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EXECUTIVE SUMMARY OF THE THESIS

Experimental study of eutectogels for drug delivery

TESI MAGISTRALE IN BIOMEDICAL ENGINEERING – INGEGNERIA BIOMEDICA

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1. Introduction

The study of drug delivery systems (DDS) is a field of science that is constantly evolving and poses new challenges to current and future scientists. By using materials that are non-toxic and have good biocompatibility, it is possible to create drug delivery systems in a specific way that can retain the drug by chemical bonding or host-guest interactions. Their peculiarity consists in encapsulating an active pharmaceutical ingredient and then allowing for its controlled release over time. Additionally, polymeric carriers can be designed to deliver the drug to a target area within the human body. This has a wide variety of clinical applications, such as cancer treatment, infections, wound healing and tissue engineering applications [1]. An example of these materials are hydrogels, which have been extensively investigated in recent years for both target release and controlled release as an alternative to conventional drug formulations.

A new class of bio-materials is represented by eutectogels, which are physical gels based on deep eutectic solvents (DESs). Although both deep eutectic solvents and eutectogels still require extensive analysis and research, they could become suitable drug delivery systems, competing with hydrogel and bijel systems. This is due to their promising properties, such as high biocompatibility, low volatility, and high thermal stability, other than the low cost of DES components [2] and their easy preparation protocols. Their 3D network, based on hydrogen bonding, makes them a suitable system for loading both hydrophilic and lipophilic drugs, therefore eutectogels can be a good alternative to expensive traditional gels used in controlled drug delivery [3]. The appropriate selection of the DES components and gelling agent, hence their properties and local intermolecular interactions, is very important due to the influence they play in the formation of the 3D network of eutectogels.

The aim of this thesis is to prepare novel hydrophobic, non-ionic, eutectogel systems based on menthol-thymol 1:1 mixture and 1,3:2,4-dibenzylidene-D-sorbitol as gelling agent. The morphology, structure and dynamics of these new materials have been investigated by several analytical techniques such as: scanning electron microscopy (SEM), infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy

(NMR). The macroscopic release capabilities of eutectogels loaded with ethosuximide, an anti-epileptic medication, under different physical condition are also studied.

2. Materials and methods

2.1. Materials

D,L Menthol (M=157.27 g/mol, racemic ≥ 98.0%, CAS 89-78-1, by Sigma Aldrich, Germany), Thymol (M=150.22 g/mol, ≥ 98.5%, CAS 89-83-8, by Sigma Aldrich, Germany), Ethosuximide (ESM) (M=141.17 g/mol, CAS 77-67-8, by Sigma Aldrich, Germany), 1,3:2,4-Dibenzylidene D-Sorbitol (DBS, > 95%, CAS 32647-67-9, by Accela ChemBio), 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid) from TSP (98 atom % D, CAS 24496-21-8, by Isotec™. Solvents and additives: Distilled water, Deionized water (milliO water, ultrapure deionized water), PBS (Dulbecco's phosphate-buffered saline tablets, pH 7.2-7.6, by Sigma Aldrich, Germany), Deuterium Oxide (99.9 atom % D, CAS 7789-20-0, by Sigma-Aldrich, Germany). All reagents and solvents were used without further purification. The reactions were carried out under atmospheric pressure.

2.2. Preparation of eutectogels

Eutectogels-drug loaded were prepared according to the following four steps procedure. *1)* 0,3g of both menthol and thymol were mixed and heated at 30-40°C under stirring until a transparent solution was obtained. *2)* While still stirring, ESM was added to the DES in concentrations of 10, 40 and 100 mg (different concentrations used for different experiments). *3)* The gelling agent (DBS) in concentrations of 2% or 5% w/w, was added after complete dissolution of the drug. Here, heating until 150°C and stirring was necessary for complete dissolution of the DBS. *4)* When the mixture became transparent, it was poured into a metal cylinder and left to solidify and fix the structure during the weekend.
Eutectogels-blank (without drug) were also prepared following steps 1, 3 and 4. A representative structure of eutectogel is shown in the figure 1. At this point, it was possible to proceed with the physicochemical characterization of all the prepared gels.

Figure 1: Prepared eutectogel with fixed structure.

2.3. Analysis methods

In this work, several analytical techniques were used to investigate morphology, structure, and dynamics of the eutectogels blank, and drug loaded. All the experiments were performed on both systems prepared with 2% and 5% w/w DBS to study the effect of gelator concentration. The morphology of eutectogels with 5% w/w DBS (blank and drug loaded) was analysed using SEM technique. Adequate sample preparation was required and therefore menthol and thymol had to be removed from the gel and replaced with ethanol. FTIR spectroscopy was performed on eutectogels blank and loaded with 100 mg of ethosuximide using a Varian 640-IR spectrometer from Agilent technologies. ^{1}H HR-MAS NMR experiments were recorded on Bruker 500 NEO operating at 500 MHz proton frequency, equipped with a dual ^{1}H/^{13}C HR MAS probe. Diffusion-ordered spectroscopy (DOSY) was used to study the diffusion of both drug and DES components in the gel phase. For NMR measurements eutectogels blank and loaded with 40 mg and 100 mg content of ethosuximide were used.
Gels loaded with 10 mg of drug were used for macroscopic releases experiments. Measurements were carried under different temperature (room temperature and 37 °C) and pH (4,2 and 7,2) conditions to study the eutectogels' response. The samples (reported in table 2.1) were immersed in 2 ml of PBS. 1 ml of the release medium was withdrawn at 5min, 30min, 1h, 1h30, 2h, 3h, 6h, 8h, 24h and 30h. Thereafter, PBS was collected every 24h for 18 days (432h). Each time, fresh PBS solution was added to compensate the collected samples. Release samples were examined though quantitative NMR on a Bruker 400Advance, thanks to its ability to clearly separate the different molecules (drug and DES components) inside the release medium (Figure 2). All release experiment has been carried out in triplicate.

Table 2.1 Samples for release studies.

Sample name	Experimental parameters
S1	5% w/w DBS, Room T, pH 7.2
S2	5% w/w DBS, Room T, pH 4.2
S3	5% w/w DBS, T = 37 °C, pH 7.2
S4	5% w/w DBS, T = 37 °C, pH 4.2
S5	2% w/w DBS, Room T, pH 7.2
S6	2% w/w DBS, Room T, pH 4.2
S7	2% w/w DBS, T = 37 °C, pH 7.2
S8	2% w/w DBS, T = 37 °C, pH 4.2

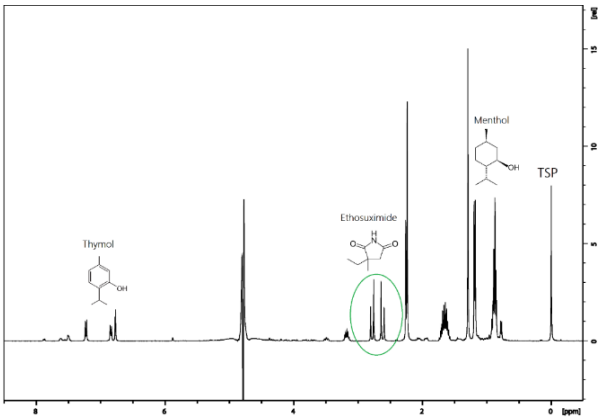

Figure 2: ^{1}H NMR spectrum of ethosuximide in release medium.

3. Results and discussion

3.1. FTIR analysis

FTIR graphs are represented on the figures below. Figure 3 shows the analysis on the eutectogel (blank and drug loaded) prepared with 5% w/w DBS and figure 4 with 2% w/w DBS. The main spectral region – the hydroxyl stretching region (3000-3700 cm^{-1}) -OH can be seen in all systems, showing the presence of hydrogen bonds that form the gel. The addition of ethosuximide has no noticeable effect on the 5% system. The gel with 2% of DBS, on the other hand, shows some variation of the -OH peak, suggesting that the drug takes part in the hydrogen bond network at lower concentration of gelator.

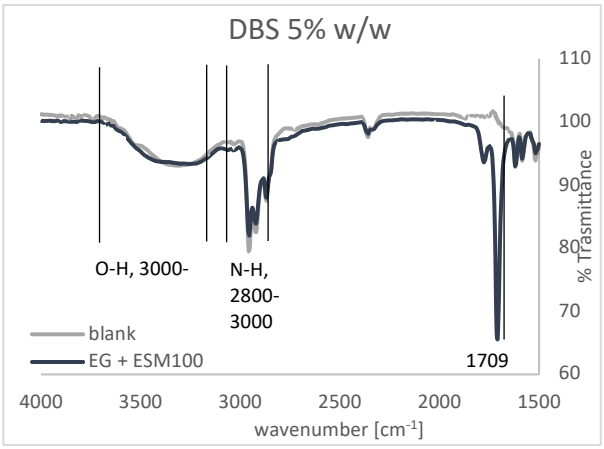

Figure 3: FTIR spectra of eutectogels with 5% w/w DBS.

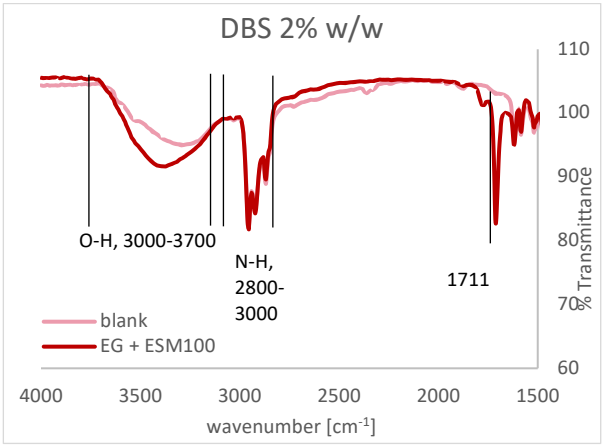

Figure 4: FTIR spectra of eutectogels with 2% w/w DBS.

3.2. SEM analysis

The scanning electron microscopy images of eutectogels with 5% w/w DBS are shown in Figure 5 (5A eutectogel without ethosuximide and 5B gel loaded with ethosuximide).

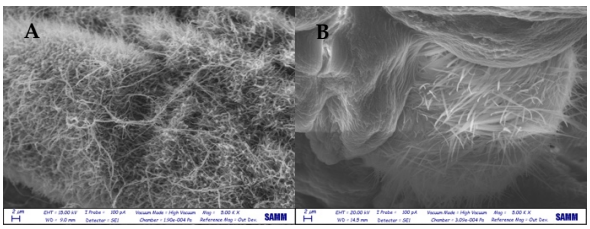

Figure 5: SEM analysis of eutectogels with DBS 5% w/w.

SEM images show a fibrillar network that is typically formed by molecular gelators, which self-assemble in three-dimensional networks consisting of fibres connected by noncovalent

intermolecular interactions such as hydrogen bonding [4]. These non-covalent interactions are also what allow these structures to trap small molecules within them.

3.3. NMR analysis

NMR measurements were performed on different samples, to study both possible DES-drug interactions and the diffusion motion of ethosuximide and the DES components inside the gel 3D network.

Figure 6 shows ^{1}H HR-NMR spectra acquired on three different gels (no drug, 40 mg and 100 mg drug loading) with 2% w/w of gelator. In these spectra we can separate peaks corresponding to menthol (>4 ppm), thymol (6-8 ppm) and ESM (2.1 ppm) which will be used for DOSY measurements. By comparing these spectra, it is also possible to see how the addition of ESM affects the -OH peak of menthol (3.3 ppm), which shifts to higher ppm values (dotted green line figure 6). This can be attributed to hydrogen bonding interactions between menthol and ethosuximide, which is consistent with the results from FTIR measurements.

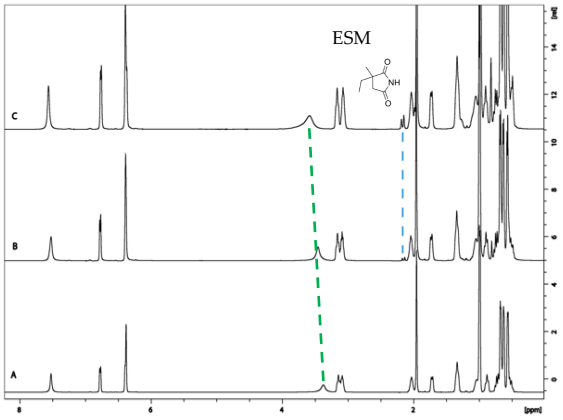

Figure 6: A) ^{1}H HR-MAS spectrum of blank eutectogel with 2% w/w of DBS; B) ^{1}H HR-MAS spectrum of eutectogel loaded with 40 mg ESM; C) ^{1}H HR-MAS spectrum of eutectogel loaded with 100 mg ESM.

The results of the diffusion studies are reported in Figure 7. Figure 7A shows diffusion results of ethosuximide in the two systems 5% w/w and 2% w/w DBS. The diffusion motion is slower for ESM in the 5% DBS gel system than in the 2% DBS gel. This is due to a more entangled 3D fibrillar network formed by the higher concentration of gelator. The diffusion coefficient values are in the range ($3.5 \cdot 10^{-11}$-$5 \cdot 10^{-11}$ m^2/s) which is typical of a viscous liquid.

Moreover, increasing the drug concentration, its diffusion coefficient decreases in both systems prepared with different content of gelling agent. For eutectogels with 5% w/w DBS content, there is some decrease in ESM diffusion, but the variability is smaller than the system prepared with 2% w/w DBS due to a less mobility caused by the higher amount of gelling agent. In conclusion, the effect of the drug and the concentration of DBS are competitive, and the gelator effect becomes more relevant as its concentration increases.

The behaviour of DES components (menthol and thymol) was also investigated (Figure 7B and C), HR-MAS NMR measurements clearly show the intra-gel mobility of both these molecules.

Both menthol and thymol show a slower diffusion in going from 2% to 5% w/w amount of DBS, which is consistent with the idea of a tighter mesh size and stronger fibrillar network with the increase of the gelator, which hinders the molecules movement. It is also interesting to note the effect of drug concentration on Men/Thy mobility, which decreases with increasing amount of drug. The variation of the motion of the DES components with the drug concentration also confirms the hypothesis of the DES-drug interaction in the formation of hydrogen bonds.

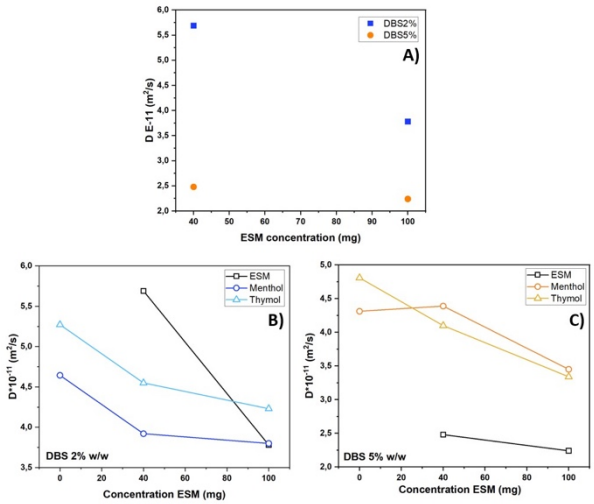

Figure 7: Diffusion coefficients of A) ESM only in 2% DBS and 5% DBS eutectogels. Comparison of DES components and ESM in B) 2% DBS and C) 5% DBS eutectogels.

3.4. Release measurements

Figure 8 below shows four ESM release profiles from eutectogels with 5% w/w DBS content under different experimental conditions (pH and temperature). The slowest rate of drug release is obtained at room temperature and pH 7.2, reaching 89% of mass released after 18 days. At room temperature and pH 4.2 drug is released faster than for pH 7.2, and after 18 days, the mass released reaches 87%. 100% of mass released is achieved only at 37 °C, and pH 7.2.
Furthermore, at 37°C, an initial burst effect is observed simultaneously in both neutral and acidic conditions, reaching 60% of the mass released within 24 hours. At pH 4.2, the process slightly slows down compared to pH 7.2 and after 18 days 87% of drug mass is released.

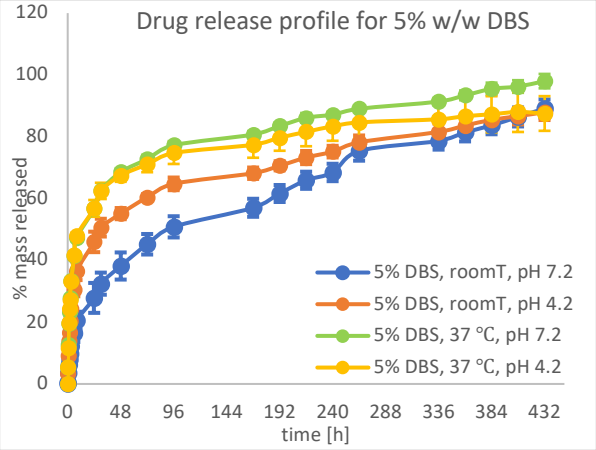

Figure 8: Release profiles of ethosuximide from eutectogels with 5% w/w DBS

Figure 9 reports four drug release profiles from eutectogels with 2% w/w DBS content under different pH and temperature conditions. The slowest rate of drug release is seen at room temperature and pH 7.2, reaching 95% of mass released after 18 days, for pH 4.2 - 96% of drug mass is released. Almost 100% of mass released is achieved in all cases. At 37°C and neutral-acid pH drug release profiles overlap, initial burst effect (up to 60% mass release) is achieved simultaneously after 24 hours.

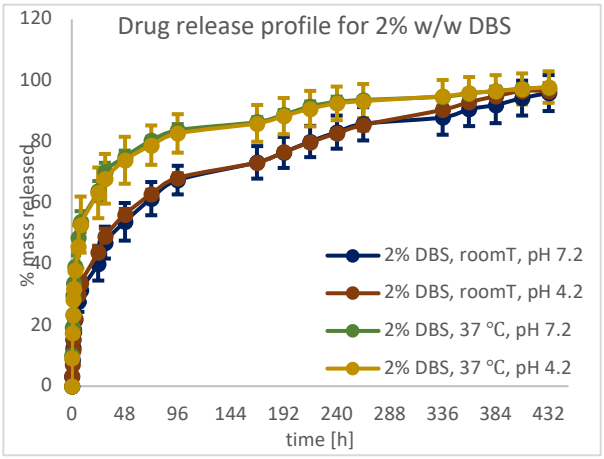

Figure 9: Release profiles of ethosuximide from eutectogels prepared with 2% w/w DBS.

A comparison of all release results shows that higher values of released mass were obtained for eutectogels with lower gelling agent content, which means that the amount of drug released from the eutectogels is influenced by the internal structure of the gel network. The initial burst release can be correlated to the drug being weakly bonded to the surface of the eutectogels.
An important observation is the thermo-responsive and pH-responsive feature of these systems, with different behaviour depending on the concentration of DBS. For systems with 2% w/w DBS added, the major effect on release kinetics is seen with a change in temperature, while a change in pH does not appear to have a large effect.
In the case of gels prepared with 5% w/w DBS, the relationship seems to be more variable. Again, a faster release is observed at higher temperature, but the pH effect is evident at room temperature where at pH 4.2 the release is faster than at neutral pH. These results can be related to the fact that an acidic environment influences the strength of hydrogen bonds, weakening them and allowing a higher mobility of the species entrapped in the fibrillar system.

3.5. Mathematical modelling

Mathematical models of drug release profiles provided useful information about the mass transfer mechanism responsible for the sustained release of the drug. The mechanism of drug release from eutectogels (for all experimental conditions) was analysed up to 60% of mass released and fitted

with the kinetic model – Korsmeyer-Peppas power equation:

$$f_t = \frac{M_t}{M_\infty} = kt^n \qquad (3.1)$$

where M_t and M_∞ are the amount of drug released at time t and at infinite time, respectively, k is the rate constant, and n is the release exponent which indicates the transport mechanism. Table 3.1 shows the fitting results along with the correlation coefficient R^2 for all samples (S1-S4 prepared with 5% DBS and S5-S8 prepared with 2% DBS). All results show that the correlation coefficient is close to 1 indicating a good fit of the experimental data. The diffusion exponent n, for all samples, is in the range 0.16-0.32 (< 0.45) thus indicating that the release of the drug is dominated by a quasi-Fickian diffusion mechanism. The constant K, related to the release rate, has values in the range 0.043-0.076 and 0.092-0.12 for the gels-5% and gels-2% DBS respectively. These results show that the release rates, being lower for eutectogels obtained with 5% DBS, are in agreement with the NMR diffusion data.

Table 3.1 The summarized values of the correlation coefficient (R^2), the constant K and the diffusion exponent n.

Korsmeyer-Peppas modelling			
Sample name	**R^2**	K	n
S1	0.99578609	0.05907786	0.21648865
S2	0.98984493	0.04329311	0.32133957
S3	0.98611717	0.07676123	0.27523084
S4	0.98416317	0.07624857	0.27703054
S5	0.99330675	0.11228687	0.15901942
S6	0.99259168	0.11662362	0.15777656
S7	0.99607741	0.09747633	0.26407556
S8	0.99577753	0.09202179	0.26800335

4. Conclusions and future perspectives

Eutectogels prepared using menthol-thymol, with different concentrations of gelling agents and loaded with ethosuximide at various concentrations, were successfully prepared and analysed using different analytical techniques. FTIR analysis allowed obtaining the unique and characteristic IR spectrum of eutectogels. The morphological and structural information was verified by SEM images, which proved the fibrillar network, typically formed by molecular gelators. The diffusion coefficients of ethosuximide and menthol/thymol were obtained by ^{1}H HR-MAS NMR technique. Finally, drug release measurements were performed, which showed both the effect of different gelator concentration and the thermo-responsive and pH-responsive character of these eutectogels, proving that different environments influence the strength of hydrogen bonds, weakening them and allowing a higher mobility of the molecules entrapped in the highly entangled 3D network.

After all studies have been performed, it would be interesting to make a proposal for a series of tests as a future plan to complete this research project. Research could focus on the effect of the gelling agent, increasing its content and study how this affects the release of the drug, along with the study the effect of pH at even higher acidities, such as 1.2 for example, to gain a better understanding of its effect on the hydrogen bond system. Changing the active pharmaceutical ingredients encapsulated in the systems would also be a future goal. For example, lipophilic drugs can be loaded into menthol-thymol gels and studied for structure and release profile. Finally, it can be concluded that eutectogels could be promising systems in the field of drug delivery in the future, but a long way of research and analysis is needed before this happens.

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