International Journal of Pharmaceutics 647 (2023) 123553

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Cyclodextrin-based supramolecular deep eutectic solvent (CycloDES): A vehicle for the delivery of poorly soluble drugs

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ARTICLE INFO

Keywords:
Cyclodextrins
DES
BCS class II
Solubility
Choline Chloride
Drug delivery

ABSTRACT

The aim of this work was to develop a new class of deep eutectic solvent (DES) composed of a complexation agent, namely hydroxy-propyl-β-cyclodextrin (HPβCD), to exploit a synergic solubilization-enhancing approach. For this purpose, cyclodextrin-based supramolecular DES (CycloDES) were physical–chemical characterized and loaded with three different BCS class II model drugs, specifically Cannabidiol, Indomethacin, and Dexamethasone, evaluating the influence of different factors on the observed solubility and permeation compared with the only HPβCD/drug complexation.

Hence, CycloDESs were presented as a possible vehicle for drugs and represent a novel potential approach for solving BCS class II and IV solubility issues, demonstrating at least a 100-fold improvement in the investigated drug solubilities. Furthermore, CycloDESs demonstrated a significantly improved resistance to dilution preserving a high percentage of drug in solution (i.e. 93% for Indomethacin) when water is added to the DES if compared with a glucose-choline chloride DES, used as a standard. This evidence guarantees the solubility-enhancing effect useful for the delivery of BCS class II and IV drugs converting solid raw material to advantageous liquid vehicles bypassing the rate-determining dissolution step.

1. Introduction

Since their discovery in 2003, eutectic mixtures characterized by a drastic decrease in their melting point, namely deep eutectic solvents (DES), have attracted increasing interest owing to their potential role as next-generation green solvents (Abbott et al., 2003). These mixtures are prepared by heating and mixing two or more compounds, usually solids at room temperature (RT), at a specific molar ratio, resulting in a clear crystal liquid even at low temperatures. The significant reduction in melting temperature when compared to the individual components is due to interactions established between a hydrogen-bond donor (HBD) and a hydrogen-bond acceptor (HBA) (Zainal-Abidin et al., 2019).

The advantages of DES can be easily resumed in nonreactivity with water, low- or nontoxicity, biodegradability, ease of preparation, and inexpensive and readily available constituents, often belonging to the generally recognized as safe (GRAS) list (Emami and Shayanfar, 2020). The proper selection of compounds can indeed modify the characteristics of the DES in terms of viscosity, surface tension, density, and melting temperature (Smith et al., 2014). Over the past two decades, the development of these innovative systems for possible new applications in various fields, including the pharmaceutical industry.

Among the other applications, DESs have been studied and exploited for the delivery of many drugs belonging to the biopharmaceutical classification system (BCS) classes II and IV (Amidon et al., 1995; Takagi et al., 2006), characterized by poor solubility in water. In fact, an oral liquid DESs formulation in which the drug is solubilized could avoid many problems associated with the use of solid formulations, for instance bypassing the steps of disaggregation and dissolution in the body.

In fact, eutectic formation technology could represent an approach alternative to lipid-based formulations (Racaniello et al., 2022), amorphous solid dispersions (Pistone et al., 2023, 2022), to be combined with other tools such as self-emulsifying DDSs (Nazzal et al., 2002) and cyclodextrins (El Achkar et al., 2020a; Kfoury et al., 2022; McCune et al., 2017).

Specifically, cyclodextrins (CDs) are cyclic oligosaccharides consisting of (α-1,4)-linked D-glucopyranose units. The CDs used in pharmaceutical products are αCD, βCD, and γCD, which consist of six, seven,

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https://doi.org/10.1016/j.ijpharm.2023.123553
Received 5 July 2023; Received in revised form 23 October 2023; Accepted 23 October 2023
Available online 24 October 2023

and eight D-glucopyranose units, respectively (Jansook et al., 2018).

Water-soluble inclusion complexes between CDs and lipophilic poorly soluble drugs are formed in an aqueous environment encapsulating some lipophilic moiety of the drug in CDs' central cavity showing also low toxicity profile (Lopedota et al., 2016). Native αCD, βCD, and γCD have limited solubility in water, which hinders their application as solubilizing complexing agents, especially βCD. Hence, new modified CDs, such as hydroxy-propyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin, were developed through chemical modifications showing improved solubility in water and, therefore, broader drug delivery performance. (Saokham et al., 2018).

The oligomeric glucopyranose structure of CDs exposes a relevant number of eligible hydrogen-bonding moieties that identify CDs as suitable compounds for generating DESs based on the results already obtained for different carbohydrates previously listed.

Different methods have been developed for CDs and DESs and can be divided into two main categories: using CDs in an already prepared DES (Cantelli et al., 2021; Cecone et al., 2020; Georgantzi et al., 2017), or making CDs a component of DES itself, generating a new system defined as a supramolecular deep eutectic solvent (SUPRADES) (Di Pietro et al., 2020; El Achkar et al., 2020b). Namely, the term supramolecular refers to all non-covalent interactions between molecules, and herein, it finds a double involvement in both the drug-CD complex and DES formation (Lehn, 1993).

Based on the advantages of the above-mentioned systems, in this work CDs and DES were combined to obtain a new vehicle for the delivery of poorly soluble drugs, called CycloDES. The focus of this research was to investigate the permanence of the drug in an aqueous solution after dilution using CD-based DES, which has posed a challenge for eutectic mixtures. This study aimed to address this DES-related soft spot by exploiting the herein demonstrated synergy of the adopted technologies. The work explored the potential of utilizing different compounds to generate DESs presenting the same CD. In particular, novel components for eutectic mixtures were explored, focusing our attention mainly on the choline chloride, used for the first time in pharmaceutical field as partner for hydrogen bond formation with HPβCD and not as a solute in a DES. This modified CD was chosen for its improved water solubility and lack of ionic groups that could affect hydrogen bonding and interact with other components or excipients in a formulated product. In fact, in the case of sulfobutylether-β-cyclodextrin, the presence of the negative charge may not only influence complexation but also induce electrostatic interactions that could lead to ionotropic gelation or impart a salty taste due to the presence of the counter ion (István Puskás et al., 2015; Ricci et al., 2022). Moreover, for a prospective scale-up of this technology, HPβCD represents a more cost-effective alternative for the production of CycloDES. In the end, CycloDES were tested in terms of solubility and permeability performances using three different BCS class II model drugs, namely cannabidiol (CBD), dexamethasone (DEX), and indomethacin (IND), characterized by different physical–chemical characteristics. The results obtained have confirmed that HPβCD-based CycloDESs are an effective and innovative system for improving the solubility of low aqueous solubility solid drugs, without the addition of other solvents.

2. Materials and methods

2.1. Materials

Hydroxy-propyl-β-cyclodextrin (DS = 7.5, MW = 1480 Da) and Dexamethasone were gifted by Farmalabor s.r.l. (Canosa di Puglia, Italy). Cannabidiol crystals 99 % were purchased by Enecta (Amsterdam, Netherlands). Indomethacin, choline chloride (≥99 %, ChCl), citric acid (≥99.5 %, CA), lactic acid (80 %, LA), tartaric acid (≥99.5 %, TA), D-(+)-glucose (≥99.5 %), phosphoric acid (≥85 %, triethylamine (≥99.5 %) acetic acid glacial (≥99 %) and solvent for HPLC (water, methanol, acetonitrile) were purchased by Sigma Aldrich Italy (Milan, Italy). MilliQ water was obtained through a Milli-Q instrument by Millipore Sigma (Burlington, Massachusetts, USA).

2.2. Preparation of CycloDES

Different CycloDESs were prepared following the procedure described by Dai et al. (2013) by vigorous magnetic stirring the raw materials at 80 °C using the molar ratio shown in Table 1 and Table S1. Considering samples containing only solid raw materials such as HPβCD, CA, and ChCl, the influence of water was studied by evaluating the percentage of water of 0, 5, 10 and 20 %. On the other hand for samples containing LA had a percentage of water depending on the amount of LA used and its degree of purity as water solution at 80 %. The prepared samples are shown in Table 1. The adopted parameters concerning water quantity, heating temperature, and time for further studies were set to a heating time of 1 h at 80 °C. After the preparation, samples were left to return to RT.

Drug-loaded CycloDES were obtained by adding the needed quantity of the 3 solid state model drugs to the mixture and then mixing through magnetic stirring at 37 °C for 3 h to promote the agitation taking advantage of the reduced viscosity at this temperature. The complete solubilization of the drug was assessed and the samples were stored at RT.

2.3. Density

Density was assessed via gravimetric analysis at 25 °C: 10 μL of CycloDES were withdrawn using positive displacement pipettes (MICROMAN® E pipette, Gilson Italia, Italy), with disposable capillary piston tips and weighed with an AS 82/220.R2 Analytical Balance. The test was conducted in triplicate.

2.4. Rheology

Flow curves were obtained using HAAKE^{TM} MARS iQ rheometer (Thermo Fisher Scientific, Waltham, US) with an aluminum cone-plate geometry (20 mm diameter, 1° cone angle, 0.1 mm truncation gap). The acquisition was conducted in the range between 0.1 s^{-1} and 2,500 s^{-1} following a steady-state procedure, with the shear rate increasing after a stable value in shear stress was reached. The temperature was maintained at 30 °C for all the samples. Measurements were performed in triplicate at least for each sample.

Table 1
List of the combination of the raw materials adopted, their respective molar ratios for the preparation of CycloDESs, and the amount of water in each sample.

CycloDES composition	Molar ratio	Water content % wt
CA:HPβCD	14:1	0
		5
		10
		20
LA:HPβCD	35:1	14.5
ChCl:HPβCD	35:2	0
		5
		10
		20
LA:ChCl:HPβCD	35:17.5:2	8.5
CA:LA:HPβCD	14:35:2	8.2
CA:ChCl:HPβCD	14:17.5:2	0
		5
		10
		20
TA:HPβCD	7:1	0
		5
		10
		20

2.5. Solubility of the model drugs in CycloDES

The maximum solubility of the model drugs in the prepared CycloDESs was determined by adding an excess of the drug to 1 g of each mixture. As a control, two samples were prepared, water (1 mL) and a water solution with an HPβCD concentration of 314 mg/mL (1 mL, 212 mM). For the HPβCD containing control sample, the amount of HPβCD was in the range of the maximum quantity contained in CycloDESs. All obtained samples were put under constant stirring in an orbital shaker for 5 days at 37 °C. After this time, all samples were centrifuged in an Hettich MICRO22R Eppendorf centrifuge at 10,000 rcf or 10 min at 37 °C. Afterward, 50 μL was withdrawn from the supernatant, diluted with methanol to 1 mL, and used as a stock solution for HPLC analysis.

The ChCl:HPβCD CycloDES was selected for further in-depth analysis for the versatility in improving the solubility for all three model drugs and the low viscosity.

2.6. After-dilution stability

After solubility analysis, drug-loaded ChCl:HPβCD CycloDES were tested to assess the capacity of the system to preserve the concentration of the drug after dilution. As a reference, a DES composed of Glucose and ChCl (1:2 M ratio, 20 % wt water content) was prepared and saturated with the 3 model drugs following the procedure previously described. Briefly, 100 μL of samples were diluted in 900 μL of water and subsequently prepared for HPLC analysis. Data were plotted as percentage of the drug remained in solution after the dilution. All the assay was done in triplicate.

2.7. HPLC quantification

To quantify DEX, IND, and CBD 3, slightly modified HPLC methods were developed and adopted using Shimadzu HPLC Nexera series, equipped with SPD-M40 photodiode array detector, SIL-40C autosampler and CTO-40C column oven. For DEX, a Zorbax Eclipse C18 plus 250 × 45 mm, pore size 5 μm with guard column was used with MeOH:H_2O 0.6 % TEA pH 2.6 H_3PO_4 (65:35) as the mobile phase at 1.2 mL/min, T = 35 °C λ = 254 nm, retention time 5.7 min (Heda et al., 2011). The calibration curve was obtained in a range of concentration between 5.09x10^{-3} and 1.2 mM ($R^2 = 0.9999$). For IND a Zorbax Eclipse C18 plus 150 × 45 mm pore size 5 μm was used with ACN:H_2O pH 2 (40:60) obtained with H_3PO_4 as mobile phase at 1.5 mL/min, λ = 220 nm, retention time 2.9 min (Pai and Sawant, 2017). The calibration curve was obtained in a range of concentration between 3.35x10^{-2} and 3.35 mM ($R^2 = 0.9999$). For CBD, a Zorbax Eclipse C18 plus 150 × 45 mm pore size 5 μm with a guard column was used with MeOH:ACN:H_2O pH 4.5 AcOH (52:30:18) as the mobile phase at 1.8 mL/min, λ = 228 nm, retention time 3.2 min (Analakkattillam et al., 2022). The calibration curve was obtained in a range of concentration between 1.90x10^{-2} and 1.90 mM ($R^2 = 0.9996$). Further information are available in Figure S3.

2.8. Phase solubility

The interactions between the single model drugs and the HPβCD were evaluated with phase solubility studies, according to the method reported by Higuchi and K.a.c (1965) on the inclusion complexes of CBD, IND, and DEX with HPβCD, using a modified previously described protocol (Racaniello et al., 2021). Briefly, an excess of the drugs was added to deionized water or aqueous solutions of HPβCD (0–212 mM) for 72 h until solubility equilibrium was achieved. 1 mL of each suspension was placed in a 1.5 mL Eppendorf, vortexed, and maintained in an orbital shaker at a constant temperature of 37 ± 0.2 °C for the entire experiment. After 72 h, the samples were centrifuged at 10,000 rcf in an Eppendorf centrifuge and filtered through a 0.22 μm membrane filter. The prepared samples were analyzed by HPLC after appropriate dilution. The experiments were performed in triplicates. The following *Equation (1)* was used to calculate the aqueous solubility at 25 °C and the inclusion constant of the drug with CD:

$$K_{1:1} = \frac{Slope}{Intercept(1 - Slope)} \quad (1)$$

where the slope and intercept (S_0) were obtained from the phase solubility diagram.

2.9. Differential scanning calorimetry (DSC)

DSC was used to assess the CycloDESs generation and the correct solubility of the model drugs in the systems. The analysis was conducted using a Mettler Toledo DSC822 instrument. Thermograms were obtained for the raw materials, CycloDES, and the drug-loaded CycloDES. Samples were prepared in an aluminum pin weighing 5–10 mg of pure substances, 10–15 mg of CycloDES and analyzed in the range of 0 – 300 °C. increasing temperature at a rate of 5 °C/min under nitrogen flow (50 mL min^{-1}).

2.10. Thermogravimetric analysis

TGA was performed to verify CycloDES stability at increased temperatures with a PerkinElmer Thermogravimetric Analyzer Pyris 1 TGA. An open platinum pan was calibrated at the temperature of 50 °C and the samples were placed within it. Thereafter, the prepared pan was suspended in the furnace. The weight of material was reported during heating from ~~50 to 300 °C at a heating rate of 5 °C/min~~ 25 to 800 °C at a heating rate of 10 °C/min, starting from an initial weight of the sample of 10–15 mg. Nitrogen flow was imposed at 30 mL/min.

2.11. Nuclear magnetic resonance (NMR)

NMR spectroscopy was used to control the establishment of hydrogen-bonding interactions and drug incorporation in the systems. Experiments were performed by dispersing CycloDES, drug-loaded CycloDES, and a physical mixture of the components not heated in deuterated water. The spectra were recorded using an Agilent VNMRS 500 MHz spectrometer, and the ^{1}H NMR spectra were analyzed using the residual solvent signal of deuterated water at 4.79 ppm for all the samples except for CBD-loaded CycloDES, that showed better solubility in DMSO-d_6 with a reference peak at 2.48 ppm.

2.12. In vitro permeability studies

The permeation features of the CycloDES were assessed using an already reported procedure (He et al., 2004; Santos et al., 2019). In vitro permeation studies were performed using Franz cells with 0.6 cm^2 lumen and a Cuprophan membrane with a 10 kDa cut-off to mimic a topical epidermal application. For the analysis, the IND, CBD, and DEX-loaded CycloDES were prepared at 4 mg/mL of the drug concentration, while, as control, 3 different HPβCD aqueous solutions were saturated with one of the model drugs for each solution. In particular, a 100 mM HPβCD solution was used for CBD and DEX, and a 200 mM HPβCD solution for IND, due to its lower observed solubility. 500 μL of the so-made samples were placed in the donor compartment. The pH of the donor compartment was 6 for both the CycloDES and control. The receiving compartment consisted of 9.5 mL of a solution of ethanol: phosphate-buffered saline (PBS) at pH 7.4 (1:1) (Bourdon et al., 2016; Fang et al., 2001; Tabboon et al., 2022). Withdrawals were performed at 15-minute intervals for the first 2 h and at 60-minute intervals for the next 6 h, and final withdrawal was performed 24 h after the beginning of the assay. The concentrations of the withdrawn samples were evaluated by HPLC.

The permeability coefficient (*P*) was calculated from the slope of the following equation (Equation 2).

$P(t) = -\frac{V}{2A} \times \ln\left(1 - 2\frac{C_t}{C_0}\right)$ (2).

Where C_t is the concentration in the receptor compartment at time t, C_0 is the initial concentration in the donor compartment, V is the volume in two chambers, and A is the effective area of permeation.

The diffusion coefficient (D) across the membrane was calculated according to Fick's Law of diffusion, following the equation (Equation 3):

$D = \frac{V_1 V_2}{V_1 + V_2} + \frac{h}{A} + \frac{1}{t} \ln\left(\frac{C_f - C_i}{C_f - C_t}\right)$ (3).

where D ($cm^2\ s^{-1}$) is the diffusion coefficient, C_i and C_f are the initial and final concentrations, C_t (mol/L) is the concentration at time t in the receptor compartment, V_1 and V_2 (cm^3) are the volume of liquid in the donor and receptor compartment, respectively, h (cm) is the thickness of the membrane and A (cm^2) is the effective diffusion area of the membrane.

2.13. Ex vivo permeability studies

An ex vivo investigation was conducted on pig ear skin. Fresh hairless pig ears from pigs aged 10–12 months were procured from a local slaughterhouse in Bari, Italy, by Siciliani S.p.A., and treated in accordance with Lopedota et al. (2018).

The adopted experimental design consisted of a Franz cells with 0.6 cm^2 lumen. The donor compartment contained the samples previously described in section 2.12. Briefly, the receiving compartment consisted of 9.5 mL of a solution of ethanol:phosphate-buffered saline (PBS) at pH 7.4 (1:1) thermostated at 37 ± 0.5 °C. Withdrawals were performed at 30-minute intervals for the first 2 h and at 60-minute intervals for the next 6 h, and final withdrawal was performed 24 h after the beginning of the assay. After the experiment, the skin was recovered and appropriately processed to determine the quantity of drug entrapped within it (Lopedota et al., 2015).

The concentrations of the samples were evaluated by HPLC. The results obtained were then compared to the quantity of the drug loaded in the donor compartment and evaluated as a percentage.

The study was conducted in triplicate.

2.14. Statistical analysis

Permeability experimental data were reported as Mean ± SD (standard deviation). Statistical analysis was conducted by Graph Prism version 8.0 (GraphPad Software Inc., La Jolla, CA, USA). In detail, an unpaired t-test was applied and statistically significant differences were set at probabilities of $*p < 0.05$ (p-value style: $***p < 0.001$, $**p < 0.01$, $*p < 0.05$).

3. Results and discussions

3.1. Preparation of CycloDES

The appropriate molar ratio between constituents useful for hydrogen bond formation, water content and the time required to complete the preparation were the key factors considered in the selection of parameters required for preparing CycloDES. The molar ratios were adjusted based on a protocol previously published by Dai et al. (2013) concerning natural DES (NADES). This protocol was modified to create CycloDES, taking advantage of the oligomeric glucopyranose structure of HPβCD and resulting in a sevenfold increase in the required number of moles for the interaction. This strategy resulted in the formation of three binary systems made of LA, CA, ChCl, and three ternary systems, which were obtained by combining the previously mentioned. The ternary systems were studied to evaluate the impact of each component on the final CycloDES. To date, the role of the HPβCD in this study has been assumed as HBD, mainly for the analogy with other carbohydrates and the component here described as HBA (Florindo et al., 2017), even if other authors reported HPβCD as HBA (El Achkar et al., 2020b). Differences in the process were observed in terms of the time required for preparation completion, particularly when the water amount was considered. In fact, the water could influence the generation of the system playing a relevant role in DESs, especially NADES, owing to the important contribution to the hydrogen bond interactions, and the final solubility properties (Dai et al., 2015). The parametrical analysis conducted on the water amount useful for proper CycloDES preparation highlighted the enrollment of water in the hydrogen bond formation. Samples with 0 and 5 % of water were found not capable of turning into crystal clear liquid at all (Fig. 1A). The samples with 10 and 20 % of water both resulted successfully in accomplishing the preparation with the samples at 10 % of water reaching the liquid state after 4 h, while the samples with 20 % of water in less than an hour. These evidences can be explained by the influence of water on the viscosity, with higher quantity of water inducing a reduction of this property resulting in easier mixing and preparation. Further, the already observed risk of over-reducing CycloDES solubilizing capacity has been avoided at the chosen water percentage, also ensuring the rapid formation of the studied systems (Dai et al., 2015). Therefore, further studies were conducted by fixing the same water content at 20 % wt for all the CycloDESs to exclude it as a cause for the different solvent performances. The only exception was the TA:HPβCD mixture, which did not accomplish the preparation under the selected conditions and, for this reason, was excluded.

The temperature was set to 80 °C for all the samples, ensuring the generation of the crystal fluid. In particular, the sample composed of CA:HPβCD was found the hardest to obtain since only the high temperature could guarantee the generation in a compatible time. This could be attributed to the high viscosity of the systems, hindering adequate magnetic stirring. Nevertheless, the preparation of the ternary mixtures comprising CA in the composition demonstrated better behavior than the binary systems, with ChCl and LA capable of reducing heat input and, therefore, the time needed to generate CA containing CycloDES.

The heating process was maintained for 1 h at 80 °C visually establishing the formation of a clear crystal liquid as an endpoint. The possible presence of air bubbles in the samples at the end of the process was automatically removed by the system during the cooling process.

3.2. Rheology

This study was essential to guarantee the generation of systems suitable for different applications, and the influence of water content played a major role in tailoring the viscosity, as previously explained (Dai et al., 2015). In fact, the possibility of selecting the appropriate mixture based on the administration route characteristic increases the number of applications in formulations for dermal, ophthalmic, and mucosal applications, where the viscosity could guarantee the success of the formulation, and in encapsulation processes where a low flowability could affect the production itself. All the mixtures studied exhibited a Newtonian fluid behavior for shear rates below 2,500 s^{-1} with dynamic viscosity values remaining stable for all the range. The dynamic viscosities (η) resulted to be related to the presence and the quantity of CA with the $\eta_{HP\beta CD:CA}$, $\eta_{HP\beta CD:CA:LA}$, and $\eta_{HP\beta CD:CA:ChCl}$ ranking at the 3 higher values, while the $\eta_{HP\beta CD:ChCl}$ and $\eta_{HP\beta CD:LA}$ were the lowest (Fig. 1B). Hence, the systems are able to display viscosities suitable for different applications guaranteeing lower or higher viscosity depending on the CycloDES composition.

3.3. Phase solubility studies

Phase solubility studies of the different drugs investigated in the presence of HPβCD were performed, which allowed the evaluation of the host–guest interaction by calculating the complexation constant (Laquintana et al., 2019). DEX and IND presented an A_L-type, which suggested and 1:1 HPβCD-drug interaction, while for CBD an A_P profile

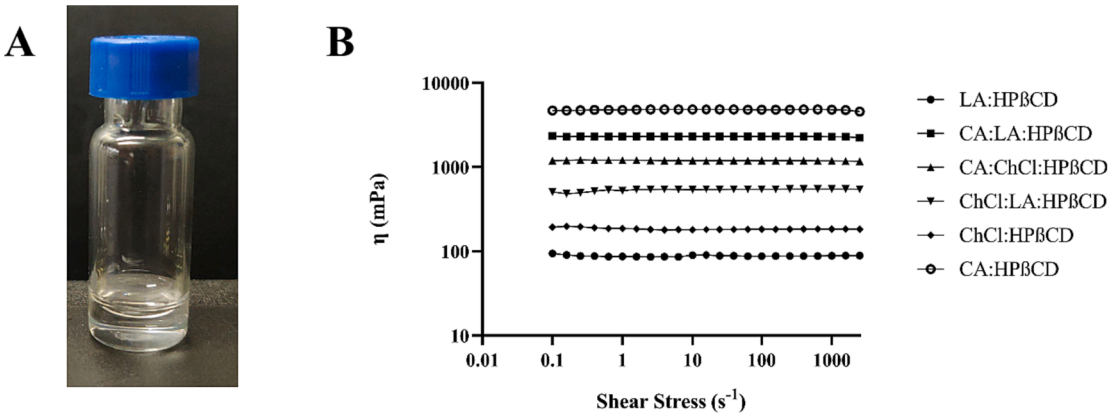

Fig. 1. A. Representative picture of CycloDES after the heating process. **B.** Viscosity of CycloDESs evaluated at increasing values of shear rate.

could be identified with an high order complex formation (Saokham et al., 2018). The calculated inclusion constants shown in Table 2 indicate a good interaction between the drugs and HPβCD. In fact, all the values obtained are included in the range from 50 to 2,000 M^{-1}, values considered optimal for drug/CD complexation (Pistone et al., 2022). From the results shown in Fig. 2, it is possible to assess how DEX benefits from interaction with HPβCD, showing a 142-fold improvement over its intrinsic aqueous solubility (S_0 = 0.12 mg/mL) at 212 mM of HPβCD; similarly, IND showed a 147-fold increase in its intrinsic aqueous solubility (S_0 = 0.014 mg/mL) at 212 mM of HPβCD. In the case of CBD, a 45-fold improvement if compared to S_0 of 0.076 mg/mL was reached at 212 mM of HPβCD.

3.4. Solubility of the model drugs in CycloDES

The solubility data for the three model drugs on the different CycloDES and control samples, reported in Table 3, showed an overall improvement in terms of drug-loaded by the systems with a significant enhancement compared to the solubility of the drug in water. Density data were used to transpose the results obtained for 1 g of CycloDES in molar concentrations.

Although DEX solubility was already greatly improved from HPβCD alone, the binary system consisting of LA and the ternary system with CA and LA achieved a comparable performance in terms of improvement against the solubility in water of 158 and 230-time fold respectively (Fig. 3A). All investigated IND-containing CycloDES showed better solubility values than the control sample represented by a solution of drug and HPβCD. This behavior may be attributed to the properties given by the CycloDES in improving solubility, with a limited inclusion effect of HPβCD due to the low $K_{1:1}$ shown in the phase solubility study. One explanation for IND's solubility in aqueous systems including eutectic solvent solutions might include hydrogen bonding interactions between CycloDES and IND, which serves as the hydrogen bond acceptor thanks to its carboxylic group. More particular, the acidic environment induced by the components of CycloDES has enhanced and strengthened hydrogen bonding interactions and, then, the solubility of IND (Akbarzadeh Gondoghdi et al., 2023; Khorsandi et al., 2020). At last, ChCl:HPβCD was the only eligible CycloDES for solubilization of CBD for its already documented instability in acidic environment, comprising the gastric juice, that could lead to the conversion of different THC regioisomers and isoTHC (Franco et al., 2022; Marzullo et al., 2020). Hence, this evidence excluded LA and CA, and their CycloDES, as useful excipients for a CBD formulation, nevertheless showing the ChCl:HPβCD CycloDES as a potential vehicle capable of loading CBD with a 45-fold and 175-fold improvement in its solubility if compared to HPβCD water solution and water, respectively.

The liquid form obtained for the drugs represents an appealing solution to avoid both polymorphism and improve low-water solubility constraints. The pharmaceutical industry has relied on eutectic mixtures mainly for reducing organic solvents use, shortly exploring other options for commercialization (Pedro et al., 2020; Shamshina et al., 2015). In this context, the chance to generate liquid formulations of an active pharmaceutical ingredient started with the introduction of THEDES and has the potential to improve the absorption and speed of drug delivery, while also decreasing its harmful effects (Aroso et al., 2016).

This result made the ChCl:HPβCD CycloDES the more versatile system among the produced with the possibility to incorporate a higher number of molecules without inducing acid-related instability.

Table 2
Phase solubility studies obtained parameters.

Sample	Slope (x 10^{-3})	Intercept (x 10^{-4})	$K_{1:1}$ (M^{-1})
IND	14.6	0.5	296.3
DEX	207.2	0.2	1306.8

3.5. After-dilution stability

The conducted analysis was essential for understanding the possible behavior of the CycloDES after dilution in water. As described above *(section 3.1)*, an increased amount of water could lead to the loss of the H-bond network and drug precipitation. The adopted dilution allowed to observe the reduction in terms of drug concentration always remaining above the intrinsic solubilities of the model drugs and therefore excluding its influence in the process.

For this experiment, Glucose:ChCl DES was chosen as a standard for the structural analogy with the ChCl:HPβCD DES and to check in deep the influence of the synergy between HPβCD and DES. First of all, the ChCl:HPβCD CycloDES showed an improved solubility for all the model drugs if compared to the Glucose-ChCl DES (data not showed). After the dilution, the CycloDES demonstrated an improved ability to preserve a significantly high percentage of drug in solution, despite the possible loss of the H-bond network, observed in the ChCl:Glucose with a drastic decrease of the drug in solution after the dilution (Fig. 3B). These results could be explained with different mechanisms starting with the influence of the HPβCD and its intrinsic solubility-enhancing features, with the amount of drug contained in the CycloDES preserved after dilution thanks to complexation in the HPβCD cavity. Another possible mechanism is the development of multicomponent complex in diluted aqueous environment made of drug-HPβCD-ChCl in analogy with what already described in literature for molecules such as amino acids and hydroxy acids (Mura, 2003; Redenti et al., 2000).

Following this observed synergy, it is groundbreaking to highlight that the presence of HPβCD could overcome one of the main DES-related issues when dilution is considered. On the other hand, while applying

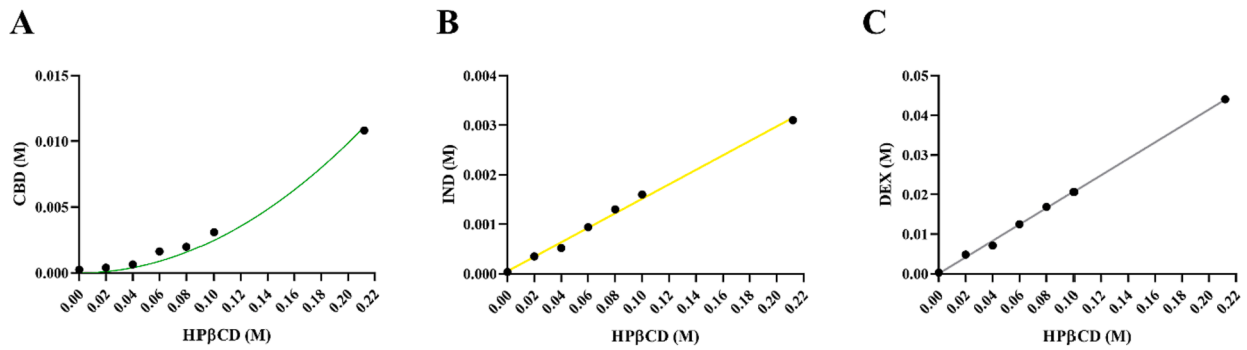

Fig. 2. Linear regression obtained from phase solubility studies. A: CBD, B: IND, C: DEX.

Table 3
Density of CycloDES and measured solubility.

Sample	Density	Concentration (mM)		
	(g/mL)	DEX	IND	CBD
Water		0.31	0.04	0.24
Water:HPβCD		44.08	3.72	10.83
LA:HPβCD	1.15	48.41	23.18	/
CA:HPβCD	1.36	17.31	24.72	/
ChCl:HPβCD	1.11	21.42	12.51	38.34
CA:ChCl:HPβCD	1.23	11.89	8.20	/
CA:LA:HPβCD	1.29	70.47	12.58	/
LA:ChCl:HPβCD	1.18	34.46	12.94	/

CycloDES, it could be possible to deliver poorly soluble drugs maintaining them in solution after their administration and, therefore, improving their adsorption once arrived to the target site.

3.6. Differential scanning calorimetry

The calorimetric analysis conducted using DSC was mainly exploited to verify the correct preparation of CycloDES and subsequently to assess the complete incorporation of the drugs into them.

Therefore, thermograms for all raw materials were obtained and compared with those of the generated CycloDES (Figure S1 A). The absence of melting peaks related to a single substance was used as evidence for the establishment of the characteristic DES interactions. To prove this concept, further analyses were conducted on the sample

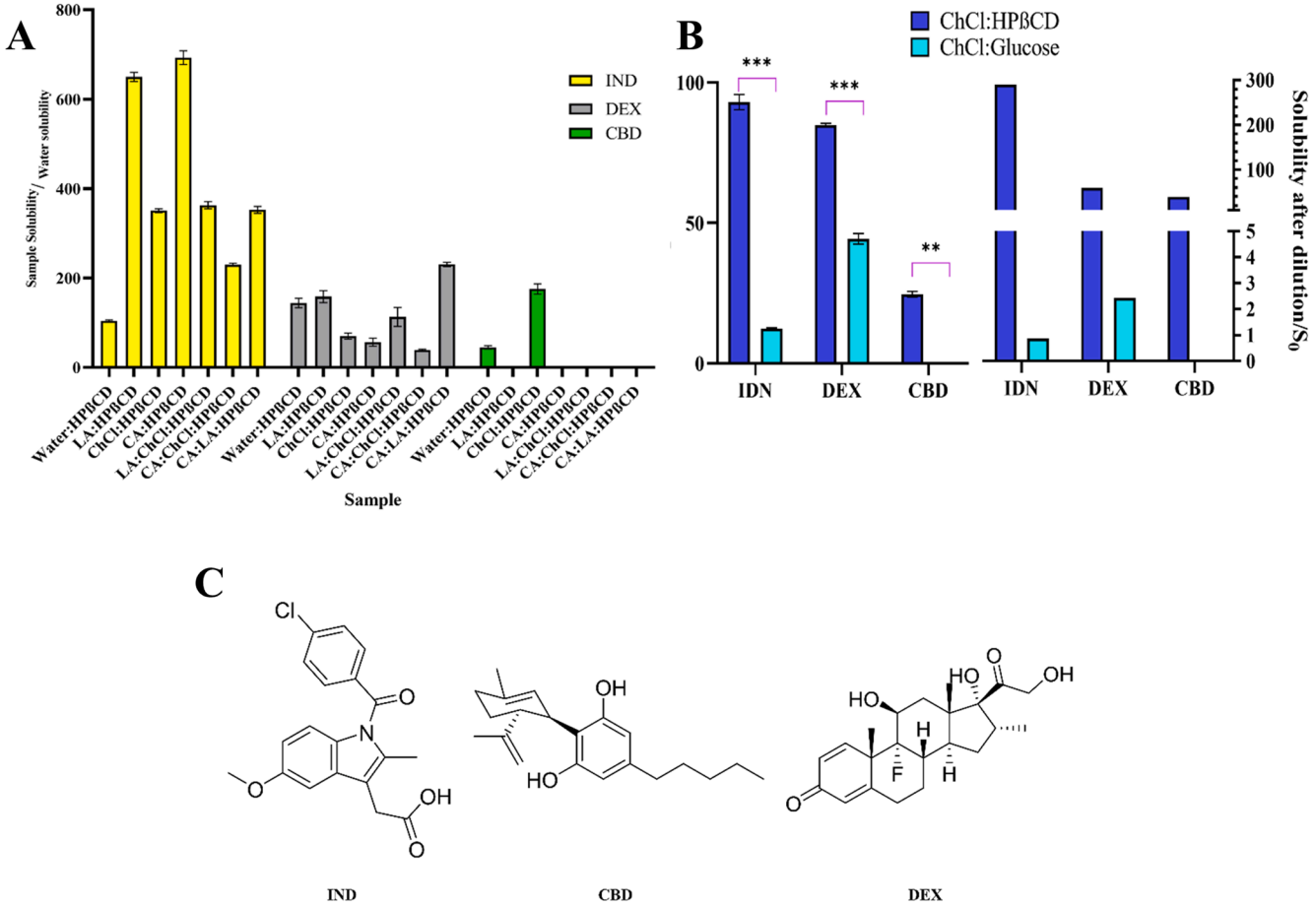

Fig. 3. A. Model drugs solubility improvement in HPβCD water solution and CycloDESs if compared to water solubility. **B.** Percentage of drug remained in solution after dilution of ChCl:HPβCD and ChCl:Glucose DES in water. **C.** Structure of the adopted model drugs.

composed of ChCl:HPβCD, which was chosen as the best solubilizer for the three drugs. Briefly, the samples previously analyzed for CycloDES formation were compared with a physical mixture formed by the same proportion of the components, not exposed to the heating process. The results (Figure S1 B) showed that the melting peak relative to ChCl at ≈70 °C was preserved in the physical mixture, whereas it was absent in CycloDES.

The thermograms obtained for the drug-loaded CycloDES samples (Fig. 4) showed the complete absence of the characteristic endothermic peaks of CBD and IND at 264, 70 and 165 °C, respectively, indicating drug solubilization within CycloDES. The endothermic peak of DEX at 262 °C resulted overlaid to the degradation peak registered for the ChCl:HPβCD CycloDES. Therefore, evidence of drug solubilization was provided using other techniques.

3.7. TGA

TGA results, in Fig. 5, affirmed the different behaviors of CycloDES in regard to their composition. In fact, CA-containing samples showed a constant weight loss until 220 °C, hypothetically related to a loss in water (González-Rivera et al., 2022). Furthermore, the presence of more components in the eutectic mixtures increases the number of steps related to the different observed T onset. On the other hand, the samples ChCl:HPβCD and LA:HPβCD presented single T onset values related to the HPβCD and LA melting and evaporation point, with the latter showing more pronounced weight loss of 27 % due to the evaporation phenomena. Interestingly, the LA:ChCl:HPβCD curve presented its T onset in a range of temperatures averaged by the presence of the 2 components with a weight loss of 15 %. In the end, the ChCl-based CycloDES resulted being the more stable to the increasing temperature, showing an important weight loss at 270 °C, hypothetically related to the degradation of the entire system.

3.8. Nuclear magnetic resonance

A study of the chemical shifts of protons was performed at different levels to support the evidence found through DSC regarding CycloDES formation and to evaluate the incorporation of the model drugs in the ChCl:HPβCD mixture. As shown in Fig. 6, the values of chemical shifts highlighted the interaction among the components with higher chemical

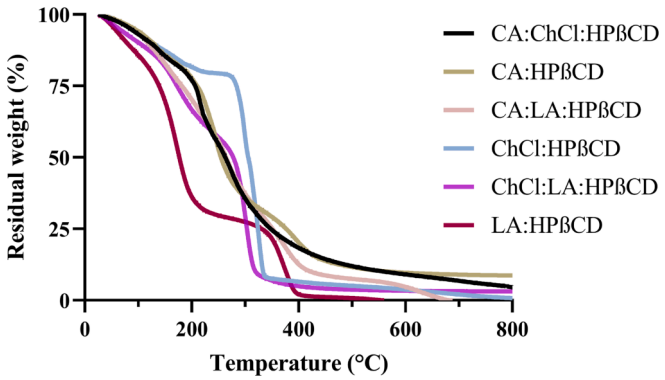

Fig. 5. TGA curves for the prepared CycloDES.

shift values, evidence of hydrogen bonding formation (Duarte et al., 2017). The spectra for the loaded samples demonstrated that the drug structure was preserved during the incorporation process and the chemical shift demonstrated the same displacement as that observed for the component, but to a reduced extent, which suggested the inclusion of the drug in CycloDES, but not their involvement in the structure of the mixture. This evidence confirms that CycloDESs could exploit interaction for increasing the solubility of the loaded compounds, likewise with IND, with a strategy that differs from THEDES formation, highlighting its possible application in a broad variety of BCS class II drugs.

3.9. In vitro permeability studies

In order to evaluate the efficacy of CycloDES for a possible dermal application and, therefore, for crossing a model artificial skin membrane, in vitro permeability tests were performed. As already reported by Duarte et al. (2017), DESs have shown an overall improvement in terms of permeability, mainly due to an increase in drug solubility in the different mixtures evaluated (Santos et al., 2019). Therefore, for this analysis, instead of comparing CycloDES with the drug in water, another experimental model was chosen, consisting of HPβCD water solution. This positive control, especially when compared with the previous negative control of the drug in water, represented a real benchmark for the CD-based system CycloDES.

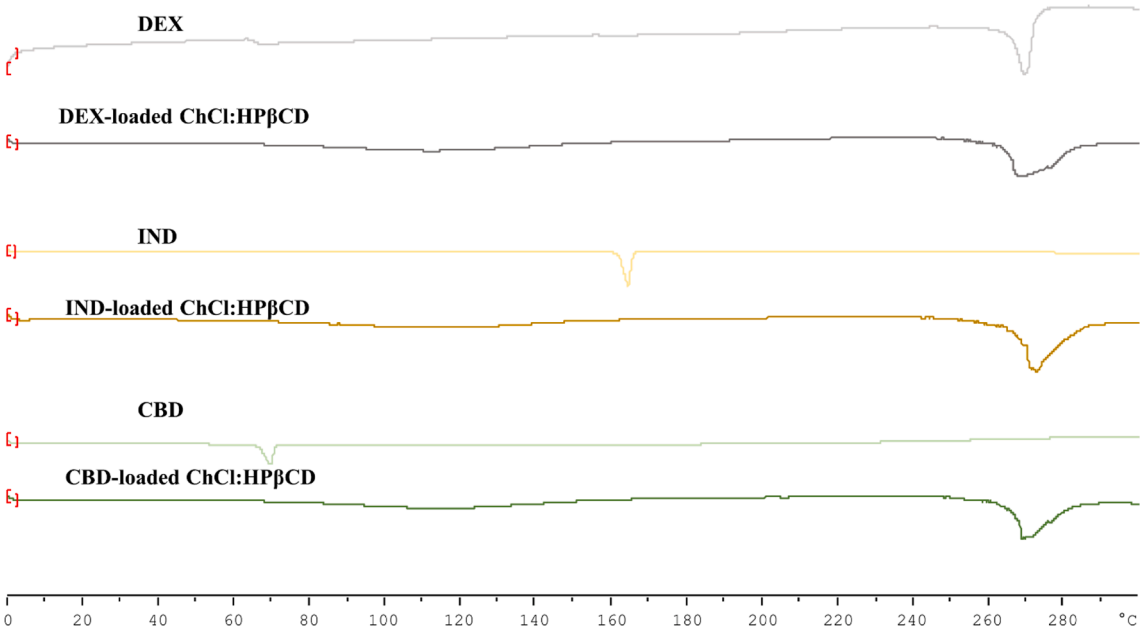

Fig. 4. DSC of drug-loaded ChCl:HPβCD CycloDES.

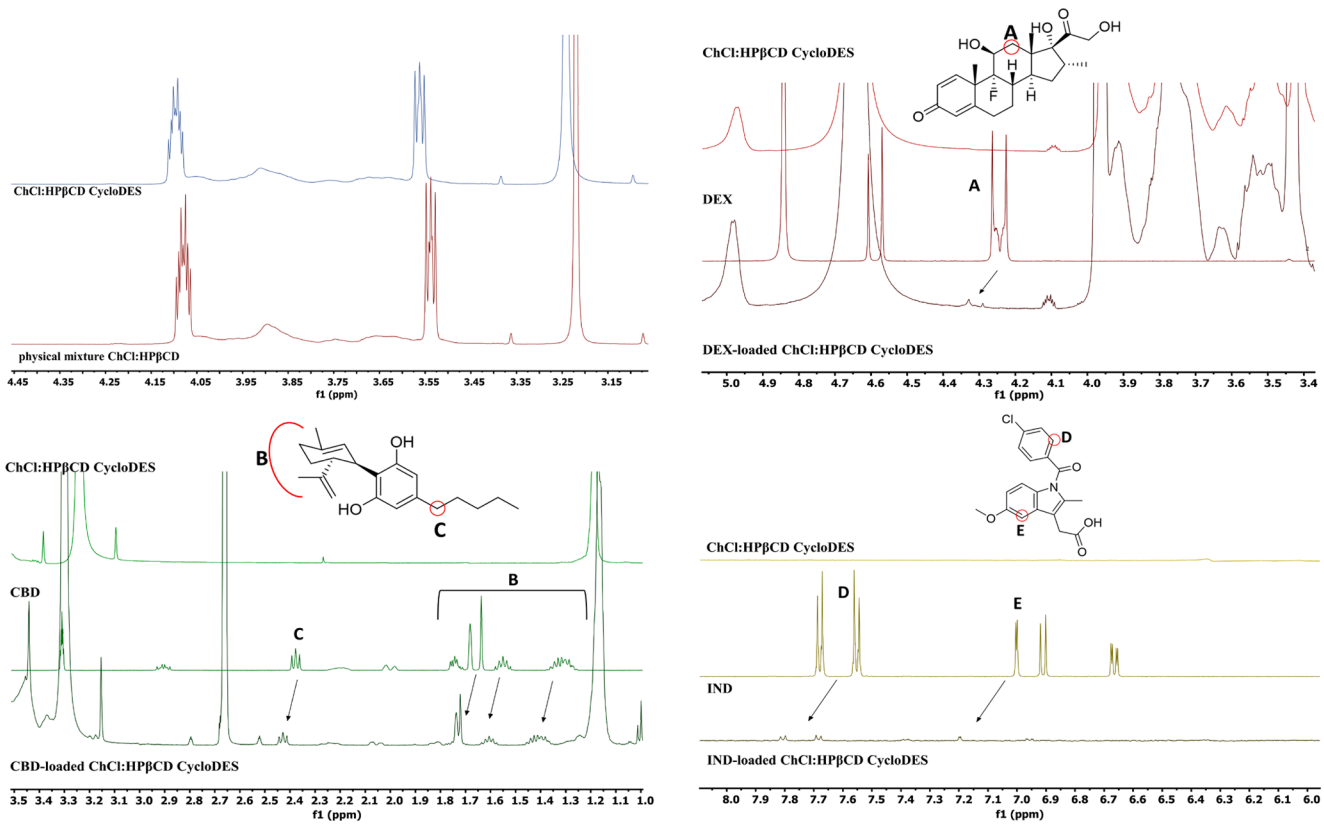

Fig. 6. Focused zoom on the observed shift in terms of ppm and relative drug structures. The relevant drug characteristic peaks were highlighted in the figure as follow. For DEX: A. 4.24 ppm; For CBD: B. 1.2–1.8 ppm, C. 2.36; For IND D. 7.62, E. 6.98. Full spectra are available in Figure S2.

Indeed, the obtained data showed a reduced velocity in permeability for CycloDES, manifested by sample permeation curves below those describing the controls (Fig. 7), with the P coefficient calculated from the linear region (Caridade et al., 2013).

Although all the permeabilities of the samples are described by P coefficient above 10^{-6}, demonstrating a good permeability, the

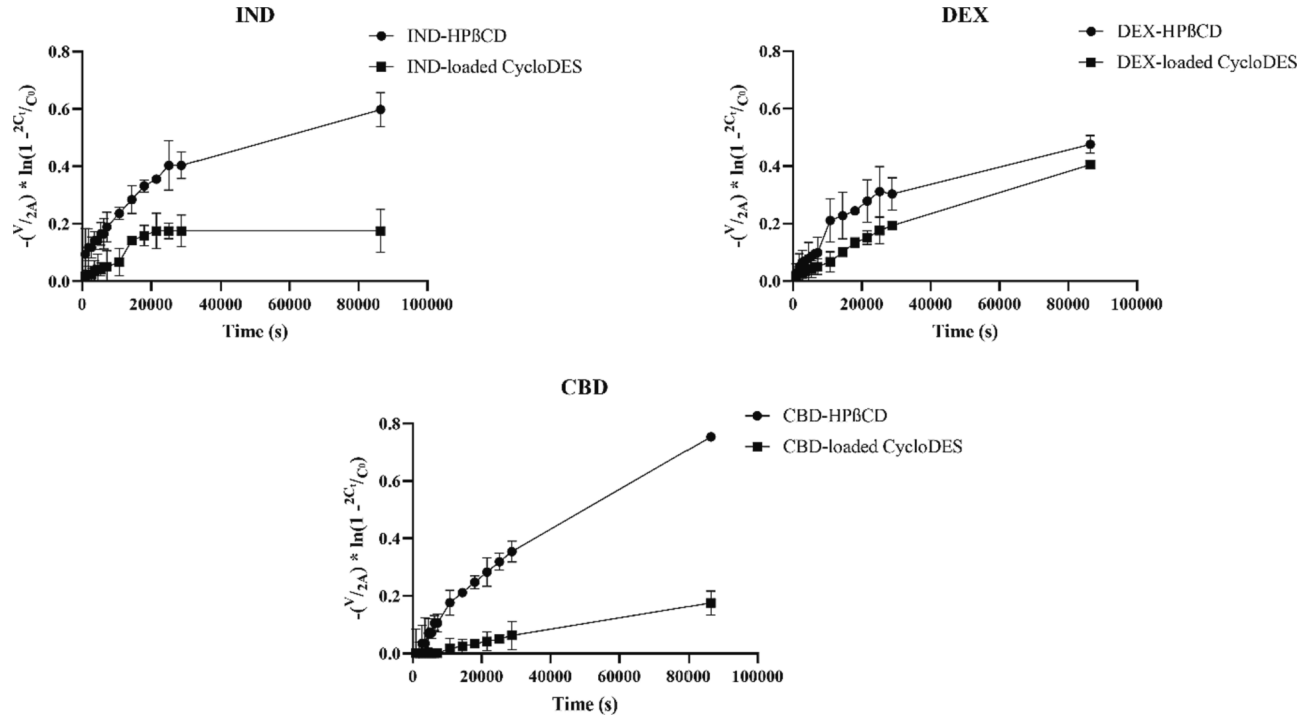

Fig. 7. Permeation curve of samples and control. A significant difference between the permeation of CBD-loaded and IND-loaded CycloDES with their positive control was find with P-value < 0.01 (**) and < 0.001 (***), respectively.

observed profiles could be attributed to the difference in the supramolecular structure manifested by the higher viscosity of CycloDES and the lower diffusion coefficient (Table 4).

The samples indicated as IND, CBD and DEX represent the control samples. All the data, and relative standard deviations, are the results of triplicate experiments.

For CBD, a lag time of 30 min was observed for the control and 2 h for CycloDES, possibly related to the higher lipophilicity of the molecule ($XlogP_{CBD} = 6.5$) and consequently higher affinity for the membrane, manifested by the lower diffusion coefficient of the CBD-loaded CycloDES. On the other hand, the difference in the lag time could be explained by the lower affinity to the phase in the acceptor chamber of CBD, for the presence of an aqueous solution. In the case of IND, a plateau in the permeability was reached after 6 h and maintained until 24 h, which could hypothetically be correlated with an equilibrium between the donor and the acceptor chamber.

Nevertheless, the permeability obtained through the model membrane suggests a balanced permeation improvement, compatible with the use of CycloDES in sustained release reservoir systems, where CDs diluted in water cannot be applied.

3.10. Ex vivo permeation studies

The ex vivo studies that were conducted showed that no drugs were released into the receiver compartment even after 24 h, mainly due to the thickness of the skin membrane.

This indicates that CycloDES may be suitable for local treatment as a component of topical formulations. To determine the quantity of each compound present in the ear skin after 24 h, the drugs were extracted from the skin and the results were measured in μg/cm^2 (Table 5).

The results obtained demonstrate that the HPβCD-induced enhancement of penetration, previously reported (Murgia et al., 2013), is not hindered by its use in an eutectic mixture. Comparable performances versus the controls were observed for IND and DEX while for CBD-loaded CycloDES a reduced permeability was observed after 24 h. The similar behavior between CycloDES and DEX control is due to the comparable solubilizing capacity of the systems adopted. The results obtained by IND and CBD when observed in terms of the amount of drug present within the membrane showed an higher concentration of drug. Nevertheless, the data regarding IND are influenced by the ionization that molecule is subject to, in fact at the observed pH of 6 of both the donor compartment, IND is present in its less permeable, ionized form as already reported (Chiang et al., 1991; Hayashi et al., 1992). Thus, the improved drug loading capacity of the CycloDES could act as a driving force toward the membrane, potentially enhancing the therapeutic treatment. These evidences suggested the need for individual evaluation of each possible CycloDES in combination with a loaded drug to assess permeability behavior. However, the utilization of CycloDES could lead to improved drug concentrations in complex topical formulations, decreasing the amount of CD required. Additionally, generating CycloDES from various components ensures the necessary elasticity to achieve the optimal mixture, solubilizing the targeted active ingredient and exhibiting the desired traits of the components themselves. Further, the proper characteristics of a complete medicinal product could be guaranteed by the excipient selection, likewise in EMLA cream, where the THEDES lidocaine-prilocaine had been included in a topical anesthetic formulation (Ehrenström-Reiz et al., 1983; Gajraj et al., 1994).

Table 4
CycloDES permeability data.

Sample	Permeability coefficient (10^{-6} cm/s)	Diffusion coefficient (cm^2/s)
IND-HPβCD	13.89 ± 1.15	6.67E-07 ± 3.75E-07
IND-loaded CycloDES	5.53 ± 0.46	6.44E-07 ± 2.59E-07
CBD-HPβCD	7.59 ± 0.24	1.59E-07 ± 6.96E-08
CBD-loaded CycloDES	2.08 ± 0.08	4.54E-08 ± 5.36E-08
DEX-HPβCD	6.00 ± 0.78	3.98E-07 ± 9.25E-08
DEX-loaded CycloDES	6.98 ± 0.46	2.13E-07 ± 6.62E-08

Table 5
Amount of drug extracted by the skin membrane.

Sample	Concentration of entrapped drug in membrane (μg/cm^2)	$\frac{\text{Drug in the membrane}}{\text{Total drug loaded}} \times 100$
IND-HPβCD	1.83 ± 0.61	0.26 ± 0.02
IND-loaded CycloDES	7.69 ± 1.75	0.39 ± 0.05
CBD-HPβCD	70.93 ± 9.23	14.93 ± 2.1
CBD-loaded CycloDES	119.92 ± 7.52	5.99 ± 0.83
DEX-HPβCD	293.83 ± 20.23	14.76 ± 1.76
DEX-loaded CycloDES	287.51 ± 15.88	14.18 ± 0.94

4. Conclusions

The CycloDESs developed and characterized have widened the pool of compounds capable of generating DES with HPβCD and demonstrated to be a further step in addressing solubility-related issues of BCS Class II and IV drugs. In fact, taking advantage of the synergic approach induced by the eutectic nature of the system and the presence of HPβCD the solubility of the herein-used model drugs was improved by at least 100-fold if compared to water solubility. The obtained results confirmed our former hypothesis with at least one of the proposed CycloDES capable of overcoming the performance shown by the water solution of HPβCD. The prepared liquid vehicle allows skipping the rate-determining steps of other pharmaceutical solid dosage forms such as disintegration and dissolution. The observed increased drug solubilization could reduce the use of CDs in the formulation, decreasing the overall cost of the preparations and possible CDs-related side effects. Among the newly introduced, ChCl:HPβCD CycloDES solubilized directly all the solid-state active principle ingredients without any extraction step from an already drug-loaded liquid media likewise oils. The data from DSC, TGA, and NMR guarantee the proper solubilization and no degradation of the model drugs in the ChCl:HPβCD mixture, resulting in a useful tool for the delivery of pH-sensitive drugs (i.e. CBD). Further, CycloDESs synergic approach was successfully proven to maintain the drug in solution in percentage significantly higher than the model Glucose:ChCl DES when dilution occurred. As a result, this capability marks an improvement over other DESs' current state, which sees a dramatic decrease in solubilizing capacity as dilution rises. These features, accompanied by the different observed viscosities and slow permeation through the membranes, allow their enrollment in many other elaborate DDS, such as microparticles or emulsions, with applications, not only with dermal formulations but also targeting the mucosal route.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Gennaro Balenzano is supported by Italfarmacy srl (Trani, Italy). Authors acknowledge Farmalabor srl. (Canosa di Puglia, Italy), Centro Studi e Ricerche "Dr. S. Fontana 1900-1982" (Canosa di Puglia, Italy)

and ALFATESTlab (Milano, Italy) for their technical support. Authors thank Mr. Nicola Di Masi (University of Bari, Bari, Italy) for their skillful technical assistance in recording NMR spectra. Authors also acknowledge Siciliani SpA (Palo del Colle, Italy) for providing the samples for ex vivo experiments. The University of Bari "Aldo Moro" (Bari, Italy) is also gratefully acknowledged for its financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2023.123553.

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