# THEDES- THERAPEUTIC DEEP EUTECTIC SOLVENTS: NEW SOLVENTS FOR THE FUNCTIONALIZATION OF NATURAL-BASED POLYMERS

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## ABSTRACT

It is known that there are a number of deep eutectic solvents (DES) that can be constituted by bioactive substances or even active pharmaceutical ingredients (APIs). The synthesis of bioactive DES or therapeutic DES- THEDES, based on ibuprofen, a non-steroidal anti-inflammatory agent, and *R*-(-)-mandelic acid, known for its antibacterial activity, was performed.
In this work, a polymeric blend of starch and poly-ε-caprolactone (SPCL 30:70) doped with THEDES in different amounts of 10 and 30 wt %, was submitted to foaming with $scCO_2$ at 20.0 MPa and 40 °C. The THEDES prepared were analysed by Karl Fisher to control the percentage of water present and by differential scanning calorimetry (DSC). The morphological characterization of the SPCL samples doped with THEDES was by scanning electron microscopy and micro-computed tomography. The studies carried out suggest that the proposed approach provide interesting cues for the development of structures for drug delivery.

## INTRODUCTION

Natural-based biopolymers are now seen as safe and green alternatives for traditional plastics, and find applications in areas ranging from biomedicine to electronics. Their major drawback is still related to their low processability. In recent years, there has been extensive work in the development of plasticizing agents for these polymers, mainly the use of ionic liquids (ILs) [1–3]. Recently, we established that the combination of alternative solvents such as supercritical $CO_2$ and ILs, has the ability to enhance the porosity and the mechanical properties of SPCL blends [4][5]. This is mainly attributed to the fact that the interactions established between the IL and the polymer promotes the flexibility of the material.
Nevertheless, ILs present challenges when the applications of the foamed materials intend to be in the biomedical area, since their toxicity for humans is not yet fully understood.

In recent years a new type of solvents called deep eutectic solvents (DES) found applications where ILs were traditionally the solvents of choice [6–8]. These solvents result from the mixture of components which at a particular composition present a high depression in the melting point, becoming liquid at room temperature [9]. Most of the known DES are composed by quaternary ammonium salts such as choline chloride (ChCl) and a hydrogen bond donor such as urea, succinic acid or even glycerol [10]. Recently, Dai and co-workers found that a number of primary metabolites was also able to behave as DES. These can be constituted by sugars like glucose and organic acids such as citric acid, which are now referred to as natural deep eutectic solvents (NADES) [6]. An increasing number of research groups are dedicated to the characterization of DES and NADES, and their possible applications, as they present greener, more sustainable and cheaper alternative to traditional ILs.

One of the areas where DES can have interesting applications, is in the development of bioactive solutions or materials. DES can be formed by one or more bioactive compounds, with different biological activity. Stott *et al.* [11] and Carriazo *et al.* [12] have reported the formation of a DES composed by menthol (anesthetic and permeation enhancer compound) and ibuprofen (a model anti-inflammatory drug). Ruβ *et al.* [13] also reported the formation of a DES composed by ChCl and *R*-(-)-madelic acid (known for its antimicrobial activity).

In this work, and following a previous approach on the processing of biopolymers [14], a biocompatible polymeric blend (starch:poly-ε-caprolactone, SPCL) was used. The main goal was to use bioactive or therapeutic DES (THEDES) as foaming agents of the biopolymer blend to be applied in controlled drug release systems. The same approach used by Duarte *et al.* was used to obtain materials with high porosity and pore interconnectivity [4].

Ultimately, the bioactive compounds will remain in the porous matrix obtained by supercritical fluid foaming, and, since it is a polymeric and biocompatible porous matrix, the controlled release of the compound into a medium will be achieved. This has one major advantage, which is the possibility of using the therapeutic agent in its liquid form, overpassing the problems related with the polymorphism and solubility of the crystalline state [15].

## MATERIALS AND METHODS

### Materials

The polymer used in this work was a commercial blend of corn starch with poly- ε-caprolactone (SPCL 70 wt %) in granular form obtained from Biocycle. The reagents used in the preparation of THEDES were choline chloride (ChCl ≥98 %, CAS 89-78-1, Aldrich), *R*-(-)-mandelic acid (98 %, Alfa Aesar) and (+/-)-menthol (≥98 %, CAS 67-48-1, Sigma). Ibuprofen (CAS 31121-93-4, Sigma) was obtained in the sodium salt form. This reagent was washed and dried in order to obtain the isolated ibuprofen. Carbon dioxide (99.998 mol%) was supplied by Air liquid.

## THEDES preparation

THEDES solutions were prepared according to previously reported papers [12,13]. In the case of the eutectic mixture ChCl:mandelic acid, the two components were mixed in a molar ratio of 1:2. A small amount of distilled water was added to ensure complete dissolution, and the mixture was then heated at 40 $^{o}$C under constant stirring for at least 1 hour. The mixture was then dried using a rotary evaporator at 50 °C under vacuum, until a clear viscous liquid was obtained. The solution was kept under vacuum until further use. The eutectic mixture menthol:ibuprofen was prepared in the molar ratio of 3:1. The mixture was heated at 40 ºC and became a clear liquid without addition of water. Following the procedure of Stott *et al.* [11], the mixture was stored at -20 ºC.

## Sample preparation

The SPCL-THEDES samples were prepared by mechanical mixing in a mortar, weighing the appropriate amounts of SPCL and each THEDES, to obtain final SPCL samples with 10 and 30 wt% of each of the THEDES. The final mixtures were all homogeneous blends and no phase separation was observed. Disc shaped samples were prepared by compression moulding using stainless steel rings (12x2 mm) as a mould, at 50-60 ºC, 7 MPa for 10 minutes.

## Supercritical fluid foaming

The compressed moulded samples of SPCL and SPCL doped with THEDES were foamed using supercritical carbon dioxide at 20.0 MPa, 40 ºC for one hour. A high pressure 20 ml stainless steel cell was used. The sample was loaded in the high pressure cell, thermostated in a water bath until the desired temperature is reached. At this point $CO_2$ is compressed into the cell up to 20 MPa and the system is maintained pressurized for one hour. Thereafter, the vessel is depressurized and the sample recovered.

## Characterization

THEDES mixtures were characterized by differential scanning calorimetry (DSC) and the water content was determined by Karl-Fischer titration. The morphology of the structures was analyzed by scanning electron microscopy (SEM) and micro-computed tomography (micro-CT).

## RESULTS

The resulting eutectic mixtures- THEDES- were homogeneous and presented no phase separation after storage. DSC studies were performed in order to have a better understanding

of the thermal behaviour of the THEDES mixtures and to ensure that the samples did not suffer degradation upon processing.

**DSC**

DSC of the pure compounds that form the two studied DES were collected. All pure compounds exhibited the thermal events of melting and crystallization, and were in accordance with tabled values (as well as glass transition in the case of menthol, ibuprofen and mandelic acid). It is very interesting to notice that the THEDES, ChCl:*R*-(-)-mandelic acid in a molar proportion of 1:2 and menthol:ibuprofen in a molar proportion of 3:1, the mixture no longer presents the characteristic thermal events of the pure compounds. This proves that the components of the THEDES are in fact combined and present a different physical state than its crystalline form.

**SCF foaming**

SPCL samples doped with the two different THEDES were also homogeneous. Before and after foaming the samples were characterized using several by scanning electron microscopy and micro-computed tomography.

*SEM and Micro-CT analysis*

SEM micrographs were collected for samples of SPCL doped with THEDES, before and after supercritical carbon dioxide foaming. It is clear that the presence of THEDES enhances the foaming processes, which is easily proved by the formation of porous structures upon THEDES doping. As reported in previous works SPCL per se is not able to undergo the foaming processes in such as large extent rendering approximately 14% of porosity under the same foaming conditions.[14]

Micro-CT experiments were carried out in order to evaluate the morphological parameters characteristic of the samples. Table 2 presents the summary of the results obtained. It was observed that the supercritical foaming does increase porosity, regardless of the THEDES used for the doping, being more significant for the case of ChCl:mandelic acid. In the case of ChCl:mandelic acid the porosity, mean pore size and interconnectivity were higher when using 10 wt% for the doping of SPCL. When doping SPCL with menthol:ibuprofen, the same trend was obtained using 30 wt% of the THEDES. This shows that the foaming process of SPCL can be tuned by the choice of the THEDES for the doping and the extent of foaming is related with the interactions that are established between the THEDES and the SPCL, similarly to what happens when the polymer is dopped with conventional ionic liquids.[14]

**Table 1:** SEM micrographs of the SPCL samples doped with different amounts of THEDES ChCl:mandelic acid and menthol:ibuprofen in different weight percentages, before and after the foaming process.

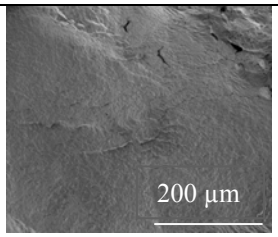
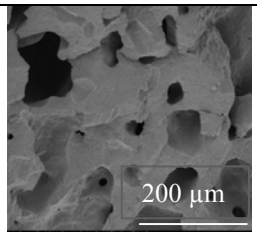
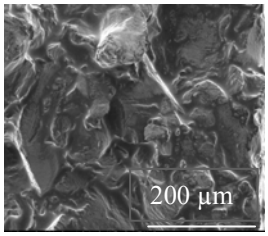
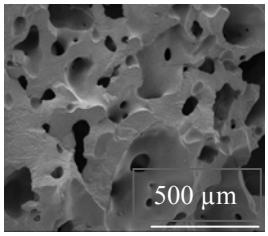
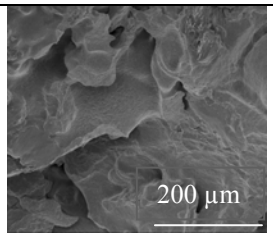
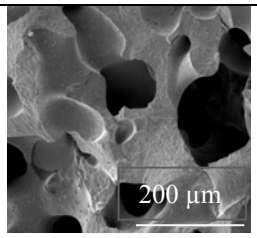
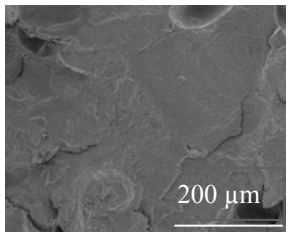
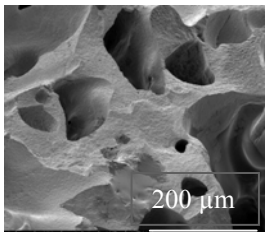
| THEDES | wt % | No foaming | Foaming |
|---|---|---|---|
| ChCl:mandelic acid | 10 |   |   |
| | 30 |   |   |
| Menthol:ibuprofen | 10 |   |   |
| | 30 |   |   |

**Table 2:** Summary of micro-CT analysis of the samples of SPCL doped with the THEDES mixtures, with and without foaming.

| Sample | Porosity% | Mean Pore size µm | Interconnectivity% |
|---|---|---|---|
| 10ChCl:mandelic acid_no foaming | 0 | - | 0 |
| 30ChCl:mandelic acid_no foaming | 0 | - | 0 |
| 10ChCl:mandelic acid | 31.5±1.4 | 258±33 | 20.2±9.5 |
| 30ChCl:mandelic acid | 16.6±0.6 | 199±23 | 12.2±6.8 |
| 10mentol:ibuprofen_no foaming | 0 | - | 0 |
| 30mentol:ibuprofen_no foaming | 0 | - | 0 |
| 10mentol:ibuprofen | 25.5±3.2 | 253±45 | 10.0±5.5 |
| 30mentol:ibuprofen | 22.9±1.4 | 285±31 | 13.3±5.7 |

## CONCLUSION

The preparation of deep eutectic solvents with bioactive or therapeutical compounds was demonstrated in this work following an easy and simple route. The supercritical foaming process of biopolymers is a feasible process to obtain porous materials and it is also enabled by the presence of THEDES. The presence of these deep eutectic solvents enhances porosity, pore size and interconnectivity of the samples and it is dependent on the THEDES used for the SPCL doping. Moreover, there is the advantage of obtaining a porous biopolymer matrix that is impregnated with therapeutic agents, which can later be used as alternative controlled drug release system. The compounds when mixed and in eutectic mixture form become less crystalline and therefore an improvement of its therapeutic action can be expected.

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