Solubility enhancement of caffeine and furosemide using Deep Eutectic Solvents formed by choline chloride and xylitol, citric acid, sorbitol or glucose

Laura Lomba[a,b], Alejandra Polo[a], Julia Alejandre[a], Nuria Martínez[a], Beatriz Giner[a, b*]

[a]Facultad de Ciencias de la Salud, Universidad San Jorge, Campus Universitario, Autov. A23 km 299, 50830, Villanueva de Gállego, Zaragoza, Spain.

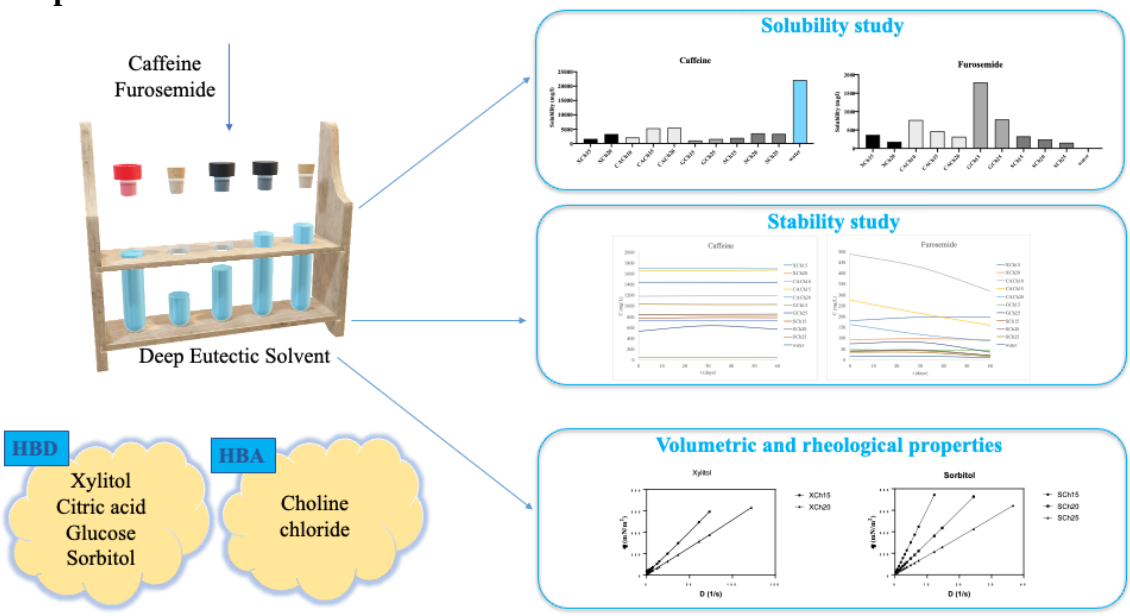
[b]Instituto Agroalimentario de Aragón-IA2 (Universidad de Zaragoza-CITA), Zaragoza, Spain

[*]Corresponding author: bginer@usj.es

Abstract: The low solubility of active pharmaceutical ingredients (APIs) in aqueous medium hinders the formulation of drugs in liquid solvents. Several methodologies are used to improve their solubilities being one of them the use of Deep Eutectic Solvents (DES). In this work 10 DES formed by xylitol, citric acid, sorbitol and glucose (HBD) and choline chloride as HBA have been prepared. The solubility of caffeine and furosemide in these DES and their stability have been studied. Additionally, their volumetric and rheological properties have been analysed. Results show that in the case of caffeine, the increase of water content, increase the solubility. This trend is the opposite in the case of furosemide. The stability study reveals that the concentrations of API in DES have been stable during the test time. Finally, the rheological study shows that all studied DES are non-Newtonian fluids, with viscosities decreasing when water content increases.

Keywords: DES, active pharmaceutical ingredients, solubility, stability.

Graphical abstract



1. Introduction

Active pharmaceutical ingredients (API) are, in general, poor soluble in water and present low dissolution rates and bioavailability (Goeke *et al.*, 2018). Approximately, 40% of approved drugs and almost 90% of those in development are poorly soluble (Kalepu & Nekkanti, 2015; Loftsson & Brewster, 2010; Papich & Martinez, 2015). Not having oral liquid pharmaceutical forms can be a severe problem for all those patients with swallowing problems or dysphagia, pediatric or geriatric population. Often, issues of adherence to treatment or erroneous dosages arise when the medication must be manipulated to facilitate administration (Chin & Joos, 2016; Zajicek *et al.*, 2013).

The increase of solubility of poorly soluble drug has been addressed on many occasions and normally, modifications in drug formulations are used to increase the oral bioavailability: new forms of dosage, changes of drugs forms (esters or salts), prodrugs, active metabolites, or use of different administrations routes. As the vast majority of drugs are ionizable drugs (weakly acids or basis), the modification of the pH medium is also a common strategy for modulating the solubility of the APIs (Serajuddin, 2007). Some other techniques involve the use of co-solvents and surfactants, polymeric micelles or solid lipid nanoparticles (Kalepu & Nekkanti, 2015), reduction particle size, hydrotropy, cocrystal, amorphous compound formation or nanosuspension technology (Ainurofiq *et al.*, 2021).

During the last years, a new perspective has emerged to improve the drug formulation and solubility problems: the use of Deep Eutectic Solvents (DES) for increasing drug solubility. Several studies have shown that these solvents can be used for this purpose and the preliminary results are promising (Abdkarimi & Haghtalab, 2021; Chakraborty *et al.*, 2021; Golgoun *et al.*, 2021; M. Mokhtarpour *et al.*, 2019; Masumeh Mokhtarpour *et al.*, 2020; Palmelund *et al.*, 2019; Palmelund *et al.*, 2020; Pedro *et al.*, 2019).

Let us pay attention to this type of solvents: DES are mixtures formed by hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The typical components are chemicals such as: sugars, alcohols, urea, natural metabolites, organic acids, choline chloride (Nystedt *et al.*, 2021). The charge delocalization occurring during the complexation between HBA and HBD produces a unique molecular structure that significantly reduces the melting point of the mixture, which is much lower than that of either individual chemical forming part of the moiety. Large combinations of components

and compositions can be proposed, and thus, new eutectic mixtures are almost unlimited. This is a great advantage, since a mixture can be designed on demand for a specific application, modulating its properties as desired (Paiva *et al.*, 2014).

But not only this; thanks to the sustainable source of raw materials, DES have been considered an important green alternative to some other traditional solvents (Dai *et al.*, 2013; Garcia-Alvarez, 2014; Ge *et al.*, 2017; Wagle *et al.*, 2014). There is a type of DES, named Natural Deep Eutectic Solvents (NADES), that specifically include the sustainable source, comprising natural compounds. According to the Green Chemistry precepts, the main characteristics of green solvents must be (Anastas & Eghbali, 2010; Kudlak *et al.*, 2015; Zuriaga *et al.*, 2018): high biodegradability, low (eco)toxicity both in the chemical itself and the degradation products, high accessibility, low volatility, safe use throughout the whole life cycle (design, synthesis, application, recycling, and environmental fate/disposal). Certainly, the environmental and green properties of DES are quite interesting and, although there is still a lot to study, DES deserve the green label: several studies have demonstrated low (eco)toxicity for a number of DES in different biomodels and cellular models (Ahmadi *et al.*, 2018; Benlebna *et al.*, 2018; Giner *et al.*, 2020; Lomba *et al.*, 2021; Z. Yang, 2019) and high biodegradability rates (Costa *et al.*, 2017; Hou *et al.*, 2013; Lapena *et al.*, 2021). Furthermore, DES are usually non-volatile at room temperature and chemically and thermally stable (Sekharan *et al.*, 2022).

Depending on the solubility in aqueous systems, DES can be classified as hydrophilic and hydrophobic. As mentioned before, for both types of DES, the strong ability of dissolving diverse types of molecules has been demonstrated (Bergua *et al.*, 2021; Faggian *et al.*, 2016; Garcia *et al.*, 2016; Tatar *et al.*, 2017). With this regard, another type of DES, having an API in the composition is THEDES (Therapeutical Deep Eutectic Solvent) (I. M. Aroso *et al.*, 2015; Ivo M. Aroso *et al.*, 2016). THEDES have been used to improve pharmaceutical conditions of formulations: increasement of solubility, permeability, and absorption (Tuntarawongsa & Phaechamud, 2012).

With all of this in mind, in this work, in this work, we explore the use of sustainable DES with the aim of increasing the solubility of different APIs. The final goal is the oral liquid formulation of poorly soluble drugs using green and safe eutectic solvents.

In this case, we have selected several DES formed by choline chloride as HBA and xylitol, citric acid, glucose, and sorbitol as HBDs, with different water contents. All

the components can be considered sustainable and come from renewable sources of raw materials.

We have studied the solubility and stability of two APIs, caffeine, and furosemide, in the selected DES. Since none of these drugs are commercially found in any oral liquid form, the hypothesis of our study is the following: it is possible to prepare stable liquid solutions at therapeutical concentration of furosemide and caffeine using hydrophilic DES. To cover different casuistry, one of the drugs is a typical non soluble API (furosemide) while caffeine is a water-soluble substance. In Table 1, details of the commercially available pharmaceutical forms, dosage and basic chemical information of these APIs are shown.

Table 1. Pharmaceutical forms, dosages, and pKa of the studied APIs.

APIs	**Commercial oral dosage form**	**Maximum adults dose per day (mg)**	**Maximum children dose per day (mg)**	**pK**
Furosemide	Tablet	80	40	pKa1 3.8 pKa2 7.5*
Caffeine	Capsule	300	-	8.3 (Couto *et al.*, 2015)

*Hazardous Substances Data Bank (HSDB). Available online: https://pubchem.ncbi.nlm.nih.gov/source/hsdb/3086 and https://pubchem.ncbi.nlm.nih.gov/compound/2519

Furosemide is a widely used diuretic API, applied for the treatment of hypertension and edema associated with pulmonary, heart, renal and liver disease (Chaulang *et al.*, 2009). This drug increases the excretion of cations Na^+ and water in the kidneys by inhibiting the reabsorption from the proximal and distal tubules, as well as the loop of Henle. This chemical is a weak acid with low solubility (Granero *et al.*, 2010). The aqueous solubility of furosemide can be increased depending on the pH. According to the Biopharmaceutics Classification System (BCS), furosemide can be classified as Class IV; that means, poor water solubility and poor permeability (Gulsun *et al.*, 2018). Despite of the importance of this drug, there are no commercially available oral liquid pharmaceutical forms of furosemide.

There have been an important number of attempts to increase the solubility of furosemide. For instance, Gulsun *et al.* prepared nanosuspensions of furosemide using Tween 80 to improve the solubility, and therefore, the permeability of furosemide. Different solutions were characterized and the permeability using Caco-2 cells was also

studied, concluding an important increase of both solubility and permeability (Gulsun *et al.*, 2018). The complexation of furosemide using some complexants such as cyclodextrins or calix[n]arenes has also been studied (Kreaz *et al.*, 1998; W. Z. Yang & de Villiers, 2004); in all cases, solubility, and permeability increased, depending on several factors: the complex preparation method used, or the type on complex agent. Other strategies used involved the modification of the molecular structure through the formation of esters; results showed sufficient water solubility and lipophilicity. However, a fast rate of enzymatic transformation to furosemide during or following absorption was needed (Mork *et al.*, 1990). Although all these methods have demonstrated a successful improvement of the solubility, the goal depends on the used technique, and it can be concluded that the dissolution rate of the API clearly depends on the quantity of solubility enhancer, the type of enhancer and dissolution medium (Murtaza *et al.*, 2014).

Caffeine is a methylxanthine that stimulates the central nervous system (CNS), increase alertness, and can cause restlessness and agitation. It relaxes smooth muscles and stimulates the contraction of the cardiac muscle. Additionally, it stimulates the gastric acid secretion and increase the gastrointestinal motility. It can be combined with analgesics and ergot alkaloids decreasing headaches (Grzegorzewski *et al.*, 2022). Although caffeine is an hydrosoluble API, there are only solid pharmaceutical forms commercially available. It is worth mentioning that caffeine is often used in cocrystallization techniques for improving cocrystals due to their ability to improve solubility, dissolution, bioavailability, stability and processability of some hydrophobic APIs (Chen *et al.*, 2011; Rodrigues *et al.*, 2018).

2. Experimental Section

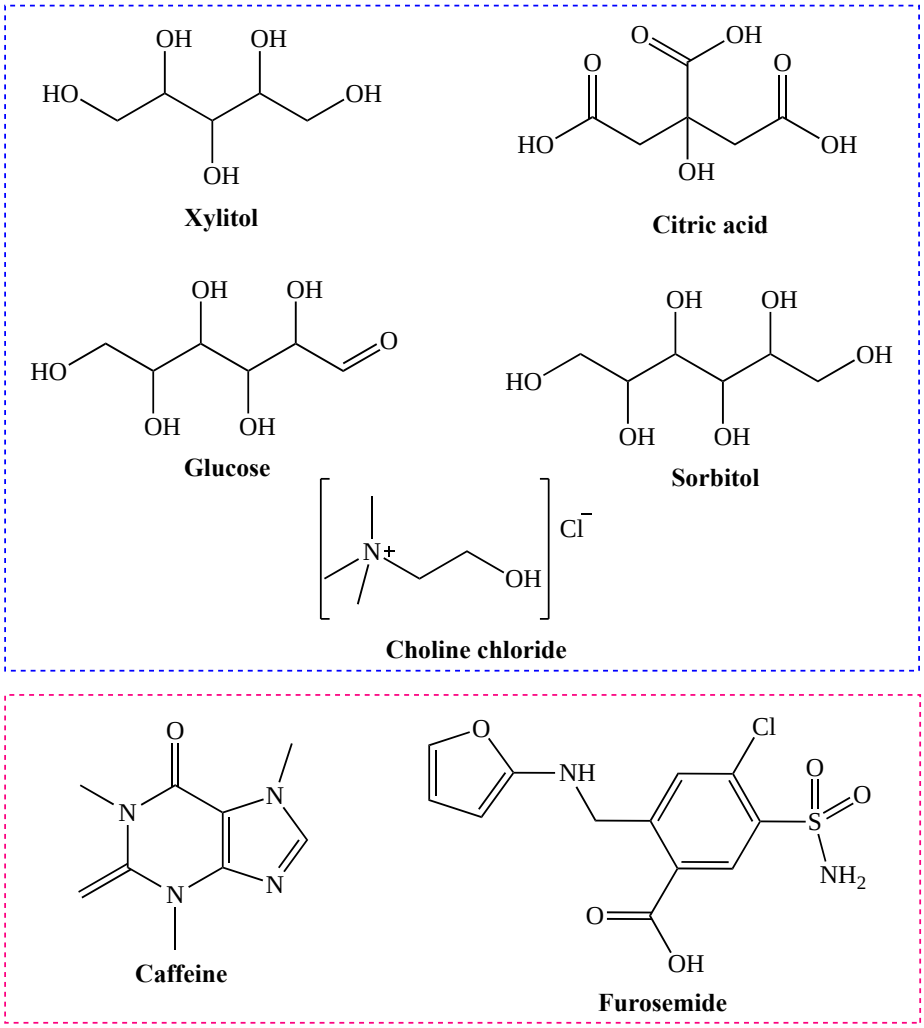
2.1 Chemicals

Some chemical information of the pure chemicals used in this study is gathered in Table 2. Additionally, chemical structures are shown in Figure 1. Chemicals have been dried under vacuum for 24 hours prior to use.

171 Table 2. Information of the pure chemicals used in this study.

Chemical	CAS number	Molar mass (g/mol)	Supplier	Formula
Xylitol	87-99-0	152.15	Fagron	$C_5H_{12}O_5$
Citric acid anhydrous	77-92-9	192.12	Acofarma	$C_6H_8O_7$
Glucose anhydrous	50-99-7	180.16	Acofarma	$C_6H_{12}O_6$
Sorbitol	50-70-4	182.17	Sigma-Aldrich	$C_6H_{14}O_6$
Choline Chloride	67-48-1	139.63	Panreac	$C_5H_{14}ClNO$
Caffeine anhydrous	58-08-2	194.19	Acofarma	$C_8H_{10}N_4O_2$
Furosemide	54-31-9	330.74	Acofarma	$C_{12}H_{11}ClN_2O_5S$

172



173

174 Figure 2. Chemical structure of pure compounds.

2.2. Preparation of Deep Eutectic Solvents

In this study, several DES have been prepared (100 g) by the combination of citric acid, xylitol, glucose and sorbitol with choline chloride and water at different proportions. Compounds have been weighed using a Sartorius Entris 5201-1S balance (uncertainty $\pm 10^{-1}$ g). The reagents have been introduced in the appropriate proportions in a jar with constant stirring and heat in a water bath at 60-70ºC until a mixture of transparent and homogeneous appearance has been obtained. Finally, all the mixtures have been stored in darkness until their use. The information related to the composition and the final molar mass of the mixture can be found in Table 3. The average molar mass of each DES has been calculated according to the following equation (Ahmadi *et al.*, 2018):

$$MW_{DES} = X_{HBA(ChCl)} \cdot MW_{HBA(ChCl)} + X_{HBD} \cdot MW_{HBD} + X_{water} \cdot MW_{water.} \quad \text{(Eq. 1)}$$

where X is the mole fraction and MW the molar mass.

Table 3. Information of prepared DES.

DES	**Abbreviation name**	**Mole proportion**	Molar mass **(g/mol)**
Xylitol:Choline Chloride: Water	XCh15	1:2:15	38.96
	XCh20	1:2:20	34.41
Citric Acid:Choline Chloride: Water	CACh10	1:2:10	50.11
	CACh15	1:2:15	41.19
	CACh20	1:2:20	36.15
Glucose:Choline Chloride:Water	GCh15	2:5:15	60.38
	GCh25	2:5:25	47.14
Sorbitol:Choline Chloride:Water	SCh15	3:2:15	54.79
	SCh20	3:2:20	47.43
	SCh25	3:2:25	42.52

2.3. Solubility study

The solubility of the two APIs, caffeine and furosemide in the studied DES has been obtained using a modification of the shake-flask method (Kalepu & Nekkanti, 2015).

To quantify the solubility of each API, a spectrum scan has been obtained at different wavelengths to select the maximum absorbance to be used for each drug in ethanol as solvent. The scan has been performed between 190-450 nm using a Shimadzu UV-1800 spectrophotometer model PGT600 with ±1 nm precision and connected to UV WIN version 5.2 software. Maximum absorbance, Abs_{max}, has been plotted against the weight concentration (g solute/g solvent) to obtain the calibration curves. The used

concentrations for the calibration curve for each API have been 2.5, 5, 10, 15, 20, 30 and 40 mg/l for caffeine and 2.5, 5, 7.5, 10, 15, 30, 50 y 60 mg/l for furosemide.

The solubility study has been carried out in both ultrapure distilled water and in the studied DES. The supersaturated solutions were prepared in first place, following the general rules for solutions preparation and supersaturation was checked visually. Then the solutions were stirred during 24 hours at controlled temperature. Afterwards, samples have been protected from light using aluminium foil and rested during 24 h at 25ºC. After this, supersaturation of mixtures was checked visually again. This study has been carried out using a J.P. Select heater.

Then, the samples have been centrifuged using Biofuge Primo R centrifuge for 5 min at 5000 rpm. Supernatants have been filtered using PES syringe filter of 0.22 µm and analysed using UV-Vis T60U spectrophotometer. Concentrations have been obtained by interpolation of absorbance in the calibration curves obtained before. The experiment has been carried out in triplicate.

2.4. Stability study

Three solutions of each API have been prepared in the studied DES and ultrapure water at concentrations below the maximum solubility found in the previous experiments. The mixtures have been kept under stirring until the API has been totally dissolved. Next, solutions have been stored in a Memmert ICH260L climatic chamber at constant temperature (25ºC) and 60% humidity. The pH and concentration of each solution have been measured at 0, 30 and 60 days.

The pH has been measured through a Sesion +PH3 pHmeter high performance electrode 5012. The concentration of the APIs has been measured by High Performance Liquid Chromatography (HPLC) using a HPLC Agilent 1100 with automatic injector. A Kromasil C18 (250 mm x 4.8 internal diameter x 5 µm particle size) column has been used with an injection volume of sample loop of 20 µm, flow 1.0 ml/min. The temperature was controlled during the measurements at 30ºC. Before sample injection, 0.1 g of each sample was diluted in 10 ml of methanol and filtered through 0.45 µm filter. A gradient elution was carried out. The starting eluant was H_2O pH=2: acetonitrile (90:10) to reach after 20 min of flowing 10:90. Analytes have been detected at 254 nm for caffeine and at 341 nm for furosemide.

2.5 Volumetric and rheological properties

The density of the studied DES, at 25ºC, has been measured with Anton Paar DSA 5000 densimeter (uncertainty 0.1 kg/m^3).

To carry out the rheological study, the viscosity of the studied DES has been measured via a rotational viscosimeter Brookfield (DV-E). A shear rate sweep has been performed, starting with the highest speed (normally 60 rpm) down to the lowest (0.3 rpm). The temperature was controlled at 25ºC with an immersion bath Termotronic JPSELECTA with precision ± 0.1 ºC. Viscosity measurements were repeated twice.

Shear stress, τ, obtained experimentally from viscosity data were correlated using the Herschel-Bulkley model for non-Newtonian fluids:

$$\tau = \tau_0 + kD^n \quad (2)$$

In this model, the relationship between shear stress and shear rate is characterized through the parameters τ_0 (yield shear stress), *k* (consistence factor) and *n* (flow index).

2.6 Statistical analysis

Statistical analysis has been carried out using GraphPad Prism 9.0 program. One-way ANOVA method and Tukey-Kramer Honest Significant Differences model have been used. In the null hypothesis (H_0), it is considered that there are no statistically significant differences between the groups and therefore they are equal, and in the alternative hypothesis (H_1) it is assumed that there are differences between groups. A 95% confidence interval is chosen, so if $p < 0.05$, the null hypothesis is rejected, and the alternative is accepted.

3. Results and discussion

3.1. Preparation of DES

Several methods for preparing DES have been previously described such as evaporation, heating of freezing-drying. In this case, the heating method has been used because it is sample and common (Nam *et al.*, 2015). The studied deep eutectic solvents (XCh, CACh, GCh and SCh) have formed a homogeneous and transparent solution. No precipitate has been appreciated at any time at room temperature.

3.2 Calibration curves

In Table 4, the results obtained for the calibrations curves are gathered. These results include validation parameters such as coefficient of determination, R^2, limit of detection, *LD*, and limit of quantification, *LQ*. Ethanol have not shown interference in the spectra.

Table 4. Calibration equation caffeine and furosemide in ethanol. Wavelength of maximum absorbance (Abs), $\boldsymbol{\lambda\, Abs_{max}}$, and validation parameters: coefficient of determination, R^2, limit of detection, *LD*, and limit of quantification, *LQ*. [a] $LD = \frac{x+3S}{m}$; [b] $LQ = \frac{x+10S}{m}$ being m the slope, and x and S, the average and the deviation of the blank.

API	Slope calibration line c in c (mg/l)	λ (Abs_{max}) (nm)	R^2	LD[a]	LQ[b]
Caffeine	0.04336	254	0.998	$1.31 \cdot 10^{-7}$	$3.55 \cdot 10^{-7}$
Furosemide	0.01574	341	0.999	$3.61 \cdot 10^{-7}$	$9.78 \cdot 10^{-7}$

3.3 Solubility studies

In Table 5, values of the mean of the experimental maximum concentration of caffeine and furosemide and the standard deviation, are shown. Additionally, a graphical comparison of the solubilities can be found in Figure 1.

Table 5. Solubility, *s* (mg/l), and pH results with their corresponding standard deviation for caffeine and furosemide in studied DES and water.

Solvent/API	Solubility (mg/l)		pH	
	Caffeine	Furosemide	Caffeine	Furosemide
XCh15	1616 ± 130	365.0 ± 13.0	4.51 ± 0.3	3.42 ± 0.1
XCh20	3287 ± 60.7	184.7 ± 35.4	4.54 ± 0.3	3.78 ± 0.2
CACh10	2102 ± 63.0	773.2 ± 86.1	0.19 ± 0.1	0.23 ± 0.1
CACh15	5333 ± 327	468.5 ± 64.4	0.40 ± 0.1	0.39 ± 0.1
CACh20	5476 ± 178	318.9 ± 32.2	0.48 ± 0.1	0.49 ± 0.1
GCh15	1063 ± 58.9	1796 ± 173	6.91 ± 0.3	3.78 ± 0.3
GCh25	1585 ± 75.3	787.0 ± 7.07	6.22 ± 0.2	3.72 ± 0.2
SCh15	1962 ± 126	327 .1± 15.0	6.12 ± 0.3	3.86 ± 0.2
SCh20	3567 ± 84.2	247.4 ± 7.47	5.81 ± 0.1	3.74 ± 0.3
SCh25	3349 ± 117	156.1 ± 5.62	6.21 ± 0.3	3.95 ± 0.2
Water	22142 ± 370	17 ± 2.50	7.35 ± 0.3	5.24 ± 0.3

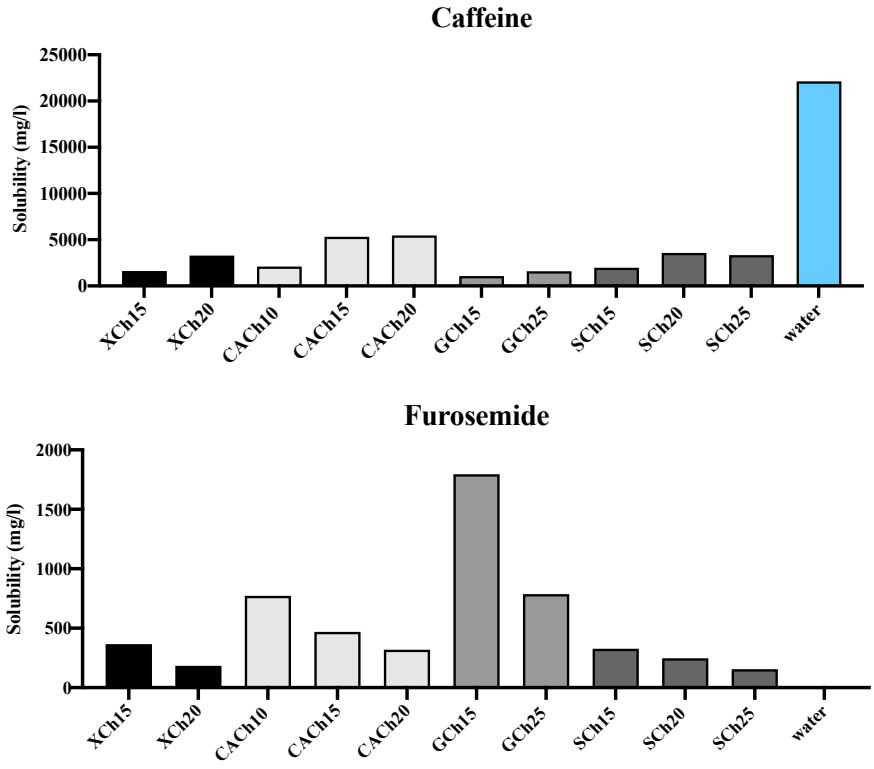


Figure 1. Comparison of the results of solubility obtained for caffeine and furosemide in studies DES and water.

In Table 6, the statistical analysis is shown. In the case of caffeine, there are statistical differences among the studied DES and water. However, there are not statistical differences among DES but the one containing citric acid. For furosemide, there are statistical differences among the studied DES and, in general, for all DES except in the case of: water and SCh25 and some DES with xylitol.

In the case of caffeine, the higher values of solubility are found for DES containing citric acid followed by DES with sorbitol, xylitol, and glucose. The most soluble solvent is CACh20. The pH of the resulting solutions is in all cases lower than the pKa of caffeine, thus this molecule should be mostly in its neutral form. Due to this, caffeine is more soluble in acidic mediums such as citric acid DES. However, it should be remarked that the solubility of caffeine increases as the content of water does in all cases excepting for sorbitol DES (solubility decreases slightly in SCh25 compared to SCh20). This behavior is similar to the observed for citric acid DES; the solubility of caffeine in CACh10 is considerably lower than for CACh15 or CACh20, being for these two quite similar. This could be due a salting in effect; caffeine and DES components could be competing for water molecules in their solvation process.

Shumilin *et al.* studied the effect of mono and disaccharides on caffeine self-association and solubility. They found that sugars increase the concentration of caffeine in its monomeric state but decrease the solubility in all oligomeric forms. Sugars act as selective hydrotropes (Shumilin *et al.*, 2019). Additionally, Oliveira *et al.* studied the solubility of caffeine in aqueous and solutions of deep eutectic solvents. They observed that the solubility of caffeine was reduced compared to solubility in pure water; this can also be seen in our study. Considering the high hydrophilic character of caffeine, it is expected a competition between the DES and caffeine to form hydrated complexes with water molecules. Thus, caffeine solubility could be reduced due to the presence of the DES components (Oliveira *et al.*, 2021).

It is important to note that, sorbitol can interact with the purine face through the non-polar hydrogen atoms in contact, and the hydrogen bonding stablished with hydroxyl groups pointing off to the sides to form hydrogen bonds with the solvent. However, it has been observed that there is a little tendency for the sorbitol molecules to hydrogen bond to the caffeine (Tavagnacco *et al.*, 2013).

Paying attention to furosemide, the higher values of solubility are found for Glucose DES, followed by acid citric, xylitol and sorbitol DES. Furosemide is a weak acid with pK_a=3.8 and practically insoluble in water. In addition, its aqueous solubility increases as the pH increases (Gulsun *et al.*, 2018). In the cases of our study, a diminution of solubility can be observed for all the studied DES when water is included being more soluble in CACh10, GCh15 and SCh15.

Markovic *et al.* have shown that the solubility of furosemide presents a pH-dependent solubility, in accordance with its acidic nature being more soluble at pH near 7-8 than at 1-4. At lower pH values, this drug is poorly soluble in tamponade aqueous solutions. This pH dependance agrees with the results obtained in this study. Additionally, a significant regional-dependent permeability has been observed diminishing as the intestinal segments are more basics (more distal) (Markovic *et al.*, 2020).

The solubility is normally related with the ability of mass transfer from the solute to solution. Thus, similar solvents with lower viscosity will dissolve more easily. However, in this case, the DES with lower viscosity are not better solubilizers for furosemide. This is mainly because the presence of water: the increase of water content in DES enhances the polarity of DES-water mixtures that decrease the solubility of hydrophobic molecules, overcoming the effect of viscosity. Additionally, water can easily penetrate the strong hydrogen bonding pattern in a DES on reaching a certain threshold,

and thereby solubilize HBA/HBD by forming simple aqueous solution, which results in reduction or loss of dissolving capacity held by a DES (Lu *et al.*, 2016).

The analysis of the molecular structure and the hydrogen bonds that can be formed is fundamental when it comes to explaining the increased solubility of the active ingredients in DES (Abdkarimi & Haghtalab, 2021). When caffeine is added in water, several hydrogen bonds are formed between the API and water molecules, which lead to solvation and dissolution of the compound. When the solubilization medium is a DES, some changes can appear in the system; water molecules can enter in the structure of DES preventing the drug from solubilizing in the DES. For caffeine, the solubility increases as the amount of water does. This can occur because of the intermolecular interactions formed between the drug, the DES and water. In this case, API molecules present more affinity for water than for the DES. This could be due to the number of hydrogen bonding sites: on the one hand, the caffeine molecule, with several acceptor sites (nitrogen and oxygen atoms) and no donors, captures water molecules very easily and caffeine is solvated. On the other hand, DES, with multiple hydrogen bond sites, both donors and acceptors, have a well-structured network that somehow does not allow the release of water molecules to solvate caffeine.

Furosemide has a higher solubility in DES than in water, signifying a higher chemical affinity. The molecular structure of the API that gives rise to hydrogen bond interactions is formed by two nitrogen atoms, in sulfonamide and amine groups, both donors and acceptors, and three other hydrogen atoms, in an acid group (donor and acceptor) and ether (acceptor only). Water is made up of small molecules, which are not capable of solubilizing all of furosemide, with non-polar parts in its structure. The much larger DES molecules, with multiple hydrogen bonding sites, facilitate solubility through interactions with polar groups on furosemide.

Another important point to consider in the solubility of drugs when DES are used is the pH of the solution. On many occasions, in addition to taking into account the intermolecular solute-solvent interactions that can be generated, it is necessary to consider the pH values. According to Avdeef (Avdeef, 2007), the charge state dramatically affects apparent solubility and thus pKa values, that are used to calculate the charge state at a particular pH, are considered fundamental.

In this case, all the DES, are formed by acidic or weak acidic substances, and all the solutions formed are acid or slightly acid. On the other hand, caffeine has a pKa = 8.3 and furosemide has two pKa1=3.8 and pKa 2 =7.5 (Table 1). These pKa values explain

the differences in pH solutions (Table 5) for a given API in the different solvents, DES and water. At these pH solutions, both caffeine and furosemide should be ionized, excepting in the case of furosemide in citric acid DES, with a low pH. For DES containing caffeine, the influence of pH becomes apparent; given its pKa, the solubility must decrease with increasing pH, this fact being especially visible for the most acidic DES, containing citric acid, with substantially higher solubility values than for the rest of DES, whose pH values are similar. Therefore, for these DES with similar pH, other factors, already mentioned, could be more important. In the case of furosemide, the solubility mechanism and its relation to pH is not clear. The natural tendency, taking into account the pKa values of this substance, would be to appreciate an increase in solubility with pH. However, this effect is not appreciated, not even in the case of DES containing citric acid, with other factors, such as the previously mentioned network of hydrogen bonds, having greater weight in this case.

Table 6. *p* values for the solubility statistical analysis.

Caffeine	**Water**	**XCh15**	**XCh20**	**CACh10**	**CACh15**	**CACh20**	**GCh15**	**GCh25**	**SCh15**	**SCh20**	**SCh25**
Water	-	-	-	-	-	-	-	-	-	-	-
XCh15	<0.0001	-	-	-	-	-	-	-	-	-	-
XCh20	<0.0001	0.000	-	-	-	-	-	-	-	-	-
CACh10	<0.0001	0.9284	0.0383	-	-	-	-	-	-	-	-
CACh15	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-
CACh20	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	-	-	-	-	-	-
GCh15	<0.0001	0.7385	<0.0001	0.1023	<0.0001	<0.0001	-	-	-	-	-
GCh25	<0.0001	>0.9999	0.0002	0.8950	<0.0001	<0.0001	0.8005	-	-	-	-
SCh15	<0.0001	0.9860	0.0043	>0.9999	<0.0001	<0.0001	0.1265	0.9728	-	-	-
SCh20	<0.0001	<0.0001	0.9977	0.0049	<0.0001	0.0002	<0.0001	<0.0001	0.0004	-	-
SCh25	<0.0001	0.0001	>0.9999	0.0208	<0.0001	<0.0001	<0.0001	<0.0001	0.0021	>0.9999	-
Furosemide	**Water**	**XCh15**	**XCh20**	**CACh10**	**CACh15**	**CACh20**	**GCh15**	**GCh25**	**SCh15**	**SCh20**	**SCh25**
Water	-	-	-	-	-	-	-	-	-	-	-
XCh15	<0.0001	-	-	-	-	-	-	-	-	-	-
XCh20	0.0369	0.0449	-	-	-	-	-	-	-	-	-
CACh10	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-
CACh15	<0.0001	0.6472	0.0003	<0.0001	-	-	-	-	-	-	-
CACh20	<0.0001	0.9987	0.2829	<0.0001	0.1630	-	-	-	-	-	-
GCh15	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-
GCh25	<0.0001	<0.0001	<0.0001	>0.9999	<0.0001	<0.0001	<0.0001	-	-	-	-
SCh15	<0.0001	0.9998	0.2117	<0.0001	0.2221	>0.9999	<0.0001	<0.0001	-	-	-
SCh20	0.0017	0.4713	0.9805	<0.0001	0.0065	0.9519	<0.0001	<0.0001	0.9025	-	-
SCh25	0.1271	0.0117	>0.9999	<0.0001	<0.0001	0.0955	<0.0001	<0.0001	0.0669	0.7921	-

3.4 Stability studies

The stability of drugs in solution is essential to assure that the dose is correct and additionally, to interpret the biochemical data properly (Gomez-Sanchez *et al.*, 2020).

In this section, the stability study carried out is analyzed. This study has been based on the measurements of concentration of APIs in each solvent and pH at 0, 30 and 60 days. The concentration and pH of three solutions of each API at concentrations below the maximum solubility have been measured. These values are gathered in Table 7.

The concentration of APIs in DES formed by xylitol are stable along the days. It is noteworthy that there are no significative differences in the obtained results. However, in the case of citric acid solvents, for caffeine, it can be observed that is stable during the test period but not in the case of furosemide, which present a diminution of concentration in the last 30 days. This result can be related to the dependence of furosemide with pH. Glucose DES present stability for caffeine but not in the case of furosemide observing the same trend as citric acid (CACh20). In these cases, the concentration values at 60 days are around half than initial values. Finally, studied APIs in DES formed by sorbitol are stable in the case of caffeine but not in the case of furosemide, in which concentration values decrease as the time goes by.

Regarding to the pH, values for furosemide solutions are lower than for caffeine solutions in all cases. Solutions containing citric acid DES present in general very low values because of the acid group; being pH in all cases values between 0.19-1.13. For glucose and sorbitol DES, values of pH for caffeine vary between 5.26 and 7.04 and for furosemide 3.47 and 3.95. For xylitol DES, pH values are slightly lower, from 4.5 for caffeine to 3.5 for furosemide.

For caffeine solutions, pH remains almost constant for xylitol DES, while for glucose and sorbitol DES, pH values decreases when time goes by, being the decrease more pronounced during the first 30 days. In the DES contain citric acid, pH values, although very low in all cases, both at the beginning and at the end of the test, increase slightly.

As with caffeine, the pH of furosemide solutions in DES containing citric acid are very low, increasing slightly over time and this increase being more pronounced during the first 30 days of testing. The pH of furosemide in xylitol, sorbitol and glucose DES is almost constant during the test period (60 days). This result contrasts with the case of aqueous solution: pH decreases as the time goes. It is worth mentioning that furosemide is unstable in acid media because it suffers acid-catalyzed hydrolysis in aqueous solution.

However, in alkaline solutions furosemide presents higher stability (pH=7-10) (Sombie *et al.*, 2022). Some sweeteners (sorbitol or maltitol) are recommended to be used as pharmaceutical excipients (Raymond Rowe, 2009). However, in this study, it has been observed that the stability of some DES (sorbitol included) is not stable with time so, the next step of the use of these DES could be the formulation of liquid forms drug using some stabilizer excipients. Some studies related to thermal stability of DES indicate that they can decomposed to HBA and HBD because of breaking and weaking of their hydrogen bonds (Delgado-Mellado *et al.*, 2018). Hydrogen bonds play an important role un thermal stability of these mixtures; in general, when starting materials are stable, the higher of Tonset values of DES are obtained (Marchel *et al.*, 2022). Taking into account, for following studies some forced degradation and stability study could be explored (Blessy *et al.*, 2014).

435 Table 7. Concentrations, C, in mg/L and pH at 0, 30 and 60 days for caffeine and furosemide in studied DES and water.

Solvent	Caffeine						Furosemide					
	0 days		30 days		60 days		0 days		30 days		60 days	
	C	pH	C	pH	C	pH	C	pH	C	pH	C	pH
XCh15	736 ± 6.2	4.51 ± 0.3	729 ± 6.1	4.44 ± 0.2	723 ± 8.1	4.58 ± 0.4	180 ± 3.6	3.42 ± 0.1	196 ± 7.8	3.42 ± 0.2	197 ± 8.3	3.53 ± 0.1
XCh20	844 ± 3.9	4.54 ± 0.3	832 ± 3.7	4.42 ± 0.3	829 ± 5.3	4.50 ± 0.2	93 ± 2.1	3.78 ± 0.2	98 ± 5.2	3.75 ± 0.2	93 ± 6.3	3.83 ± 0.3
CACh10	1184 ± 8.2	0.19 ± 0.1	1189 ± 6.4	0.98 ± 0.2	1190 ± 9.3	0.97 ± 0.1	487 ± 8.6	0.23 ± 0.1	426 ± 7.3	0.96 ± 0.1	317 ± 5.6	0.95 ± 0.1
CACh15	1668 ± 7.4	0.40 ± 0.1	1666 ± 9.3	1.06 ± 0.2	1671±12.7	1.05 ± 0.1	275 ± 6.3	0.39 ± 0.1	214 ± 8.2	1.07 ± 0.2	159 ± 3.5	1.03 ± 0.1
CACh20	1698 ± 12	0.48 ± 0.1	1698 ± 10	1.12 ± 0.2	1692 ± 2.1	1.12 ± 0.1	162 ± 2.8	0.49 ± 0.1	116 ± 7.2	1.13 ± 0.2	85 ± 8.3	1.12 ± 0.2
GCh15	47 ± 2.1	6.91 ± 0.3	44 ± 1.8	7.04 ± 0.3	44 ± 1.8	5.71 ± 0.3	122 ± 1.7	3.78 ± 0.3	126 ± 5.2	3.63 ± 0.3	52 ± 4.8	3.54 ± 0.2
GCh25	535 ± 4.8	6.22 ± 0.2	637 ± 5.2	5.83 ± 0.2	572 ± 12.4	5.41 ± 0.1	75 ± 1.2	3.72 ± 0.2	82 ± 8.3	3.61 ± 0.3	36 ± 2.1	3.52 ± 0.2
SCh15	771± 6.4	6.12 ± 0.3	788 ± 5.7	5.84 ± 0.3	785 ± 9.6	5.26 ± 0.2	39 ± 0.8	3.86 ± 0.2	42 ± 4.3	3.82 ± 0.4	19 ± 1.5	3.65 ± 0.2
SCh20	843 ± 6.9	5.81 ± 0.1	846 ± 7.2	5.74 ± 0.2	849 ± 7.2	5.46 ± 0.2	41 ± 0.5	3.74 ± 0.3	45 ± 3.9	3.62 ± 0.4	20 ± 2.5	3.47 ± 0.2
SCh25	1034 ± 8.1	6.21 ± 0.3	1028 ± 7.6	5.96 ± 0.2	1028 ± 4.9	5.70 ± 0.1	36 ± 0.2	3.95 ± 0.2	35 ± 1.1	3.90 ± 0.2	17 ± 1.5	3.84 ± 0.2
Water	1446 ± 7.3	7.35 ± 0.3	1446 ± 7.1	6.48 ± 0.2	1443 ± 6.4	4.78 ± 0.3	17 ± 2.5	5.24 ± 0.3	16 ± 0.7	4.70 ± 0.2	10 ± 2.6	4.59 ± 0.3

436

3.5 Volumetric and rheological study

The DES present several advantages in the flow behavior over traditional solvents, since viscosity can be easily modulated, modifying the compositions of the components that are part of the moiety and/or adding different amounts of water, which can lead to dramatic changes in their viscous behavior. Thus, DES can be operated and used in different chemical processes and industries. However, in different occasions it has been shown that the lack of DES shear flow studies made it a difficult task to investigate the flow behaviors of different eutectic solvents (Elhamarnah *et al.*, 2019).

In this study, the flow behavior of the studied DES has been obtained. In Table 8, experimental density values at 25ºC are gathered. All the studied DES are denser than water, thus, density decreases if the amount of water in the solvent increases. Higher density values are found for DES containing sorbitol followed by glucose, citric acid and xylitol DES.

Table 8. Density experimental values, **ρ,** and adjusted parameters, τ_0, k and n (Eq. 2) with their corresponding coefficient of determination, R^2 and standard deviation, s, a t 25ºC.

Solvent	ρ **(g/ml)**	τ_0 **(mN/m²)**	k **(g/s·m)**	n	R^2	**S**
XCh15	1.123841	28.1	5.12	1.10	1.00	3.26
XCh20	1.108058	8.88	4.23	1.04	1.00	2.69
CACh10	1.146510	7.02	33.7	0.99	1.00	1.25
CACh15	1.143692	10.3	10.9	1.02	1.00	2.34
CACh20	1.125282	6.94	5.76	1.04	1.00	2.26
GCh15	1.181235	4.16	76.0	1.00	1.00	1.47
GCh25	1.158438	9.08	21.3	1.01	1.00	1.30
SCh15	1.228962	8.80	56.7	1.00	1.00	1.04
SCh20	1.210953	9.79	28.0	1.01	1.00	2.36
SCh25	1.191902	13.3	14.8	1.04	1.00	1.24

Apparent viscosity experimental values at 25ºC are graphically shown for each shear rate in Figure 2. Obtained adjusted parameters, τ_0, k and n, from Eq. 2. are gathered in Table 8 together with their corresponding coefficient of determination, R^2 and standard deviation, s. Shear stress has been also calculated from experimental results and are graphically shown in Figure 3.

The classification of these eutectic solvents has been subject to discussion on several occasions; it is generally accepted that these compounds behave as Newtonian fluids, regardless of the applied shear rate (Rita Craveiro 2015). However, there are some authors who point to a non-Newtonian behavior, solid-like behavior at room temperature (Craveiro, 2015). In this work, rheograms indicate that all the studied DES can be considered non-Newtonian fluids, showing a typical shear thinning behavior (apparent viscosity decreases with shear rate). However, as it can be seen in Figure 3, all DES tend to Newtonian fluids when shear rate increases, with apparent viscosity values almost constant. This can be also verified with the flow index parameter (n) is close to unit in all cases, being slightly away from the unit if water content increases for all the studied DES but xylitol ones.

It is also noteworthy that the yield shear stress parameters, τ_0, although are not very big, are different to zero in all cases, which is indicative of a plastic like behavior. These solvents do not begin to flow until a certain threshold value of shear stress, τ_0, has been reached. This agrees with the findings of Yan *et al.*, who pointed out that NADES are solid-like material at room temperature conditions(Yan *et al.*, 2017).

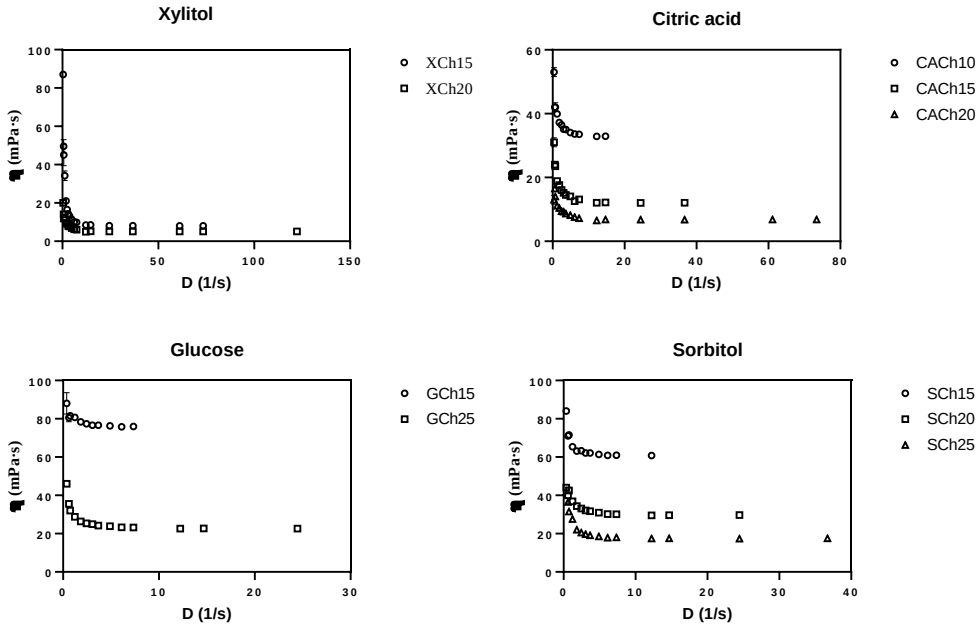


Figure 2. Apparent viscosity vs. shear rate of the studied DES at 25ºC.

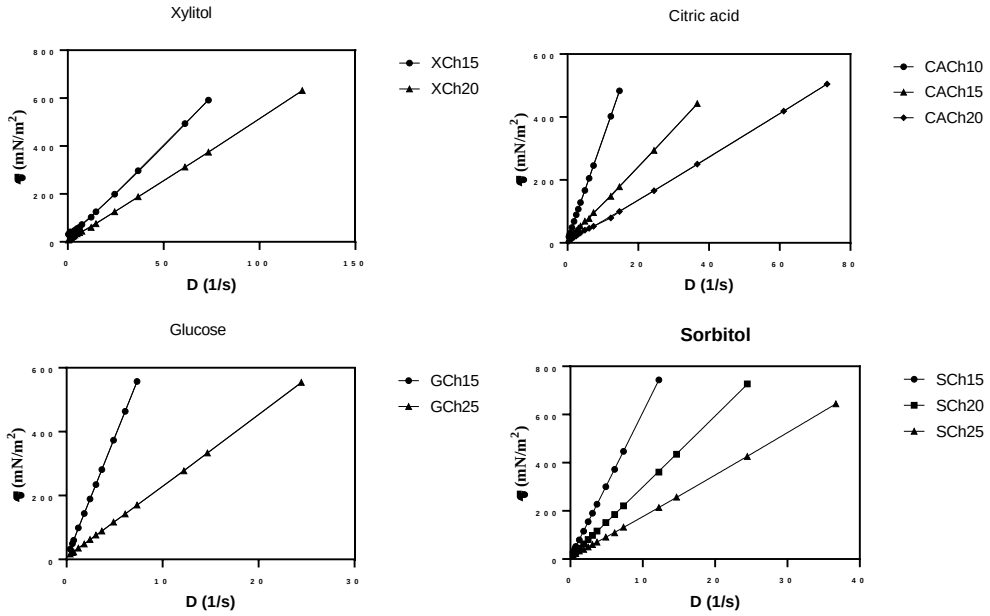


Figure 3. Shear stress vs. shear rate for the studied DES at 25ºC

5. Conclusions

In this work, ten DES formed by xylitol, citric acid, and glucose (HBD) and choline chloride as HBA have been prepared, being stable at room temperature. The solubility and stability of two APIs (furosemide and caffeine) in these DES has been also studied. The solubility has been significantly increased in the case of furosemide being the DES formed by glucose and choline chloride the moiety which increases the solubility to a greater extent. The stability of caffeine and furosemide for 60 days have been satisfactory, and the concentrations have been kept constant.

Additionally, a volumetric and rheological study of DES have also been carried out at room temperature, observing that all DES behave as non-Newtonian fluids. Furthermore, flow behaviour can be easily modulated controlling the composition of the eutectic solvents and the amounts of water.

Finally, it is necessary to formulate the final liquid system and carry out toxicological and pharmacokinetic studies to ensure the use of these systems.

Conflicts of 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.

Acknowledgements

We want to thank Universidad San Jorge for financial supporting (call for internal projects 2022).

CRediT authorship contribution statement

L. Lomba: Investigation, Methodology, Writing – original draft, Formal analysis. A. Polo: Investigation, J. Alejandre: Investigation. N. Martinez: Investigation B. Giner: Data Curation, Investigation, Methodology, Writing – original draft, Formal analysis, Writing – review & editing.

All authors have read and agreed to the published version of the manuscript.

6. References

Abdkarimi, F., & Haghtalab, A. (2021). Solubility measurement and thermodynamic modeling of sertraline hydrochloride and clopidogrel bisulfate in deep eutectic solvent of choline chloride and malonic acid. *Journal of Molecular Liquids*, 344.

Ahmadi, R., Hemmateenejad, B., Safavi, A., Shojaeifard, Z., Mohabbati, M., & Firuzi, O. (2018). Assessment of cytotoxicity of choline chloride-based natural deep eutectic solvents against human hek-293 cells: A qsar analysis. *Chemosphere*, 209, 831-838.

Ainurofiq, A., Putro, D. S., Ramadhani, D. A., Putra, G. M., & Santo, L. D. D. (2021). A review on solubility enhancement methods for poorly water-soluble drugs. *Journal of Reports in Pharmaceutical Sciences*, 10, 137-147.

Anastas, P., & Eghbali, N. (2010). Green chemistry: Principles and practice. *Chemical Society Reviews*, 39, 301-312.

Aroso, I. M., Craveiro, R., Rocha, A., Dionisio, M., Barreiros, S., Reis, R. L., et al. (2015). Design of controlled release systems for thedes-therapeutic deep eutectic solvents, using supercritical fluid technology. *International Journal of Pharmaceutics*, 492, 73-79.

Aroso, I. M., Silva, J. C., Mano, F., Ferreira, A. S. D., Dionisio, M., Sa-Nogueira, I., et al. (2016). Dissolution enhancement of active pharmaceutical ingredients by therapeutic deep eutectic systems. *European Journal of Pharmaceutics and Biopharmaceutics*, 98, 57-66.

Avdeef, A. (2007). Solubility of sparingly-soluble ionizable drugs. *Advanced Drug Delivery Reviews*, 59, 568-590.

Benlebna, M., Ruesgas-Ramon, M., Bonafos, B., Fouret, G., Casas, F., Coudray, C., et al. (2018). Toxicity of natural deep eutectic solvent betaine:Glycerol in rats. *Journal of Agricultural and Food Chemistry*, 66, 6205-6212.

Bergua, F., Castro, M., Munoz-Embid, J., Lafuente, C., & Artal, M. (2021). Hydrophobic eutectic solvents: Thermophysical study and application in removal of pharmaceutical products from water. *Chemical Engineering Journal*, 411, 128472.

Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability indicating studies of drugs-a review. *Journal of pharmaceutical analysis*, 4, 159-165.

Chakraborty, S., Chormale, J. H., & Bansal, A. K. (2021). Deep eutectic systems: An overview of fundamental aspects, current understanding and drug delivery applications. *International Journal of Pharmaceutics*, 610, 121203.

Chaulang, G., Patel, P., Hardikar, S., Kelkar, M., Bhosale, A., & Bhise, S. (2009). Formulation and evaluation of solid dispersions of furosemide in sodium starch glycolate. *Tropical Journal of Pharmaceutical Research*, 8, 43-51.

Chen, J. M., Wu, C. B., & Lu, T. B. (2011). Application of supramolecular chemistry on pharmaceutical cocrystals. *Chemical Journal of Chinese Universities-Chinese*, 32, 1996-2009.

Chin, W. W. L., & Joos, A. (2016). Moving toward a paradigm shift in the regulatory requirements for pediatric medicines. *European Journal of Pediatrics*, 175, 1881-1891.

Costa, S. P. F., Azevedo, A. M. O., Pinto, P. C. A. G., & Saraiva, M. L. M. F. S. (2017). Environmental impact of ionic liquids: Recent advances in (eco)toxicology and (bio)degradability. *Chemsuschem*, 10, 2321-2347.

566 Couto, O. M., Jr., Matos, I., da Fonseca, I. M., Arroyo, P. A., da Silva, E. A., & Simoes
567 Dornellas de Barros, M. A. (2015). Effect of solution ph and influence of water
568 hardness on caffeine adsorption onto activated carbons. *Canadian Journal of*
569 *Chemical Engineering*, 93, 68-77.
570 Craveiro, R. P. P. 2015. Engineering bio-based polymers using alternative solvents and
571 processes*Chemistry*. Repositorio Universidade Nova: Nova de Lisboa.
572 Dai, Y. T., van Spronsen, J., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2013). Natural
573 deep eutectic solvents as new potential media for green technology. *Analytica*
574 *Chimica Acta*, 766, 61-68.
575 Delgado-Mellado, N., Larriba, M., Navarro, P., Rigual, V., Ayuso, M., Garcia, J., et al.
576 (2018). Thermal stability of choline chloride deep eutectic solvents by tga/ftir-atr
577 analysis. *Journal of Molecular Liquids*, 260, 37-43.
578 Elhamarnah, Y. A., Nasser, M., Qiblawey, H., Benamor, A., Atilhan, M., & Aparicio, S.
579 (2019). A comprehensive review on the rheological behavior of imidazolium
580 based ionic liquids and natural deep eutectic solvents. *Journal of Molecular*
581 *Liquids*, 277, 932-958.
582 Faggian, M., Sut, S., Perissutti, B., Baldan, V., Grabnar, I., & Dall'Acqua, S. (2016).
583 Natural deep eutectic solvents (nades) as a tool for bioavailability improvement:
584 Pharmacokinetics of rutin dissolved in proline/glycine after oral administration in
585 rats: Possible application in nutraceuticals. *Molecules (Basel, Switzerland)*, 21
586 (11), 1531.
587 Garcia, A., Rodriguez-Juan, E., Rodriguez-Gutierrez, G., Julian Rios, J., & Fernandez-
588 Bolanos, J. (2016). Extraction of phenolic compounds from virgin olive oil by
589 deep eutectic solvents (dess). *Food Chemistry*, 197, 554-561.
590 Garcia-Alvarez, J. 2014. Deep eutectic solvents: Environmentally friendly media for
591 metal-catalyzed organic reactions. In S. O. Obare & R. Luque (Eds) *Green*
592 *technologies for the environment* pp. 37-52).
593 Ge, X., Gu, C. D., Wang, X. L., & Tu, J. P. (2017). Deep eutectic solvents (dess)-derived
594 advanced functional materials for energy and environmental applications:
595 Challenges, opportunities, and future vision. *Journal of Materials Chemistry A*, 5,
596 8209-8229.
597 Giner, B., Lafuente, C., Lapena, D., Errazquin, D., & Lomba, L. (2020). Qsar study for
598 predicting the ecotoxicity of nades towards aliivibrio fischeri. Exploring the use
599 of mixing rules. *Ecotoxicology and Environmental Safety*, 191.
600 Goeke, K., Lorenz, T., Repanas, A., Schneider, F., Steiner, D., Baumann, K., et al. (2018).
601 Novel strategies for the formulation and processing of poorly water-soluble drugs.
602 *European Journal of Pharmaceutics and Biopharmaceutics*, 126, 40-56.
603 Golgoun, S., Mokhtarpour, M., & Shekaari, H. (2021). Solubility enhancement of
604 betamethasone, meloxicam and piroxicam by use of choline-based deep eutectic
605 solvents. *Pharmaceutical Sciences*, 27, 86-101.
606 Gomez-Sanchez, R., Besley, S., Zeliku, Z., & Young, R. J. (2020). Method development
607 and application of an accelerated solution stability screen for drug discovery. *Slas*
608 *Discovery*, 25, 1191-1196.
609 Granero, G. E., Longhi, M. R., Mora, M. J., Junginger, H. E., Midha, K. K., Shah, V. P.,
610 et al. (2010). Biowaiver monographs for immediate release solid oral dosage
611 forms: Furosemide. *Journal of Pharmaceutical Sciences*, 99, 2544-2556.
612 Grzegorzewski, J., Bartsch, F., Koeller, A., & Koenig, M. (2022). Pharmacokinetics of
613 caffeine: A systematic analysis of reported data for application in metabolic
614 phenotyping and liver function testing. *Frontiers in Pharmacology*, 12, 752826.

Gulsun, T., Borna, S. E., Vural, I., & Sahin, S. (2018). Preparation and characterization of furosemide nanosuspensions. *Journal of Drug Delivery Science and Technology*, 45, 93-100.

Hou, X.-D., Liu, Q.-P., Smith, T. J., Li, N., & Zong, M.-H. (2013). Evaluation of toxicity and biodegradability of cholinium amino acids ionic liquids. *Plos One*, 8 (3), e59145.

Kalepu, S., & Nekkanti, V. (2015). Insoluble drug delivery strategies: Review of recent advances and business prospects. *Acta Pharmaceutica Sinica B*, 5, 442-453.

Kreaz, R. M. A., Dombi, G., & Kata, M. (1998). The influence of beta-cyclodextrins on the solubility of furosemide. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry*, 31, 189-196.

Kudlak, B., Owczarek, K., & Namiesnik, J. (2015). Selected issues related to the toxicity of ionic liquids and deep eutectic solvents-a review. *Environmental Science and Pollution Research*, 22, 11975-11992.

Lapena, D., Errazquin, D., Lomba, L., Lafuente, C., & Giner, B. (2021). Ecotoxicity and biodegradability of pure and aqueous mixtures of deep eutectic solvents: Glyceline, ethaline, and reline. *Environmental Science and Pollution Research*, 28, 8812-8821.

Loftsson, T., & Brewster, M. E. (2010). Pharmaceutical applications of cyclodextrins: Basic science and product development. *Journal of Pharmacy and Pharmacology*, 62, 1607-1621.

Lomba, L., Pilar Ribate, M., Sanguesa, E., Concha, J., Pilar Garralaga, M., Errazquin, D., et al. (2021). Deep eutectic solvents: Are they safe? *Applied Sciences-Basel*, 11.

Lu, C., Cao, J., Wang, N., & Su, E. Z. (2016). Significantly improving the solubility of non-steroidal anti-inflammatory drugs in deep eutectic solvents for potential non-aqueous liquid administration. *Medchemcomm*, 7, 955-959.

Marchel, M., Cieslinski, H., & Boczkaj, G. (2022). Thermal instability of choline chloride-based deep eutectic solvents and its influence on their toxicity-important limitations of dess as sustainable materials. *Industrial & Engineering Chemistry Research*, 61, 11288-11300.

Markovic, M., Zur, M., Ragatsky, I., Cvijic, S., & Dahan, A. (2020). Bcs class iv oral drugs and absorption windows: Regional-dependent intestinal permeability of furosemide. *Pharmaceutics*, 12 (12), 1175.

Mokhtarpour, M., Shekaari, H., Martinez, F., & Zafarani-Moattar, M. T. (2019). Study of naproxen in some aqueous solutions of choline-based deep eutectic solvents: Solubility measurements, volumetric and compressibility properties. *International Journal of Pharmaceutics*, 564, 197-206.

Mokhtarpour, M., Shekaari, H., Zafarani-Moattar, M. T., & Golgoun, S. (2020). Solubility and solvation behavior of some drugs in choline based deep eutectic solvents at different temperatures. *Journal of Molecular Liquids*, 297, 111799.

Mork, N., Bundgaard, H., Shalmi, M., & Christensen, S. (1990). Furosemide prodrugs - synthesis, enzymatic-hydrolysis and solubility of various furosemide esters. *International Journal of Pharmaceutics*, 60, 163-169.

Murtaza, G., Khan, S. A., Najam-ul-Haq, M., & Hussain, I. (2014). Comparative evaluation of various solubility enhancement strategies for furosemide. *Pakistan Journal of Pharmaceutical Sciences*, 27, 963-973.

Nam, M. W., Zhao, J., Lee, M. S., Jeong, J. H., & Lee, J. (2015). Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: Application to flavonoid extraction from flos sophorae. *Green Chemistry*, 17, 1718-1727.

Nystedt, H. L., Gronlien, K. G., & Tonnesen, H. H. (2021). Interactions of natural deep eutectic solvents (nades) with artificial and natural membranes. *Journal of Molecular Liquids*, 328, 115452.

Oliveira, G., Farias, F. O., Sosa, F. H. B., Igarashi-Mafra, L., & Mafra, M. R. (2021). Green solvents to tune the biomolecules' solubilization in aqueous media: An experimental and in silico approach by cosmo-rs. *Journal of Molecular Liquids*, 341, 117314.

Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R. L., & Duarte, A. R. C. (2014). Natural deep eutectic solvents - solvents for the 21st century. *Acs Sustainable Chemistry & Engineering*, 2, 1063-1071.

Palmelund, H., Andersson, M. P., Asgreen, C. J., Boyd, B. J., Rantanen, J., & Lobmann, K. (2019). Tailor-made solvents for pharmaceutical use? Experimental and computational approach for determining solubility in deep eutectic solvents (des). *International Journal of Pharmaceutics-X*, 1, 100034.

Palmelund, H., Boyd, B. J., Rantanen, J., & Lobmann, K. (2020). Influence of water of crystallization on the ternary phase behavior of a drug and deep eutectic solvent. *Journal of Molecular Liquids*, 315, 113727.

Papich, M. G., & Martinez, M. N. (2015). Applying biopharmaceutical classification system (bcs) criteria to predict oral absorption of drugs in dogs: Challenges and pitfalls. *Aaps Journal*, 17, 948-964.

Pedro, S. N., Freire, M. G., Freire, C. S. R., & Silvestre, A. J. D. (2019). Deep eutectic solvents comprising active pharmaceutical ingredients in the development of drug delivery systems. *Expert Opinion on Drug Delivery*, 16, 497-506.

Raymond Rowe, P. S., Marian Quinn (2009). *Handbook of pharmaceutical excipients*: London: Pharmaceutical Press.

Rita Craveiro , M. D., Susana Barreiros , Rui L. Reis, Alexandre Paiva , Ana Rita C. Duarte 2015. Fundamental studies on natural deep eutectic solvents: Physico-chemical, thermal and rheological properties*6th Nordic Wood Biorefinery Conference, NWBC 2015 - Helsinki, Finland* pp. 155-160): VTT Technical Research Centre of Finland.

Rodrigues, M., Baptista, B., Lopes, J. A., & Sarraguca, M. C. (2018). Pharmaceutical cocrystallization techniques. Advances and challenges. *International Journal of Pharmaceutics*, 547, 404-420.

Sekharan, T. R., Chandira, R. M., Tamilvanan, S., Rajesh, S. C., & Venkateswarlu, B. S. (2022). Deep eutectic solvents as an alternate to other harmful solvents. *Biointerface Research in Applied Chemistry*, 12, 847-860.

Serajuddin, A. T. M. (2007). Salt formation to improve drug solubility. *Advanced Drug Delivery Reviews*, 59, 603-616.

Shumilin, I., Allolio, C., & Harries, D. (2019). How sugars modify caffeine self-association and solubility: Resolving a mechanism of selective hydrotropy. *Journal of the American Chemical Society*, 141, 18056-18063.

Sombie, C. B., Guetambaba, J. E. K., Diawara, H. Z., Yameogo, J., Ouedraogo, S., Goole, J., et al. (2022). Hospital preparations of ethanol-free furosemide oral solutions: Formulation and stability study. *Journal of advanced pharmaceutical technology & research*, 13, 128-132.

Tatar, A., Barati-Harooni, A., Najafi-Marghmaleki, A., & Bahadori, A. (2017). Accurate prediction of co2 solubility in eutectic mixture of levulinic acid (or furfuryl alcohol) and choline chloride. *International Journal of Greenhouse Gas Control*, 58, 212-222.

Tavagnacco, L., Brady, J. W., & Cesaro, A. (2013). The interaction of sorbitol with caffeine in aqueous solution. *Food Biophysics*, 8, 216-222.

Tuntarawongsa, S., & Phaechamud, T. (2012). Menthol, borneol, camphor and ws-3 eutectic mixture. *Biomaterials and Applications*, 506, 355-358.

Wagle, D. V., Zhao, H., & Baker, G. A. (2014). Deep eutectic solvents: Sustainable media for nanoscale and functional materials. *Accounts of Chemical Research*, 47, 2299-2308.

Yan, Y. C., Rashmi, W., Khalid, M., Shahbaz, K., Gupta, T. C. S. M., & Mase, N. (2017). Potential application of deep eutectic solvents in heat transfer application. *Journal of Engineering Science and Technology*, 12, 1-14.

Yang, W. Z., & de Villiers, M. M. (2004). Aqueous solubilization of furosemide by supramolecular complexation with 4-sulphonic calix n arenes. *Journal of Pharmacy and Pharmacology*, 56, 703-708.

Yang, Z. (2019). Natural deep eutectic solvents and their applications in biotechnology. *Application of Ionic Liquids in Biotechnology*, 168, 31-59.

Zajicek, A., Fossler, M. J., Barrett, J. S., Worthington, J. H., Ternik, R., Charkoftaki, G., et al. (2013). A report from the pediatric formulations task force: Perspectives on the state of child-friendly oral dosage forms. *Aaps Journal*, 15, 1072-1081.

Zuriaga, E., Giner, B., Ribate, M. P., Garcia, C. B., & Lomba, L. (2018). Exploring the usefulness of key green physicochemical properties: Quantitative structure-activity relationship for solvents from biomass. *Environmental Toxicology and Chemistry*, 37, 1014-1023.

736