are also presented in Table S1. It can be observed that DL-menthol presents two melting points, at 27 and 34 °C. Corvis et al.[19] explained that DL-menthol possesses at least two polymorphs α and β, which are probably responsible for these two melting temperatures. It can be seen clearly that in all cases, the melting temperature of the mixture is different and lower than those of the pure starting components, which confirms that these DES are supramolecular complexes in the liquid state over a wide temperature range.

FTIR Analysis. Different intermolecular interactions are responsible for the formation of a eutectic mixture, according to the compounds used. In deep eutectic solvents based on choline chloride, sugars, and organic acids, the establishment of hydrogen bonds between the two compounds, one acting as a hydrogen bond donor and the other as a hydrogen bond acceptor, is responsible for the eutectic mixture formation. However, Nashiretty and Panchagnula,[20] who studied the effect of menthol in eutectic mixtures of ceramide and cholesterol, reported that menthol interacts with both the lipid alkyl tails and the polar head groups of mixture, reducing their melting temperature, and breaking the inter- and intralamellar hydrogen bonding network.

To this end, it is important to know how DL-menthol interacts with the second compound in the four eutectic mixtures prepared in this work. For that purpose, FTIR experiments were performed for the neat DL-menthol, each of the acids and the eutectic mixtures. The overlapping FTIR spectra obtained for the DL-menthol:acetic acid eutectic mixture

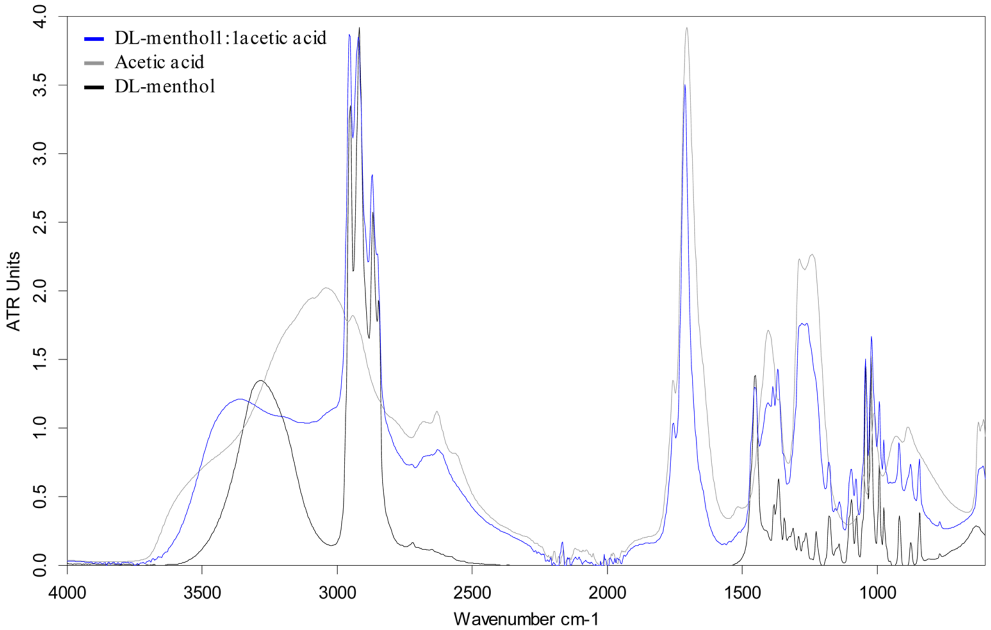

Figure 3. FTIR spectra of the pure DL-menthol, acetic acid, and the eutectic mixture of DL-menthol and acetic acid (1:1 molar ratio).

and the respective components are presented in Figure 3 as an example and the other FTIRs are presented in Figures S9−S11 of the Supporting Information.

All the hydrogen bond donors used in these eutectic mixtures have a carboxylic acid group in their structure that presents a representative band (ketone or carbonil group) around 1700 cm^{-1} in the spectra. On the other hand, in the FTIR spectra of the hydrogen bond acceptor, DL-menthol, only one representative band corresponding to the hydroxyl group, at about 3300 cm^{-1}, can be observed. Additionally, FTIR measurements were also used to confirm the formation of a new compound, through the formation of hydrogen bonds, between the DL-menthol and the hydrogen bond donors. FTIR spectra data of the eutectic mixtures show the intermolecular hydrogen bond interaction between the hydrogen bond donors and DL-menthol mostly in the carboxyl group region. It can be observed in Figure 3 that for the DL-menthol:acetic acid eutectic mixture, the carbonyl band from the HBD was originally located at low wavenumber values (~1692 cm^{-1}) and it broadens and shifts to the highest values (~1735 cm^{-1}) in the eutectic mixture. This is a strong indication of a new hydrogen bond formation, thus proving that a new compound was formed. This evidence can be further confirmed through the physical state of the compounds, for example, the eutectic mixture composed by DL-menthol and lauric acid: initially, the physical state of both these compounds is solid and after DES formation a liquid is obtained.

Thermophysical Properties. Density and viscosity are important properties of any solvent because they influence the mass transport phenomena, there by affecting their suitability for particular applications. As mentioned before, green solvents such as ILs, deep eutectic solvents and polyethylene glycols oligomers, are usually quite viscous fluids when compared to organic solvents and for that reason they have to be used in solutions with water or other solvents thus hampering their application when a hydrophobic solvent is required. As we can see in Table 2, the water content in the saturated eutectic mixtures is quite low comparing to similar eutectic mixtures produced using cholinium chloride instead of DL-menthol. For example, Florindo et al.[3] reported the water content of water saturated deep eutectic solvents composed of a salt, cholinium chloride, and several organic diacids, with values between 14 and 19 wt %, and monoacids, such as levulinic acid with water content near 10 wt %. Consequently, the hydrophobicity of the DL-menthol-based eutectic mixtures is much higher than those produced from salts, probably also due to the presence of charges in these last ones.

Table 2. Molecular Weight (MW) and Water Content, in Mass Percentage (wt %), of the Dried and Water-Saturated DL-Menthol-based Eutectic Mixtures Studied in This Work

		water content (wt %)	
eutectic mixtures	MW (g mol^{-1})	dried	saturated
DL-menthol:acetic acid	108.16	0.429	1.567
DL-menthol:pyruvic acid	110.79	1.023	1.268
DL-menthol:lactic acid	112.14	0.153	1.634
DL-menthol:lauric acid	170.95	0.276	1.237

The experimental density results for the dried and water-saturated (Table 2) samples of the four prepared eutectic mixtures as a function of temperature are plotted in Figure 4. The density data are presented in Tables S2 in the Supporting Information. It is very difficult to extract conclusions regarding the relationship between the chemical structure of the acids present in the eutectic mixtures and the respective thermophysical properties because different molar ratios were used. Only in the case of the eutectic mixtures containing pyruvic and L-lactic acids, both derived from propanoic acid, the same molar ratio (1:2) was used. However, even then for these DES, a straightforward comparison cannot be clearly established. Several explanations can be put forward, such as the possibility of dimers formation in L-lactic acid and the conformational effects between the two carboxyl groups in the pyruvic acid, to account for this behavior.[21]

Generally, the presence of water increases the density of the eutectic mixtures. The only exception is the DL-menthol:lactic acid, where a small decrease was observed. However, the effect of water in much more pronounced for the eutectic mixtures

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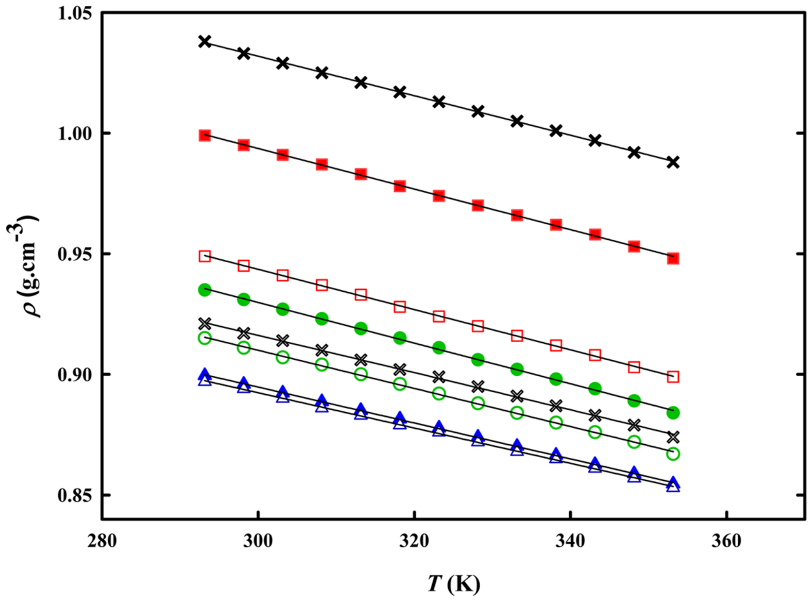

Figure 4. Experimental densities (ρ) of the dried and water-saturated eutectic mixtures as a function of temperature: DL-menthol:acetic acid (●), DL-menthol:pyruvic acid (■), DL-menthol:lactic acid (×), DL-menthol:lauric acid (▲). The filled symbols correspond to the dried samples and the empty symbols correspond to the water-saturated samples. The lines represent the fitted data calculated using eq 1.

Table 3. Fitted Values of Parameters, *a* and *b*, by eq 1 and Standard Deviation *σ*, for the Density of Dried and Water-Saturated Eutectic Mixtures as a Function of Temperature

	a (g·cm^{-3})		b (10^{-4}) (g·cm^{-3}·K)		σ[a]	
DES	dried	saturated	dried	saturated	dried	saturated
DL-menthol:acetic acid	1.183	1.147	−0.8418	−0.7901	4.7 × 10^{-4}	4.2 × 10^{-4}
DL-menthol:pyruvic acid	1.246	1.194	−0.8407	−0.8341	4.1 × 10^{-4}	2.8 × 10^{-4}
DL-menthol:lactic acid	1.278	1.149	−0.8187	−0.7749	3.2 × 10^{-4}	2.5 × 10^{-4}
DL-menthol:lauric acid	1.112	1.118	−0.7308	−0.7442	3.3 × 10^{-4}	3.3 × 10^{-4}

[a] $\sigma = (\sum(\rho_i^{exp} - \rho_i^{cal})^2/n-\nu)^{0.5}$ where n is the number of experimental points, ν the number of adjustable parameters.

containing pyruvic and lactic acids, which can be probably linked to presence of substituent groups that are able to interact with water via hydrogen bonding. The densities of the eutectic mixtures containing the linear acids, and acetic and lauric acids are less affected by the water presence. In fact, dried and saturated DL-menthol:pyruvic acid present a very small difference in the water content which is, however, reflected in the density of the two mixtures.

The density decreases linearly with temperature for all dried and water saturated samples, in the whole temperature range studied. A linear equation was used to express the correlation with the temperature:

$$\rho = a + bT \tag{1}$$

where ρ corresponds to density in g·cm^{-3}, T is the temperature in K and a and b are the fitting parameters. The adjustable parameters (a and b) were determined from the fitting of the experimental density data, from the eq 1 and are presented in Table 3, as well as the thermal expansion coefficients (α_p) were determined and are presented in Table S4 in the Supporting Information.

The thermal expansion coefficient values obtained for the eutectic mixtures vary between 7.891 and 8.998 × 10^4 K^{-1} in the case of the dried samples, and in general are up to 2–6% higher for the water-saturated samples. The highest thermal expansion coefficients were observed for the DL-menthol:acetic acid, whereas the DL-menthol:lactic acid displayed the lowest ones.

The molar volumes (V_m) of dried and water-saturated eutectic mixtures studied in this work were calculated from the density results and are presented in Table S5 in the Supporting Information. Molar volumes were determined for a range of temperatures from 293.15 and 353.15 K, and were obtained from the following equation:

$$V_m = \frac{M}{\rho} \tag{2}$$

where M corresponds to the molar mass in g·mol^{-1} and ρ is the density in g·cm^{-3}. The molar volumes for the water-saturated eutectic mixtures containing pyruvic and lactic acid are higher than those for the dried mixtures, probably due to the water present. The molar volumes increase in the following order: DL-menthol:lactic acid < DL-menthol:pyruvic acid < DL-menthol:acetic acid < DL-menthol:lauric acid. Usually, the molar volumes are in agreement with the molar mass of each compound, which does not happen in this study, because the eutectic mixture with the highest molar mass does not exhibit the highest molar volume. This can be probably be explained by chemical and structural differences in hydrogen bond donors (here the presence of dimmers might play an important role), the different proportions between DL-menthol and hydrogen bond donors and the different amounts of water. The molar volume of the dried and saturated DL-menthol:lauric acid eutectic mixture do not significantly differ from each other, due to minimal difference in their water content. As for the dried and saturated DL-menthol:acetic acid mixture the molar volumes, they are also very similar in agreement to what was observed for their density values.

The experimental viscosity data for the dried and the water-saturated samples of eutectic mixtures, as a function of temperature, are depicted in Figure 5 and reported in Table S3 in the Supporting Information. The viscosities for the dried samples of the eutectic mixtures increase in the following order: DL-menthol:acetic acid < DL-menthol:lauric acid < DL-menthol:pyruvic acid < DL-menthol:lactic acid. It can be observed that despite the similar chemical structures of the pyruvic and lactic acids and the fact that they are in same proportion in the eutectic mixtures, they display very different viscosity values. In fact, the DL-menthol:lactic acid mixture's viscosity is 1 order of magnitude higher than that of the other three eutectic mixtures studied in this work. Regarding the water content, two distinct types of behaviors can be observed, independently of the DL-menthol:carboxylic acid proportions: for the eutectic mixtures containing lauric and acetic acids, the presence of water increases the viscosity, whereas for the other two mixtures, the opposite behavior is observed, in the whole temperature range. These facts clearly illustrate the great variety of behaviors that these eutectic mixtures in particular display, which cannot be *a priori* predicted. The lack of systematization of the eutectic mixtures and deep eutectic mixtures thermophysical properties is probably due to the large amount of disparate compounds that are possible to prepare, leading to compounds with properties that are not possible to compare.

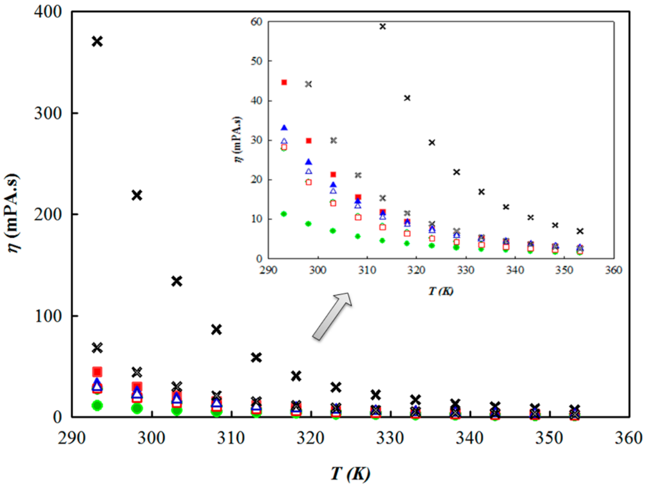

Figure 5. Experimental viscosities (η) of the dried and water-saturated eutectic mixtures as a function of temperature: DL-menthol:acetic acid (●), DL-menthol:pyruvic acid (■), DL-menthol:lactic acid (×), DL-menthol:lauric acid (▲). The filled symbols correspond to the dried samples and the empty symbols correspond to the water-saturated samples. The lines represent the fitted data calculated using eq 2.

The experimental viscosity values were fitted as a function of temperature, using the Vogel–Fulcher–Tammann (VFT) model with the following equation:

$$\ln \eta = A_\eta + \frac{B_\eta}{(T - C_\eta)} \tag{3}$$

where η is the viscosity in mPa·s, T is the temperature in K, and A_η, B_η, and C_η are adjustable parameters. The adjustable parameters were determined from the fitting of the experimental values and are listed in Table 4. The activation energy was calculated based on the viscosity dependence with temperature as follows:

$$E_a = R\frac{\partial(\ln \eta)}{\partial\left(\frac{1}{T}\right)} = R\left(\frac{B_\eta}{\left(\frac{C_\eta^{\ 2}}{T^2} - \frac{2C_\eta}{T} + 1\right)}\right) \tag{4}$$

where η is the viscosity, T is the temperature, B_η and C_η are the adjustable parameters obtained from eq 3, and R is the universal gas constant. The values of the activation energies for the four prepared eutectic mixtures are presented in Table 5.

The use of eq 4 to describe the temperature behavior of viscosity allows the discussion of the energy barrier of a fluid to shear stress, E_a values. The higher the E_a value, the more difficult it is for the molecules/aggregates to move past each other. This can be a direct consequence of the size or entanglement of the molecules/aggregates and/or the presence of stronger interactions within the fluid. Regarding the size of the hydrogen bond donors, and using the calculated molar volume calculated in Table S6, it can be observed that this parameter does not greatly influence the viscosity, because DL-menthol:lauric acid has a large V_m value and it is not the most viscous mixture. On the other hand, it can be observed that the mixtures containing lactic acid are the most viscous, which clearly indicates the relevant role of the hydroxyl substituent group in the establishment of hydrogen bonds, thus increasing the viscosity. In the case of the pyruvic acid, the carboxyl group is not as good a hydrogen bond donor as the hydroxyl, and thus its capacity to establish hydrogen bonds is much smaller than that of lactic acid. The two eutectic mixtures containing linear

Table 4. Fitted Parameters of VFT Model Given by eq 3 and Respective Correlation Coefficient (R^2) for Dried and Water-Saturated Eutectic Mixtures

		parameters			
		A_η (mPa·s)	B (K)	C_η (K)	R^2
DL-menthol:acetic acid	dried	−3.213	595.666	187.422	1
	saturated	−3.689	677.581	196.432	0.9999
DL-menthol:pyruvic acid	dried	−2.423	464.436	218.436	0.9999
	saturated	−2.782	476.198	215.341	1
DL-menthol:lactic acid	dried	−3.381	745.522	213.049	1
	saturated	−3.902	725.375	203.865	1
DL-menthol:lauric acid	dried	−3.599	815.874	178.123	0.9999
	saturated	−3.883	887.449	171.033	0.9999

Table 5. Activation Energy Values (E_a (kJ/mol)) for Dried and Water-Saturated Eutectic Mixtures as a Function of Temperature, Determined by eq 4

	DL-menthol:acetic acid		DL-menthol:pyruvic acid		DL-menthol:lactic acid		DL-menthol:lauric acid	
T (K)	dried	saturated	dried	saturated	dried	saturated	dried	saturated
293.15	38.1	51.7	59.5	56.2	83.0	65.0	44.1	42.5
298.15	35.9	48.4	54.0	51.3	76.1	60.3	41.9	40.6
303.15	33.9	45.5	49.5	47.2	70.2	56.3	39.9	38.8
308.15	32.3	42.9	45.6	43.6	65.0	52.7	38.1	37.3
313.15	30.7	40.6	42.2	40.6	60.7	49.5	36.5	35.8
318.15	29.3	38.5	39.3	37.9	56.8	46.7	35.1	34.6
323.15	28.1	36.6	36.8	35.6	53.4	44.3	33.7	33.3
328.15	26.9	34.9	34.5	33.5	50.4	42.0	32.5	32.2
333.15	25.9	33.4	32.6	31.7	47.7	40.1	31.3	31.2
338.15	24.9	32.1	30.8	30.1	45.3	38.2	30.3	30.2
343.15	24.1	30.8	29.2	28.5	43.1	36.6	29.3	29.3
348.15	23.2	29.7	27.8	27.2	41.2	35.1	28.4	28.5
353.15	22.5	28.6	26.5	26.0	39.4	33.8	27.6	27.8

Table 6. Partition Coefficients, K_i, of the Different Biomolecules between DL-Menthol-based Eutectic Mixtures and Aqueous Solutions[a]

eutectic mixtures	pH	caffeine	tryptophan	vanillin	isophthalic acid
DL-menthol:acetic acid	1.91	1.81 ± 0.01	9.05 ± 0.33	4.74 ± 0.03	8.52 ± 0.23
DL-menthol:lactic acid	1.18	37.33 ± 0.09	14.76 ± 0.17	3.03 ± 0.15	3.31 ± 0.12
DL-menthol:lactic acid	1.47	1.16 ± 0.01	2.85 ± 0.04	6.65 ± 0.04	12.94 ± 0.10
DL-menthol:lauric acid	3.74	1.09 ± 0.01	5.23 ± 0.12	7.02 ± 0.01	1.96 ± 0.03
pK_a[b]		14.0	2.38; 9.39	7.4	3.7; 4.6
water solubility[b] (mg/mL) at 25 °C		16	11.4	10	0.13

[a] The pK_a of the biomolecules and their water solubility is also shown. [b] See http://pubchem.ncbi.nlm.nih.gov/.

nonsubstituted carboxylic acids, lauric and acetic acids, have the smallest E_a values, in agreement with their small viscosity. Only in this case, the differences in V_m accounts for the differences in E_a and thus, in viscosity.

The E_a values obtained for water saturated samples are similar to each other, indicating that the presence of water, even in different amounts, and in mixtures with different molar ratios, leads an easier and similar flux of the different molecules/aggregates.

Biomolecules Partition. Four model biomolecules, namely caffeine, tetracycline, tryptophan, and vanillic acid, were used to illustrate the usage of the proposed eutectic mixtures as extraction solvents of biomolecules from aqueous solutions. Because the proposed eutectic mixtures are all partially miscible with water, no addition of salting out agents, such as salts, polymers etc., is necessary to achieve a two phase system. The partition coefficient of each biomolecule *i*, K_i, was calculated as the ratio of their concentration in both the eutectic mixture-rich phase ($C_{i,\mathrm{EM}}$) and in the water-rich phase ($C_{i,\mathrm{AQ}}$), according to the following equation:

$$K_i = \frac{C_{i,\mathrm{EM}}}{C_{i,\mathrm{AQ}}} \qquad (5)$$

The results obtained for the four biomolecules tested are presented in Table 6. It was not possible to evaluate partition data using DL-menthol:pyruvic acid due to the presence of an absorption band in UV−visible region at about 350 nm, which is due to $\pi^* \leftarrow n$ transition because pyruvic acid is a keto acid. The pH values at which extractions were carried out are included in Table 3. Comparing the pK_a of the biomolecules with the pH of the solutions, it can be concluded that this parameter has no influence extraction of the biomolecules because no correlation can be established between pH and the ionization state of the biomolecules.

Although caffeine presents a higher affinity for aqueous phase, as ascertained by its octanol−water partition coefficient (K_{ow}) equal to 0.85,[22] it is possible to extract successfully this solute to the organic solvents. For example, the extraction of caffeine from water using chloroform reached partition coefficients between 18.7 and 22.8,[23] whereas using aqueous biphasic systems (ABS) composed of imidazolium-based ILs, such as [C_7H_7mim]Cl, [Amim]Cl, [C_4mim]Cl, [C_2mim]Cl, and K_3PO_4,[22] partition coefficients higher than 120. ABS using [C_4mim][DCA] and lysine were also explored[24] for the extraction of caffeine. However, the partition coefficient obtained are much lower (K_i = 5.8) than those obtained with K_3PO_4. Louros et al.[25] also reported the partition of caffeine using ABS containing a phosphonium ionic liquid [P_{4441}]-[$MeSO_4$] and K_3PO_4, resulting in K_i = 4.75. In the present case, only when the DL-menthol/lauric acid 2:1 eutectic mixture was used as the extractant, the obtained partition coefficient was higher than 1, indicating a more favorable partition toward the eutectic mixture phase.

Tryptophan was another model biomolecule chosen, because it is one of the most hydrophobic amino acids. Nevertheless, tryptophan possesses a polar −NH group, allowing the establishment of hydrogen bonds, depending on the solvent.[25] The partition of two tryptophan analogues, *N*-methylindole and 3-methylindole, in a cyclohexane/water system, was addressed by Winley and White,[26] achieving K_i = 290 and 19.1, respectively, at 25 °C. These results are consistent with the hydrophobic nature of tryptophan. The results obtained in this work for the partition of tryptophan between an aqueous phase and each one of the prepared eutectic mixtures are higher than those obtained for caffeine. Again, no difference in the partition coefficient was observed when DL-menthol/acetic acid or DL-menthol/lactic acid were used, but for the DL-menthol/lauric acid 2:1 eutectic mixture a partition coefficients of 14.5 was obtained. Tomé et al.[27] used hydrophobic ILs to extract tryptophan from aqueous solutions and obtained partition coefficients of 4.5 and 7.8 for [C_2mim][NTf_2] and [C_8mim]-[BF_4], respectively. Neves et al.[28] and Ventura et al.[29] also studied the tryptophan partition in ABS containing imidazolium-based IL and K_3PO_4, obtaining large partition coefficients of K_i = 124 and K_i = 59 for the [Amim]Cl and [C_2mim]Cl ILs, respectively. Louros et al.[25] also reported the partition of tryptophan (K_i = 9.0) in ABS based on [P_{4441}][$MeSO_4$] and K_3PO_4 whereas Passos et al.[30] [N_{4444}]Cl and potassium citrate achieved the best result (K_i = 67).

In the case of vanillic acid, with K_{ow} = 16.3 indicating its more hydrophilic character,[31] the highest K_i (5.72) was obtained with the DL-menthol/lactic acid 1:1 eutectic mixture, probably due to a larger number of hydrogen bonds that can be promoted between the solute and the eutectic mixture. Cláudio et al.[32] studied the extraction of a similar compound, vanillin using a ABS containing imidazolium-based IL and K_3PO_4, reaching partition coefficients larger than 44 with [C_7H_7mim]-Cl, [C_4mim]Cl, and [C_6mim]Cl.

Isophthalic acid possesses very low water solubility (130 mg/L) and was chosen due to that characteristic. Buch et al.[33] tested an extraction of isophthalic acid from soil samples using dichloromethane, ethyl acetate, acetonitrile, ethanol, and propanol, and observed a larger affinity for ethyl acetate and propanol, being 80% of the compound removed from soil. Feng et al.[34] studied the partition of this compound in a system containing propyl acetate and water and obtained a partition coefficient of 40 at 30 °C. These data indicate that isophthalic acid has medium polarity, which confirms results obtained using menthol-based eutectic mixtures, with acetic and lactic acids with higher coefficients partition.

CONCLUSION

In this work, new green solvents based on eutectic mixtures with components from renewable resources are presented and studied. These new solvents are eutectic mixtures composed of DL-menthol and several organic acids in different proportions. Contrary to deep eutectic solvents, these new eutectic solvents presented low viscosities (5−100 cP) and densities lower than water, as can be evaluated by the density and viscosity values, from 293.15 up to 353.15 K and at atmospheric pressure, independently of the water content. A large diversity of behaviors in terms of the measured thermophysical properties can be observed when the different organic acids, proportions between DL-menthol and hydrogen bond donor and water contents are used making their understanding and thus prediction very difficult. Despite the fact that these eutectic mixtures are composed of naturally occurring hydrophilic acids usually also used in the preparation of deep eutectic solvents, they are immiscible with water, which confers them a privilege place among the green solvents, which are usually very hydrophilic. Using the hydrophobic character of these eutectic mixtures, partitions of selected solvents were performed to illustrate their utility in separation systems.

ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.5b00532.

> Characterization of DL-menthol-based eutectic mixtures; systems used for extraction solvents of biomolecules from aqueous solutions; experimental data of thermal properties, such as density and viscosity; the fitted of density and viscosity data for the studied eutectic mixtures; and molar volume and activation energy values calculated from experimental results (PDF).

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Notes

The authors declare no competing financial interest.

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