Deep eutectic solvents based on sugars for oral applications

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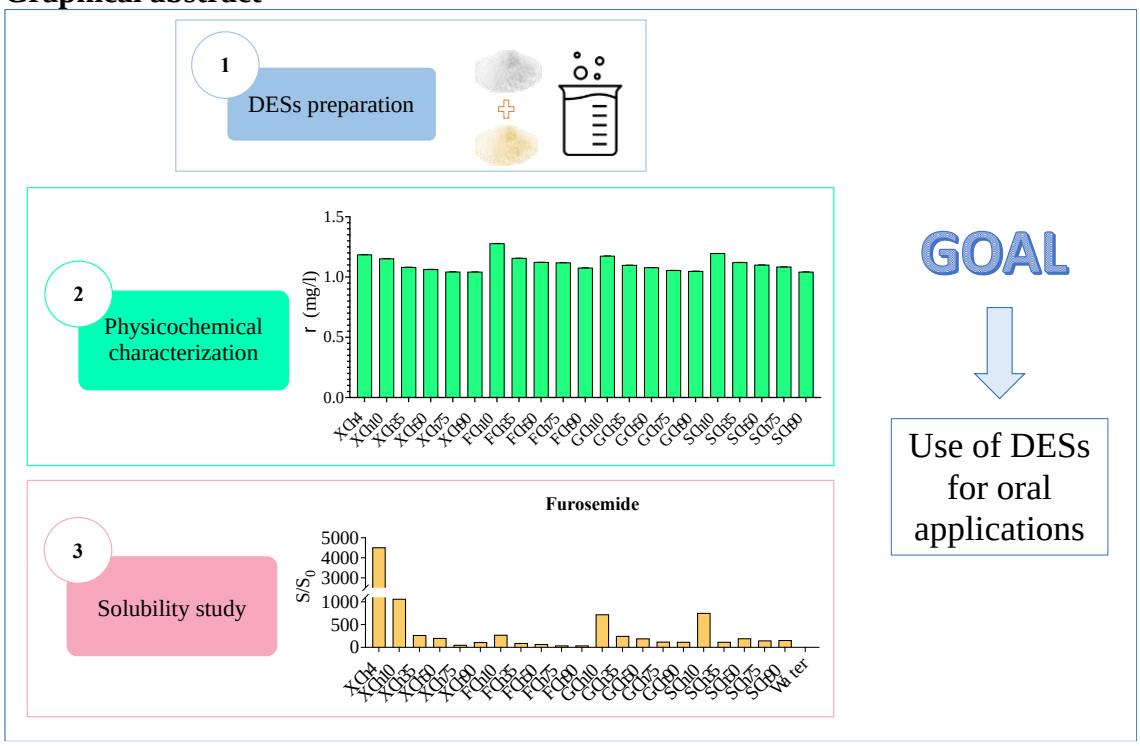
Abstract: Solubility is a critical parameter in drug formulation to achieve the desired therapeutical concentration. Most drugs are weak acids or bases and, therefore, exhibit low solubility and poor oral availability. The main aim of this work is the use of Deep Eutectic Systems (DESs) for improving the solubility of drugs in aqueous medium. In this case, we use DESs formed by choline chloride and sugars (xylitol, fructose, glucose and sorbitol) at different proportions of water. These compounds present low toxicity, and thus can be used in syrups or liquid formulations. Different physicochemical properties, such as density, refractive index, and surface tension, were obtained. In addition, a rheological study of the different systems was carried out. Finally, these DESs were applied to analyse the solubility of the following active principles: caffeine (Class I) and furosemide (Class IV) of the Biopharmaceutics Classification System (BCS). The selection of the drugs attends to different reasons. On one hand, we want to develop a new liquid formulation for model drug furosemide and, on the other hand, the study of caffeine, instead, will be used as a model for comparing purposes. Solubility results show that the systems that best solubilize caffeine are those with the highest water content; however, they do not reach the levels of solubility of pure water. On the other hand, for furosemide, a great increase in solubility was observed, especially for systems formed by xylitol and, fundamentally, in the system with the lowest water content. Obtaining an increase in solubility of up to 4530 times. These systems provide an opportunity to

improve the formulation of drugs in the liquid medium of active ingredients that are poorly soluble in an aqueous medium.

Keywords: Deep eutectic solvents, physicochemical properties, active pharmaceutical ingredients, solubility, rheological study, oral liquid formulation.

Graphical abstract



1. Introduction

Deep eutectic solvents (DESs) are mixtures composed of two or more solid or liquid components that form a eutectic mixture with a lower melting point than the individual components [1]. DESs are being widely studied since they possess excellent properties, including very low melting points, a wide range of polarity, good biodegradability, negligible vapour pressure or good toxicity profiles, among many others. As a result, these solvents can be used in numerous applications and are also safer for human health and the environment [2-6]. Specifically, due to the great solubilization capacity of these mixtures, they are used to extract high-value substances and solubilize natural products or poorly soluble drugs [7-11].

However, there are several limitations when using these solvents in a given application, and one of the principal problems is the high viscosity of the moieties. Often, these substances show viscosity values at room temperature that exceed 0.05 Pa·s in most of the DESs studied, even reaching 0.75 Pa·s [12]. Therefore, the viscosities are 50-750 times greater than that of water at room temperature. These high viscosity values have implications for the possible applications of DESs, including handling in simple operations such as the transfer of containers, and processes involving flow or cleaning becomes complex. In addition, their capacity in mass transfer processes or as a reaction medium can be reduced or limited. For this reason, in this work, the possibility of modulating the physicochemical properties of DESs, including viscosity, by adding water to the eutectic mixture was explored to develop DESs useful for pharmaceutical applications. We obtained several DESs formed by choline chloride and a sugar (glucose, sorbitol xylitol and fructose) with different water proportions and explored the effect of water content for a specific application, i.e., the use of the obtained DESs to solubilize poorly soluble drugs [13-16]. Various thermodynamic and transport properties at 25 °C were determined for these mixtures, such as density and refractive index (volumetric properties), surface tension (surface property) and viscosity (mass transport property). In total, 21 eutectic mixtures were prepared and tested.

The Biopharmaceutics Classification System (BCS) categorizes drugs into four classes (I-IV) based on their solubility and permeability [17]. Class II drugs show low solubility and high permeability, while Class IV drugs exhibit low solubility and low permeability. According to the BCS, Class II drugs possess a solubility lower than 1 mg/ml, while Class IV drugs exhibit a solubility lower than 0.1 mg/mL [18]. Examples of Class II drugs include flurbiprofen, naproxen, rifampicin or ketoconazole [19], and

examples of Class IV drugs include hydrochlorothiazide, griseofulvin, phenytoin, and spironolactone [19,20]. In general, low solubility can lead to poor bioavailability, as the drug may not dissolve well enough in the gastrointestinal fluids to be effectively absorbed by the body. Therefore, improving the solubility of drugs in these classes is an important goal in drug development [21].

The solubility of drugs can be increased through various techniques, including physical and chemical modifications of the drug, particle size reduction, crystal engineering, salt formation, solid dispersion, use of surfactants, complexation, and nanotechnology approaches, such as formulation into nanoparticles with high specific surface areas, which aids in solubility and increases dissolution rate or the use of polymers [22-25]. However, in this study, 21 eutectic mixtures were used to improve the dissolution of different pharmaceuticals. The study focused on the effect of water on the increase in solubility. In this case, the selected active pharmaceutical ingredients (APIs) are caffeine and furosemide. This selection considers the different behaviour shown by both APIs, allowing for a comparative study to be carried out; while caffeine is soluble in water (Class I) [26], furosemide belongs to Class IV of the BCS, with low aqueous solubility [27]. In addition, no commercial pharmaceutical presentations in liquid state of furosemide and caffeine-free base were found. The ultimate goal would be to develop liquid dosage forms for these active principles.

Caffeine is an alkaloid and xanthine that acts as an antagonist of adenosine receptors in the brain, which prevents the formation of adenosine, a chemical that promotes sleep, from binding to the receptors and causing drowsiness. Caffeine also increases the levels of dopamine and noradrenaline, two neurotransmitters that can improve mood and concentration [28]. This API stimulates cardiac muscle contraction and gastric acid secretion, increases gastrointestinal motility and relaxes smooth muscles [29].

Furosemide is used to treat fluid build-up and swelling caused by heart failure, liver disease, kidney disease, and other medical conditions. It belongs to a class of drugs called loop diuretics, which work by increasing the amount of urine produced by the kidneys and reducing the amount of fluid in the body [30]. It is typically taken orally in tablets and can be administered as an injection. The medication is usually taken once or twice daily, with or without food. It is important to take furosemide exactly as prescribed by a health care provider. This drug is a weak acid (pKa=3.8) and, as mentioned before, presents low solubility and permeability, so it is classified in Class IV of the BSC [31]. Its absorption can vary, and it is usually poor because of the restricted sites of absorption

[32]. The oral bioavailability is approximately 37-51% [33] or 60-70% with erratic absorption [31].

~~These drugs are very different.~~ On one hand, Caffeine is completely soluble in water, does not need to be increased in solubility and has been chosen as model of soluble drugs for comparing purposes. On the other hand, furosemide (class IV) is a very poorly soluble drug in water. In this paper we want to check if the solubility of furosemide is increased by using DESs and whether it can therefore be used in oral applications.

2. Experimental Section

2.1 Chemicals

Table 1 shows the components used to prepare the DESs that were tested later and used for the solubility study of the active principles. Chemicals were dried under vacuum for 24 hours prior to use.

Table 1. Information on the pure chemicals used in this study.

Chemical	CAS number	Molecular weight (g/mol)	Supplier	Purity (%)	Formula
Xylitol	87-99-0	152.15	Fagron	99.7%	$C_5H_{12}O_5$
Fructose	6347-01-9	180.16	Laboaragon	99-9%	$C_6H_{12}O_6$
Glucose anhydrous	50-99-7	180.16	Acofarma	99.2%	$C_6H_{12}O_6$
Sorbitol	50-70-4	182.17	Sigma–Aldrich	99%	$C_6H_{14}O_6$
Choline Chloride	67-48-1	139.63	Panreac	100%	$C_5H_{14}ClNO$
Caffeine anhydrous	58-08-2	194.19	Acofarma	99.8%	$C_8H_{10}N_4O_2$
Furosemide	54-31-9	330.74	Acofarma	99.2%	$C_{12}H_{11}ClN_2O_5S$

2.2. Preparation of deep eutectic solvents

In this study, several DESs were prepared by the combination of xylitol, fructose, glucose and sorbitol with choline chloride and water at different proportions. The components for each mixture were weighed using a Sartorius Entris 5201-1S balance (Göttingen, Germany) (uncertainty $\pm 10^{-1}$ g) and introduced in a vessel with constant stirring and heating in a water bath at 60-70 °C until a mixture of transparent and homogeneous appearance was obtained. After that, the eutectic mixtures were stored in darkness until use. Information related to the composition (molar ratio) and the final

molecular weight of the mixture can be found in Table 2. The average molecular weight of each mixture was calculated using the following equation [34]:

$$MW_{DES} = X_{ChCl} \cdot MW_{ChCl} + X_{Sugar} \cdot MW_{Sugar} + X_{water} \cdot MW_{water.} \quad (1)$$

where X is the mole fraction and MW is the molecular weight.

Table 2. Information on the prepared DESs.

DES	Abbreviation name	Composition (molar ratio)	Molecular weight (g/mol)
Xylitol: Choline Chloride: Water	XCh4	1:2:4	71.91
	XCh10	1:2:10	47.03
	XCh35	1:2:35	27.93
	XCh50	1:2:50	25.12
	XCh75	1:2:75	22.83
	XCh90	1:2:90	22.06
Fructose: Choline Chloride: Water	FCh10	2:1:10	52.30
	FCh35	2:1:35	29.74
	FCh50	2:1:50	26.41
	FCh75	2:1:75	23.72
	FCh90	2:1:90	22.80
Glucose: Choline Chloride: Water	GCh10	1:2:10	49.18
	GCh35	1:2:35	28.67
	GCh50	1:2:50	25.65
	GCh75	1:2:75	23.20
	GCh90	1:2:90	22.36
Sorbitol: Choline Chloride: Water	SCh10	1:2:10	49.34
	SCh35	1:2:35	28.72
	SCh50	1:2:50	25.69
	SCh75	1:2:75	23.22
	SCh90	1:2:90	22.38

2.3. Volumetric and rheological properties

Several properties, such as density, refractive index and surface tension, were measured at 25 °C for all studied DESs. Additionally, a rheological study was carried out with the intention of gaining more knowledge on the behaviour of these mixtures and determining how the inclusion of water affects it.

Density is an important property that provides information about intermolecular interactions in sugar-based DESs; in general, DESs present higher density values than water. In this case, this property was measured using a 10 mL pycnometer. The uncertainty in the measurement was estimated to be 0.001 mg/L.

The Abbemat-HP DR refractometer Kernchen (Gehrden, Germany) was used to determine the refractive indices at a 589.3 nm sodium D wavelength, denoted by nD. The uncertainty associated with this property is $5 \cdot 10^{-5}$.

The surface tensions (σ) were determined using a drop volume tensiometer, specifically the Lauda TVT-2 (Lauda-Königshofen, Germany). To maintain temperature consistency, a Lauda E-200 thermostat (Lauda-Königshofen, Germany) was utilized to control the sample temperature with an accuracy of ± 0.01 K. The uncertainty associated with the measured surface tensions is 0.2 $mN \cdot m^{-1}$.

To conduct a rheological investigation, the viscosity of the DESs under study was measured using a Brookfield rotational viscometer (DV-E) (Middleborough, USA). A shear rate sweep was conducted by varying the rotational speed from the highest (typically 60 rpm) to the lowest (0.3 rpm). The temperature of the sample was regulated at 25 °C using an immersion bath (Termotronic JPSELECTA) (Barcelona, Spain) with a precision of ±0.1 °C. Viscosity measurements were performed three times for accuracy.

Experimental viscosity data were utilized to obtain the corresponding shear stress (τ), which was then correlated using the Herschel-Bulkley model for non-Newtonian fluids as follows:

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

In this model, the relationship between shear stress and shear rate (D) is characterized through the parameters τ_0 (yield shear stress), k (consistency factor) and n (flow index). The coefficient of determination ($R^2 = 1 - \sum residual^2/(\bar{\tau} - \tau)^2$) and standard deviation ($S = (\sum(residual)^2/n - p)^2$) were also obtained from the mathematical model, where *n is* the number of experimental data points and *p* is the number of parameters used in the model. All properties have been measured in triplicate (n=3).

2.4 Solubility study

The solubility of caffeine and furosemide in the DESs under investigation was determined using a modified shake-flask method [35].

To quantify the solubility of the active APIs, a spectrum scan was conducted at various wavelengths to identify the maximum absorbance. Calibration curves were constructed using concentrations of 2.5, 5, 10, 15, 20, 30, and 40 mg/L caffeine and 2.5, 5, 7.5, 10, 15, 30, 50, and 60 mg/L furosemide. [36].

Next, supersaturated solutions were prepared in accordance with the standard guidelines for solution preparation, and visual inspection was used to confirm supersaturation. These solutions were stirred for 24 hours at a controlled temperature of 25 °C, protected from light, and rested for another 24 hours at 25 °C. Supersaturation was once again visually confirmed. The J.P. A select heater was employed for this study. Subsequently, the samples were centrifuged using a Biofuge Primo R centrifuge (Hanau, Germany) for 5 minutes at 5000 rpm. The supernatants were filtered using a 0.22 μm polyethersulfone syringe filter, and the concentration of APIs was measured using High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) on an Agilent 1220 DAD instrument (California, USA) and a C18 reversed-phase column Liquid Purple (ODS 5 mm x 250 x 4) from Analysis Vinílicos®. For caffeine, the isocratic mobile phase comprised 65% (v/v) acetic buffer (pH=4) and 35% (v/v) methanol. For furosemide, the isocratic mobile phase consisted of 50% (v/v) acetic buffer 1% and 50% (v/v) acetonitrile. The separation was performed using an injection volume of 20 μL at a flow rate of 1.0 mL/min. Under these conditions, the retention time was 6.5 min for caffeine and 5.5 min for furosemide. This study was carried out in triplicate (n=3).

2.6 Statistical analysis

The statistical analysis was conducted utilizing GraphPad Prism 9.0 software, employing the one-way ANOVA method and Tukey–Kramer honestly significant differences model. The null hypothesis (H0) posits that there are no significant differences among the groups, and therefore, they are equal. Conversely, the alternative hypothesis (H1) assumes that there are differences between groups. A confidence level of 95% was chosen, implying that if the p value is less than 0.05, the null hypothesis is rejected, and the alternative hypothesis is accepted.

3. Results and discussion

3.1. Preparation of DES

The studied deep eutectic solvents (XCh, FCh, GCh and SCh) formed a monophasic, homogeneous, clear and transparent solution. No precipitate was observed at any time at room temperature.

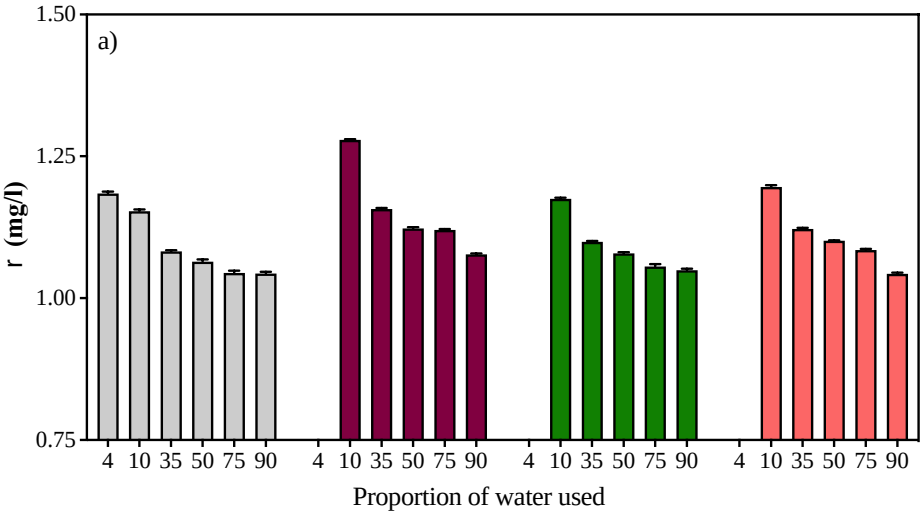
3.2 Physicochemical properties

In Figure 1, the density, refractive index, and surface tension are presented. All experimental values are gathered in Table S1 of the supplementary data. Additionally, the dependence of the three studied properties on water content expressed as weight percentage can be found in Figure S1 of the supplementary material.

Density serves as a valuable indicator of the compactness and molecular arrangement of materials and provides information for developing mass transfer processes, liquid–liquid equilibria, equations of state and predictive models [37,38]. Furthermore, the property of density plays a critical role in determining other derived thermodynamic properties and is an essential aspect of pharmaceutical formulation, as it provides critical information about the physical properties of drug substances and their formulations. This information can be used to optimize drug dosages, ensure physical stability, and detect the presence of counterfeit or substandard drugs [39]. In the context of this study, it was observed that the density of all analysed moieties at 25 °C decreased as the water content increased. Notably, the experimental density values were consistently higher than those of pure water. The highest density values are found for fructose DESs, followed by sorbitol, xylitol and glucose. A priori, higher density values are indicative of more compact molecular structures. It has been confirmed that the density depends greatly on the nature of the components of the eutectic mixture and their ability to form hydrogen bonds as well as on the composition or molar ratio. In relation to this, it appears that the presence of water, in increasing composition, profoundly affects the internal structure of the DESs and thus its network of hydrogen bonds, resulting in a decrease in its density. Previous studies have demonstrated, using NMR techniques, that the structure of the DESs is maintained at water concentrations below 50% v/v [40]. An increase in the relative amount of choline chloride in the composition leads to a decrease in solubility [12]. In this case, it is difficult to establish this correlation, since previous data on the density of DESs containing choline chloride and xylitol, sorbitol, glucose or fructose contain a different proportions of water [36] to what we studied in this work.

The refractive index is a volumetric property that provides complementary information to density. One of the primary uses of refractive index measurements in pharmaceutical formulation is to determine the purity and concentration of drug substances [41]. Furthermore, refractive index measurements can also be used to monitor the physical stability of pharmaceutical formulations [42]. Changes in refractive index can indicate the formation of crystals or other solid phases, which can affect the efficacy and safety of the drug product. In this case, all the values obtained are similar and are in the range of 1.38-1.47, presenting slightly higher values for the DESs containing fructose, followed by xylitol, glucose and finally sorbitol. As previously demonstrated, there is an inverse relationship between the refractive index and water content, in which a higher water content results in a lower refractive index [43].

Surface tension is a measure of the energy necessary to increase the surface area of a liquid by a unit amount (the force that keeps the molecules of a liquid together and prevents them from separating or spreading apart); thus, this property reflects the molecular attraction and interaction in the system [44]. Surface tension measurements are essential in pharmaceutical formulations because they are used to study the spreading and wetting behaviour of formulations on the skin surface, which directly impacts the rate and extent of drug absorption[45]. Another critical application is identifying the incompatibilities between different formulation components that could result in physical instabilities, such as aggregates, precipitation, or diminished efficacy and safety. Furthermore, surface tension measurements assist in monitoring the stability of pharmaceutical formulations by identifying any physicochemical changes that could occur over time, such as the formation of interfaces or phase separation, which could impact the drug product's long-term efficacy and stability. In this case, very similar values of surface tension are observed for DESs containing xylitol and sorbitol, while the values are slightly lower for DESs containing glucose and significantly lower if the DESs contain fructose. The behaviour with increasing water composition in the eutectic mixture does not follow a clear trend. With higher amounts of water, surface tension decreases in all cases except for DESs containing fructose, up to a certain amount of water in which interactions seem to slightly increase. In the case of sorbitol, this increase leads to values approximately equivalent to those of pure water. For DESs formed by glucose, there is no increase in the surface tension value, but rather, the values remain practically constant with a significant increase in the amount of water.



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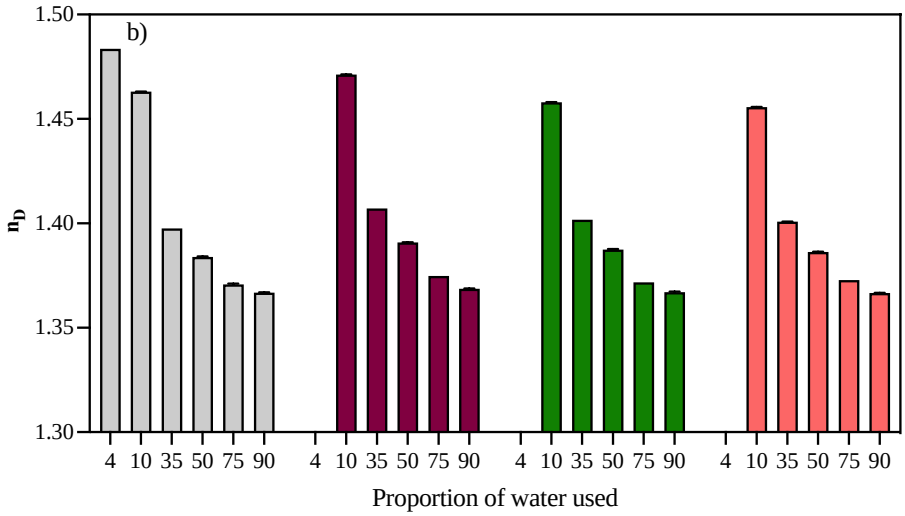

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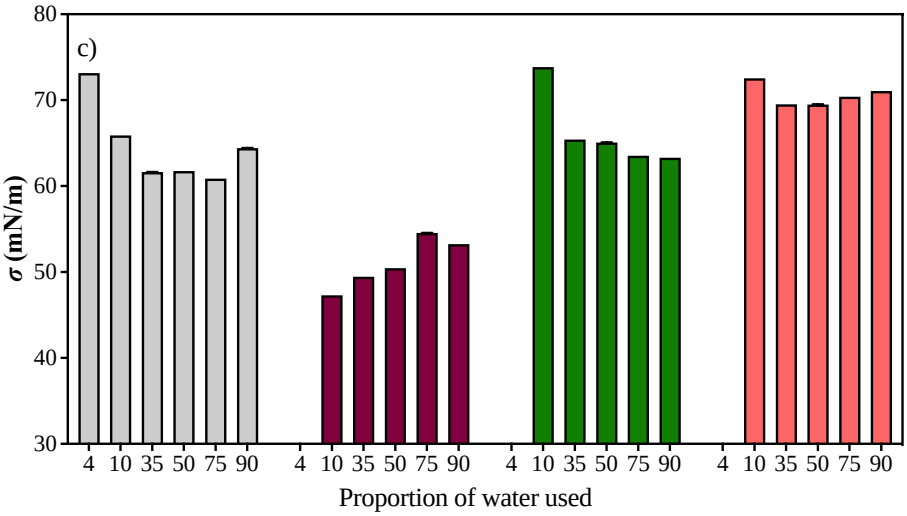

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283 Figure 1. a) Density, b) refractive index, and c) surface tension of studied DESs formed
284 by xylitol (), fructose (), glucose (), and sorbitol () with choline chloride at
285 25 °C.

Determining the rheological behaviour of DESs is of great importance in various fields, such as material science, chemical engineering, and environmental science, since the behaviour can provide insights into their structure and stability. A comprehensive evaluation of the rheological behaviour can aid in optimizing DES-based processes and developing new applications. It is well known that DESs offer numerous advantages over traditional solvents in terms of flow behaviour. The viscosity of DESs can be easily modulated by altering the composition of the components that comprise the solvent or by adding varying amounts of water. This flexibility can result in significant changes to the viscous behaviour of DESs, making them a versatile option for a range of applications [36].

The rheological behaviour of drug formulations is crucially important for a variety of reasons. Rheology provides valuable information on the flow and deformation behaviours of complex fluids, which are common in the pharmaceutical field [46]. This information is especially important during the physicochemical characterization of dosage forms at the formulation development stage. The rheological nature of a dosage form can directly affect the quality of the input (raw) material, the output (final) product, dose uniformity, filling efficiency, product stability, and overall health care cost [47].

Moreover, rheology plays a vital role in developing topical drug products in a quality by design approach [48].

Rheological measurements can also improve efficiency in processing and help formulators and end users find pharmaceutical products, including simple liquids, ointments, creams, pastes, suppositories, suspensions, and colloidal dispersing, emulsifying, and suspending agents [49] that are optimal for their individual needs.

The flow behaviour of the DESs under investigation was determined in this study. The experimental apparent viscosity of the DESs under investigation at a temperature of 25 °C is presented in Figure 2, whereas Figure 3 displays the rheograms. Table 3 presents the adjusted parameters obtained from the rheological analysis using Equation 2. Additionally, values of apparent viscosity are gathered in Table S2.

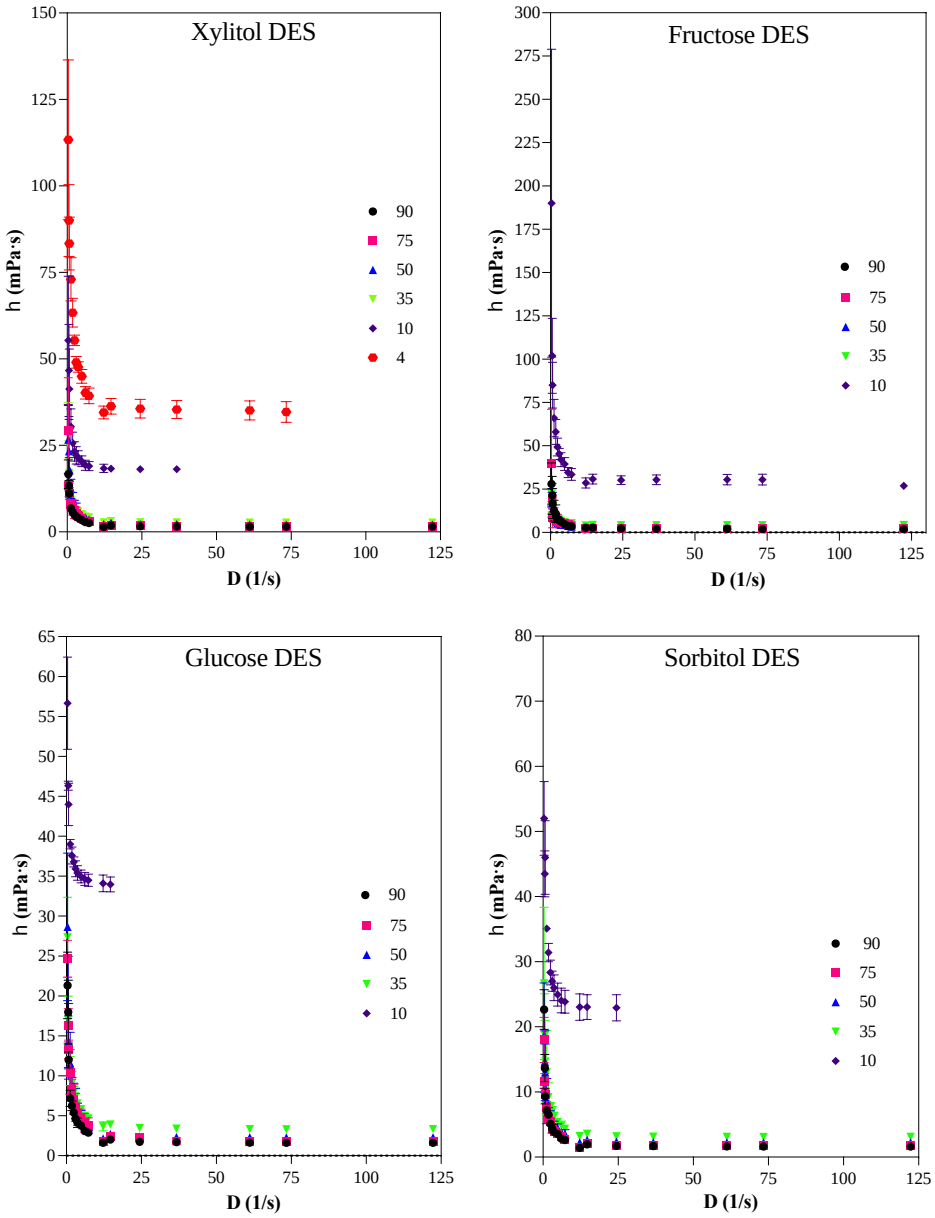

Figure 2. Apparent viscosity vs. shear rate for the studied DESs at 25 °C. 4 (●) experimental; 10 (◆) experimental; 35 (▼) experimental; 50 (▲) experimental; 75 (■) experimental; 90 (●) experimental; (—) correlated values.

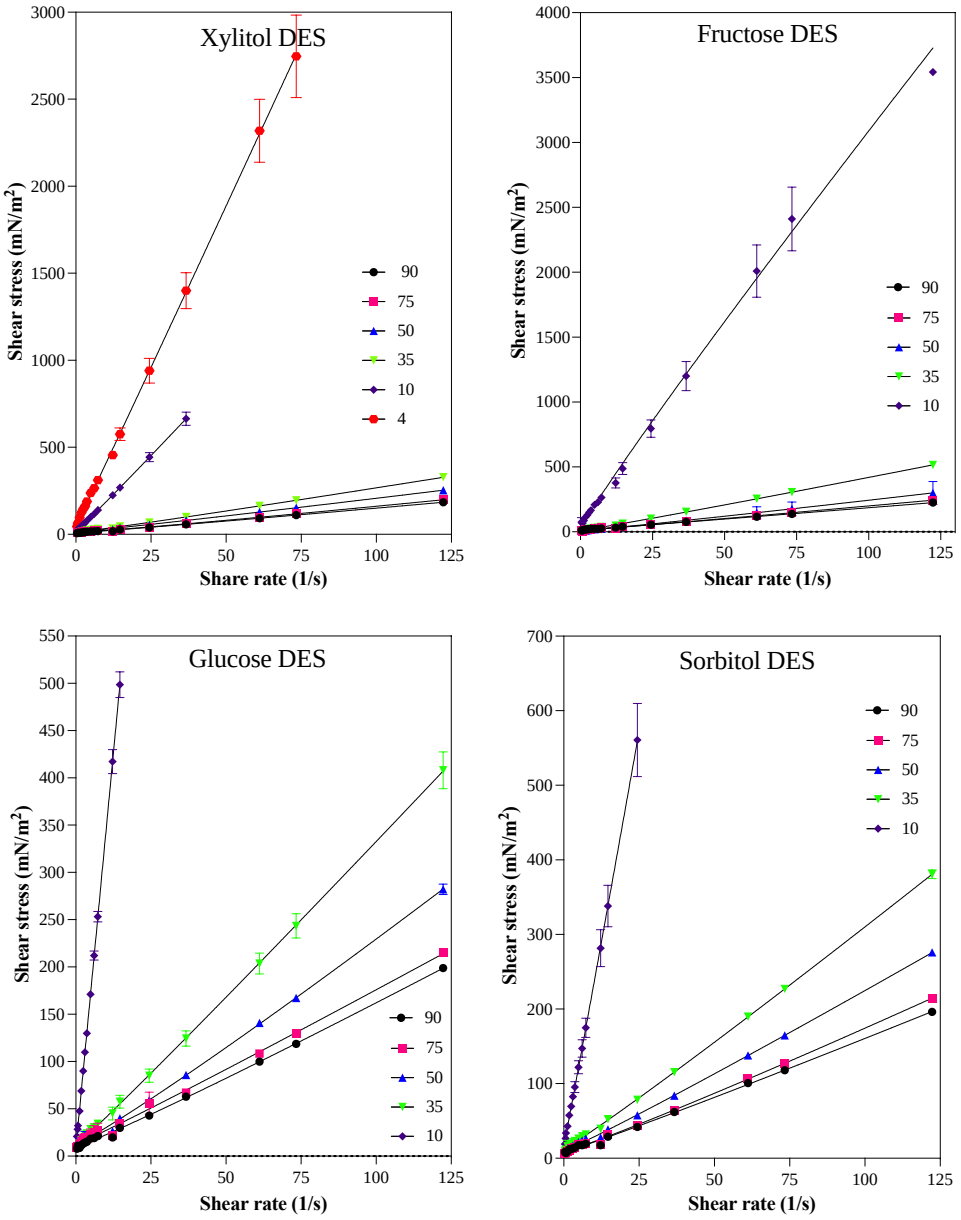

Figure 3. Shear stress vs. shear rate for the studied DESs at 25 °C. 4 (●) experimental; 10 (◆) experimental; 35 (▼) experimental; 50 (▲) experimental; 75 (■) experimental; 90 (●) experimental; (—) correlated values.

Table 3. Adjusted parameters, τ_0, k and n (Eq. 2) with their corresponding coefficient of determination, R^2 and standard deviation, s, a t 25 °C.

DES	τ_0 (mN/m^2)	k (g/s·m)	n	R^2	S
FChCl10	26.83	39.83	0.9429	0.9892	85.65
FChCl35	9.849	3.254	1.050	0.9963	8.287
FChCl50	8.400	1.710	1.069	0.9203	23.06
FChCl75	12.29	1.457	1.055	0.9912	5.894
FChCl90	13.95	1.570	1.020	0.9911	5.395
GChCl10	8.053	33.17	1.003	0.9989	5.044
GChCl35	11.32	2.715	1.037	0.9965	6.326
GChCl50	12.10	1.485	1.083	0.9968	4.082
GChCl75	12.23	1.347	1.042	0.9901	5.441
GChCl90	9.509	1.114	1.068	0.9976	2.469
SChCl10	16.33	20.75	1.021	0.9927	13.31
SChCl35	11.86	2.316	1.055	0.9985	3.793
SChCl50	9.653	1.663	1.056	0.9978	3.352
SChCl75	8.342	1.162	1.077	0.9973	2.857
SChCl90	8.283	1.178	1.056	0.9966	2.978
XChCl4	47.48	35.13	1.012	0.9930	68.28
XChCl10	17.90	15.54	1.035	0.9953	12.49
XChCl35	11.06	1.964	1.058	0.9984	3.426
XChCl50	13.48	1.413	1.068	0.9960	4.097
XChCl75	10.42	0.9420	1.102	0.9945	3.759
XChCl90	8.332	1.059	1.064	0.9975	2.354

The viscosity of the studied DESs decreases with the amount of water present in the mixture, and the highest values were found for DESs containing fructose, followed in order by those containing xylitol, glucose, and sorbitol.

The findings of this study demonstrate that all examined DESs exhibit non-Newtonian fluid behaviour, as indicated by their rheograms displaying shear thinning characteristics in which apparent viscosity decreases with increasing shear rate. Nonetheless, it was observed that as the shear rate increased, all DESs transitioned towards behaving as Newtonian fluids, with apparent viscosity values remaining almost

constant. This is further supported by the flow index parameter (n), which was found to be close to unity for all cases. Despite the amount of water introduced into the eutectic mixtures, the viscosity values of the mixtures are high. Referring to the highest shear rate values, viscosity values ranging from 34-1.6 mPa·s were found for all studied DESs, depending on the amount of water (a higher water proportion results in a lower viscosity of the eutectic mixture). This implies that for these high shear rate values, the viscosity of the mixtures with higher water content is 16 to 18 times higher than that of water at the same temperature. However, it should be noted that these are non-Newtonian fluids, unlike water, the viscosity of which does not depend on the shear force applied to induce flow.

The studied DES with a molar ratio of 10 of water can be a good alternative in liquid formulation because of a) the flow behaviour of these systems, which present a nonconstant viscosity that can be modified by the share rate. The viscosity of liquid formulations can affect several factors, such as pouring, swallowing or ease of administration [46,50]. b) Shear thinning: some non-Newtonian fluids can exhibit shear thinning behaviour, which means that the viscosity decreases as the shear rate or stress increases. This is important for liquid drugs because it can help improve swallowing and administration. When fluids are affected by shear, the formulation becomes less viscous and the flow is better through oral syringes or devices, which is better for patient compliance [46]. However, the use of non-Newtonian fluids can impact the stability of liquid formulations. Viscosity and shear thinning behaviour can affect the suspension or emulsion in liquid medium; for this reason, it is important to control the non-Newtonian behaviour to keep the distribution of drugs constant and ensure stability [51]. It must be noted that more stability studies should be carried out to determine the stability of formulations and to select adequate excipients and techniques; several excipients, such as thickeners or suspending agents, can be used to optimize the flow characteristics and improve patient acceptance [52].

3.3 Solubility study

Calibration curves were obtained representing AUC (mAU·s) versus concentration (mg/L). Additionally, the limit of detection, *LD*, and limit of quantification *LQ* were calculated using Eq. 3 and 4, respectively. These values are gathered in Table 4.

$$LD = \frac{x+3S}{m} \quad (3)$$

$$^{b}LQ = \frac{x+10S}{m} \quad (4)$$

where m is the slope and x and S are the average and the deviation of the blank, respectively.

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*.

API	**Slope calibration Line c in c** (mg/L)	**λ (Abs_{max}) (nm)**	**R^2**	**LD**	**LQ**
Caffeine	24.135	254	0.999	0.509	1.698
Furosemide	17.483	341	0.999	0.242	0.806

The main obstacle in designing oral dosage forms lies in achieving sufficient bioavailability. Various factors, such as aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, presystemic metabolism, and susceptibility to efflux mechanisms, influence oral bioavailability. Poor solubility and low permeability are the most common causes of low oral bioavailability[21].

Solubility is a crucial factor that influences desired drug concentrations in systemic circulation for both oral and parenteral dosage forms. Poor aqueous solubility is a significant challenge encountered when developing new chemical entities and generics, particularly for poorly water-soluble drugs that may require high doses to reach therapeutic plasma concentrations after oral administration. For any drug to be absorbed, it must be present as an aqueous solution at the site of absorption, and water is the solvent of choice for liquid pharmaceutical formulations. Most drugs are weakly acidic or weakly basic, and their poor aqueous solubility is a frequent cause of low oral bioavailability. Therefore, improving drug solubility is a crucial strategy in formulating pharmaceutical dosage forms for effective therapeutic outcomes [24].

In this work, several DESs were used to analyse the solubility of caffeine and furosemide with the aim of finding what systems can be used in liquid formulations.

In Table 5, values of the mean of the experimental maximum concentration of caffeine and furosemide, as well as the corresponding dose number (D_0) and pH, are shown. Moreover, $D_0 = M/V_0/S$; M is the highest single-unit dose strength of each API (80 mg for furosemide and 300 mg for caffeine), V_0 is the initial volume of water (250

mL), and *S* is the solubility; the drug is considered highly soluble if the $D_0 < 1$ [27]. Furthermore, the increase in DES solubility (S) in relation to the solubility of APIs in water (S_0) has been evidenced through the ratio (S/S_0), as shown in Figure 4.

Table 5. Solubility (n=3), *s* (mg/L), dose number and pH results with their corresponding standard deviation for caffeine and furosemide in the studied DESs and water.

Solvent/API	***s*** **(mg/L)**		***D₀***		**pH**	
	Caffeine	**Furosemide**	**Caffeine**	**Furosemide**	**Caffeine**	**Furosemide**
XCh4	658 ± 27.8	4408 ± 161	1.82	0.07	6.65 ± 0.4	2.70 ± 0.26
XCh10	1411 ± 37.3	1040 ± 1.37	0.85	0.31	6.61 ± 0.1	2.76 ± 0.09
XCh35	4003 ± 217	265 ± 17.6	0.30	1.21	6.50 ± 0.0	3.76 ± 0.16
XCh50	4232 ± 215	203 ± 8.42	0.28	1.58	6.47 ± 0.2	3.93 ± 0.03
XCh75	10805 ± 184	57 ± 1.77	0.11	5.61	6.54 ± 0.1	3.95 ± 0.06
XCh90	11691 ± 181	115 ± 1.99	0.10	2.78	5.98 ± 0.1	4.42 ± 0.09
FCh10	3015 ± 64.7	271 ± 7.77	0.40	1.18	5.08 ± 0.1	3.59 ± 0.06
FCh35	9591 ± 320	93 ± 1.00	0.13	3.44	5.15 ± 0.2	3.76 ± 0.06
FCh50	11576 ± 696	73 ± 2.52	0.10	4.38	5.47 ± 0.0	3.64 ± 0.12
FCh75	13584 ± 496	43 ± 0.58	0.09	7.44	5.62 ± 0.1	3.85 ± 0.13
FCh90	11868 ± 308	42 ± 0.58	0.10	7.62	5.69 ± 0.2	3.79 ± 0.08
GCh10	1657 ± 23.9	709 ± 16.7	0.72	0.45	5.28 ± 0.3	2.98 ± 0.07
GCh35	6479 ± 284	247 ± 11.2	0.19	1.30	6.74 ± 0.1	4.12 ± 0.11
GCh50	8029 ± 426	194 ± 3.00	0.15	1.65	5.82 ± 0.1	4.27 ± 0.03
GCh75	11487 ± 151	124 ± 3.70	0.10	2.58	6.54 ± 0.0	4.36 ± 0.03
GCh90	12473 ± 93.3	119 ± 6.50	0.10	2.69	5.98 ± 0.1	4.00 ± 0.03
SCh10	1450 ± 3.20	738 ± 51.6	0.83	0.43	6.73 ± 0.2	2.70 ± 0.01
SCh35	6454 ± 385	120 ± 8.29	0.19	2.67	6.61 ± 0.2	3.82 ± 0.12
SCh50	8416 ± 293	197 ± 3.85	0.14	1.62	6.34 ± 0.1	3.79 ± 0.29
SCh75	10774 ± 184	148 ± 1.56	0.11	2.16	6.81 ± 0.3	4.10 ± 0.08
SCh90	11833 ± 628	158 ± 9.55	0.10	2.03	6.16 ± 0.1	4.21 ± 0.38
Water	17139 ± 965	0.973 ± 0.2	0.07	328.88	5.62 ± 0.4	4.76 ± 0.03

The statistical analysis is gathered in Table S3. For caffeine, it has been observed that all DESs analysed present significant differences with water. For furosemide, several DESs do not present significant differences with water, such as XCh75, XCh90, FCh35, FCh50, FCh75, FCh90 or GCh90. These results are in accordance with the values

presented in Table 5 because the differences that were not found correspond to DESs that present lower solubility of furosemide. Additionally, it is important to highlight that, for both APIs, there are some systems in which these significant differences are not found among DESs.

For caffeine, it is observed that the highest solubility values are presented for the DESs formed by fructose, followed by glucose, sorbitol and xylitol. Furthermore, in all cases, due to the hydrophilic nature of caffeine, it is observed that the solubility values increase as the amount of water in the system increases. Although there is an increase in solubility in the systems that present more water, none of systems reach the solubility of caffeine in water. That is why all ratios of these mixtures are below that of water, as shown in the graph presented in Figure 4, and the highest ratio value was observed for the following systems: XCh90, FCh75, GCh75, GCh90 and SCh90 with values of 0.7 for all cases except for FCh75 with 0.8 value.

The effect that different sugars (mono- and disaccharides) have on the solubility of caffeine has been studied. It was found that sugars increase solubility in the monomeric state; however, they decrease solubility in oligomeric forms, that is, they act as selective hydrotropes [53]. Additionally, caffeine solubility has been studied in different DESs, and as we observed in our study, the solubility of this API decreases in DESs with respect to water[54].

All dose numbers (D_0) of caffeine present values lower than 1, which indicates that it is a highly soluble compound in water (with the lowest value, 0.07). These values vary according to the water content, and higher numbers were obtained for DESs with a lower amount of water and, therefore, those that solubilize caffeine less. The highest D0 value was found for the xylitol family, specifically for XCh4, which exhibited a lower solubility in water. Furthermore, for the pH values of the DESs and caffeine solution, all the mixtures showed pH values between 5-6.8. The pKa value for caffeine is 8.3, so this chemical should be ionized. For DESs that contain caffeine, the influence of pH becomes apparent; given its pKa, the solubility must decrease with increasing pH.

On the other hand, if the results for furosemide are analysed, the trend is completely opposite to that of caffeine, which is logical due to the more lipophilic nature of this API. The trend observed in all the groups is that furosemide reaches higher solubilities in systems with a lower amount of water. In this case, the group with the highest solubility value, which could be considered the star group for this API, is that of the mixtures formed by xylitol and choline chloride. The next group with high values is

that of sorbitol, followed by glucose and, finally, fructose. The highest ratio (S/S_0) was found for XCh4, which increased the solubility of furosemide by 4530-fold. Other systems, such as XCh4, SC10 and GCh10, can increase solubility by 758- and 728-fold.

The number dose (D0) values for furosemide are usually greater than 1. This clearly indicates that this drug shows low solubility, as indicated by the BCS. However, for several systems, this value is below 1, indicating that the solubility is increased. This occurs for XCh4, XCh10, GCh10 and SCh10, i.e, groups that possess a lower water content and show a greater solubility of this API.

Furosemide presents higher solubility in DESs than in water because of its higher chemical affinity. The molecular structure of furosemide has two nitrogen atoms that are both donors and acceptors (sulfonamide and amine) and another three hydrogen atoms (an acid that acts as a donor and acceptor and an ether that is only an acceptor). Water molecules cannot solubilize furosemide because of its nonpolar structure. The much larger DES molecules, with multiple hydrogen bonding sites, facilitate solubility through interactions with polar groups on furosemide [36].

Furthermore, for furosemide, it is essential to discuss its pH-dependent solubility due to its acidic nature (furosemide is more soluble in water at pH 7-8 than at 1-4). It presents two pKa1=3.8 and pKa 2 =7.5, and therefore, this chemical should be ionized in studies of DES. Its solubility mechanism and relation to pH are now clear.

For the pH values obtained for the DESs with furosemide, it is generally observed the pH values increase as the water content increases. In addition, there is a direct relationship between the solubility of furosemide, the pH and the water content. In all groups, a greater solubility at lower pH and less water were observed in the DESs.

For the xylitol DESs, the values vary between 2.7 and 4.4, increasing the pH as the water content increases. In addition, a greater solubility is observed for XCh4, that is, the one with a lower water content and lower pH values. For the fructose DES, there is no significant variation since they all range between 3.59-3.79. The highest solubility was observed for FCh10 with less water and lower pH. For the glucose DESs, the pH ranges between 2.98-4.36, presenting the lowest pH value for the DES with the lowest water content and greater solubility. Finally, for sorbitol, the lowest pH value was found for SCh10 and the highest for SCh90 with 4.21 showing greater solubility in the one with less water and greater solubility. This pH dependence agrees with our previous results and the results obtained in this study [27].

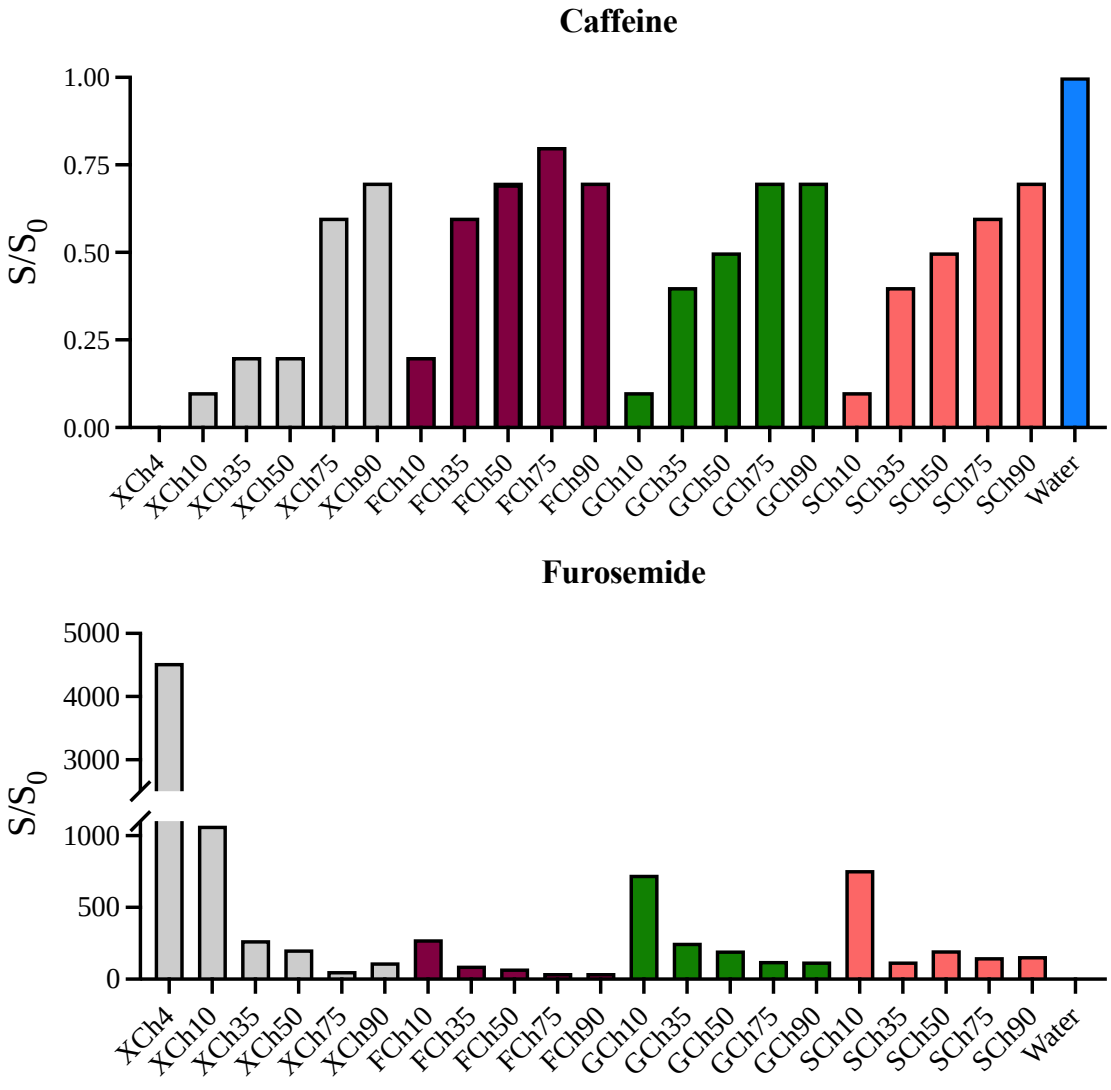

Figure 4. Comparison of the solubility results obtained for caffeine and furosemide in DESs and water.

4. Utilizing DES components in the formulation of drugs

There are many reasons that several sugars, such as xylitol, fructose, glucose, or sorbitol, are used for pharmaceutical formulations. First, sugars can be used as sweeteners, which can increase the palatable of medications and make them easier to swallow, especially for children. Second, these excipients can stabilize liquid medications since they prevent the active ingredients from breaking down or separating over time [55]. Furthermore, some sugars, such as sorbitol, can be used to keep the medication moist and prevent it from drying; thus, these sugars do not cause a decrease in effectiveness. Finally, sugars can be used to adjust the viscosity of liquid medications, which can affect the medication flow and absorption pathway [56,57].

Importantly, the use of sugars can produce different effects in the body. Normally, when drugs are formulated, one sugar or the other is selected depending on what is desired. In this case, the compounds that best solubilize furosemide are the DESs derived from xylitol, followed by those from sorbitol and glucose. Glucose or fructose are in the group of caloric sweeteners; however, xylitol and sorbitol are low-caloric monosaccharides [58]. These two, sorbitol and xylitol, do not raise blood sugar levels as much as the rest of sweeteners [59].

Considering the obtained results in this work and with our previous studies [38], these mixtures could be used in liquid formulation drugs; however, more studies are needed to develop drugs using these mixtures.

5. Conclusions

This work shows the development and characterization of 21 deep eutectic solvents (DESs) formed by sugars (glucose, sorbitol, xylitol, and fructose) at different water proportions have been prepared.

From a physicochemical point of view, it has been observed that the analysed systems vary their properties depending on the concentration of water in the medium, due to the intermolecular interactions that are formed. The rheological study shows that the systems exhibit non-Newtonian behaviour, which can be useful in oral applications. In addition, these systems have been shown to improve the solubility of furosemide (more than 4500 times with respect to solubility in water). This solubility value leads to a drug administration volume of approximately 18 ml for adults and 9 ml for paediatric doses (80 and 40 mg of API, respectively). Despite its high viscosity and considering the rest of the physicochemical properties of these DESs (density approximately 20% higher than that of water and surface tension similar to that of water) as well as the characteristics of palatability, sweetness and low caloric value, XCh4 could be a good candidate for further studies on the liquid-state formulation of furosemide.

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.

Declaration of generative AI in scientific writing

During the preparation of this work the authors used ChatGPT and Google Translator Service in order improve readability and language. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Supplementary material

Table S1. Density, refractive index and surface tension of prepared DESs at 25ºC.

DES	ρ (g/ml)	nD	σ (mN/m)
XCh4	1.186	1.483597	73.16
XCh10	1.153	1.463064	65.90
XCh35	1.082	1.397572	61.63
XCh50	1.064	1.383846	61.75
XCh75	1.044	1.370774	60.87
XCh90	1.043	1.366884	64.43
FCh10	1.279	1.471231	47.30
FCh35	1.157	1.407167	49.45
FCh50	1.123	1.390811	50.44
FCh75	1.120	1.374817	54.54
FCh90	1.077	1.368712	53.25
GCh10	1.175	1.458004	73.85
GCh35	1.099	1.401739	65.42
GCh50	1.079	1.387369	65.08
GCh75	1.056	1.371770	63.54
GCh90	1.049	1.367031	63.31
SCh10	1.196	1.455710	72.55
SCh35	1.122	1.400797	69.51
SCh50	1.101	1.386306	69.50
SCh75	1.085	1.372850	70.40
SCh90	1.043	1.366639	71.07

741 Table S2. Average apparent viscosity, η, of prepared DESs at 25ºC.

	η (mPa·s)										
Shear rate	XCh4	XCh10	XCh35	XCh50	XCh75	XCh90	FCh10	FCh35	FCh50	FCh75	FCh90
122.30			2.685	2.072	1.623	1.508	26.84	4.215	2.449	2.006	1.850
73.38	34.67		2.657	2.083	1.607	1.500	30.44	4.173	2.430	2.003	1.887
61.15	35.13		2.670	2.100	1.613	1.517	30.45	4.183	2.443	2.020	1.903
36.69	35.37	18.11	2.707	2.200	1.647	1.580	30.33	4.187	2.413	2.080	2.080
24.46	35.60	18.13	2.770	2.280	1.710	1.630	30.17	4.170	2.450	2.200	2.240
14.68	36.30	18.28	3.117	2.633	1.983	1.867	30.80	4.317	2.633	2.567	2.767
12.23	34.50	18.36	2.760	2.220	1.460	1.413	28.60	3.940	2.060	2.200	2.640
7.34	39.33	19.00	4.233	3.567	2.967	2.567	33.50	5.233	3.367	4.067	3.467
6.12	40.20	19.27	4.433	4.233	3.167	2.833	34.60	5.333	3.733	4.500	3.800
4.89	44.97	20.40	5.133	4.933	3.833	3.400	39.47	6.067	4.333	5.100	4.767
3.67	47.67	21.40	5.800	5.400	4.333	4.000	42.00	6.667	4.467	5.533	6.200
3.06	49.00	22.10	6.233	6.633	5.433	4.433	45.33	7.467	4.900	5.933	7.200
2.45	55.33	23.27	6.700	7.400	6.067	4.900	49.33	8.300	5.300	7.200	7.700
1.83	63.33	25.73	8.133	9.600	6.933	5.733	58.00	9.200	6.433	8.267	10.80
1.22	73.00	30.40	9.200	12.40	7.800	6.800	66.00	11.00	8.40	10.00	12.60
0.73	83.33	41.33	13.67	18.00	11.00	11.00	85.00	14.33	10.33	8.33	16.67
0.61	90.00	46.67	17.00	23.33	13.33	13.33	102.0	17.67	17.00	19.67	21.33
0.37	113.3	55.33	25.33	26.67	29.33	16.67	190.0	22.00	20.00	40.00	28.00

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743 Table S2. Continued.

	η (mPa·s)									
Shear rate	GCh10	GCh35	GCh50	GCh75	GCh90	SCh10	SCh35	SCh50	SCh75	SCh90
122.3		3.337	2.308	1.762	1.626		3.113	2.256	1.754	1.604
73.38		3.317	2.280	1.763	1.617		3.093	2.243	1.737	1.607
61.15		3.330	2.303	1.773	1.633		3.103	2.253	1.740	1.647
36.69		3.387	2.340	1.827	1.713		3.147	2.287	1.760	1.687
24.46		3.480	2.420	2.293	1.750	22.93	3.200	2.350	1.810	1.710
14.68	33.97	3.917	2.717	2.383	2.033	23.05	3.567	2.633	2.117	1.967
12.23	34.11	3.680	2.160	1.780	1.613	23.04	3.220	2.260	1.480	1.420
7.34	34.50	4.667	3.667	3.733	2.900	23.85	4.333	3.567	2.767	2.633
6.12	34.67	5.000	3.867	4.100	3.100	24.05	4.833	3.867	3.100	2.833
4.89	34.97	5.833	4.867	4.700	3.767	24.95	5.367	4.433	3.633	3.533
3.67	35.40	6.533	5.400	5.200	4.133	26.00	6.267	4.867	4.000	3.867
3.06	35.93	7.433	6.567	6.033	4.633	27.00	7.033	5.567	4.467	4.333
2.45	36.80	7.900	7.203	7.067	5.400	28.35	7.700	6.200	5.400	5.100
1.83	37.60	9.067	8.400	8.267	6.267	31.40	9.200	6.800	5.867	6.533
1.22	39.00	10.60	11.60	10.47	7.200	35.10	13.00	8.600	7.600	7.200
0.73	44.00	13.67	14.33	13.33	12.00	46.00	14.67	13.00	9.667	9.333
0.61	46.33	17.00	18.00	16.33	18.00	43.50	18.67	14.67	11.67	13.67
0.37	56.67	27.33	28.67	24.67	21.33	52.00	26.67	18.67	18.00	22.67

744 Table S3. *p* values for the solubility statistical analysis for caffeine and furosemide

Caffeine	Water	XCh4	XCh10	XCh35	XCh50	XCh75	XCh90	FCh10	FCh35	FCh50	FCh75	FCh90	GCh10	GCh35	GCh50	GCh75	GCh90	SCh10	SCh35	SCh50	SCh75	SCh90
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh4	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh10	<0.0001	0.6289	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh35	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh50	<0.0001	<0.0001	<0.0001	>0.9999	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh75	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh90	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3382	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh10	<0.0001	<0.0001	0.0006	0.1769	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh35	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0282	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh50	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.5874	>0.9999	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh75	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-
FCh90	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1011	>0.9999	<0.0001	<0.0001	>0.9999	0.0002	-	-	-	-	-	-	-	-	-	-	-
GCh10	<0.0001	0.1630	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0072	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-
GCh35	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-
GCh50	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	<0.0001	<0.0001	<0.0001	<0.0001	0.0010	-	-	-	-	-	-	-	-
GCh75	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.7817	>0.9999	<0.0001	<0.0001	>0.9999	<0.0001	0.9994	<0.0001	<0.0001	<.0001	-	-	-	-	-	-	-
GCh90	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.5620	<0.0001	<0.0001	0.3175	0.0688	0.9061	<0.0001	<0.0001	<0.0001	0.1794	-	-	-	-	-	-
SCh10	<0.0001	0.5389	>0.9999	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-
SCh35	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	0.0008	<0.0001	<0.0001	<0.0001	-	-	-	-
SCh50	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0399	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9993	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-
SCh75	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	0.2823	<0.0001	0.0371	0.5167	<0.0001	0.0793	<0.0001	<0.0001	<0.0001	0.7188	0.0002	<0.0001	<0.0001	<0.0001	-	-
SCh90	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1326	>0.9999	<0.0001	<0.0001	>0.9999	0.0001	>0.9999	<0.0001	<0.0001	<0.0001	0.9999	0.8561	<0.0001	<0.0001	<0.0001	0.1051	-

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746 Table S3. Continued

Furosemide	Water	XCh4	XCh10	XCh35	XCh50	XCh75	XCh90	FCh10	FCh35	FCh50	FCh75	FCh90	GCh10	GCh35	GCh50	GCh75	GCh90	SCh10	SCh35	SCh50	SCh75	SCh90
Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh4	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh10	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh35	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh50	<0.0001	<0.0001	<0.0001	0.8756	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh75	0.9504	<0.0001	<0.0001	<0.0001	0.0023	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XCh90	0.0508	<0.0001	<0.0001	0.0015	0.3382	0.9260	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh10	<0.0001	<0.0001	<0.0001	>0.9999	0.7709	<0.0001	0.0008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh35	0.2657	<0.0001	<0.0001	0.0001	0.0713	0.9997	>0.9999	<0.0001	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh50	0.7001	<0.0001	<0.0001	<0.0001	0.0114	>0.9999	0.9975	<0.0001	>0.9999	-	-	-	-	-	-	-	-	-	-	-	-	-
FCh75	0.9980	<0.0001	<0.0001	<0.0001	0.0005	>0.9999	0.6859	<0.0001	0.9815	>0.9999	-	-	-	-	-	-	-	-	-	-	-	-
FCh90	0.9985	<0.0001	<0.0001	<0.0001	0.0005	>0.9999	0.6634	<0.0001	0.9773	>0.9999	>0.9999	-	-	-	-	-	-	-	-	-	-	-
GCh10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-	-
GCh35	<0.0001	<0.0001	<0.0001	>0.9999	0.9963	<0.0001	0.0100	>0.9999	0.0011	0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-	-	-	-	-
GCh50	<0.0001	<0.0001	<0.0001	0.7153	>0.9999	0.0056	0.5247	0.5789	0.1405	0.0258	0.0013	0.0012	<0.0001	0.9725	-	-	-	-	-	-	-	-
GCh75	0.0228	<0.0001	<0.0001	0.0038	0.5324	0.7905	>0.9999	0.0021	>0.9999	0.9773	0.4790	0.4566	<0.0001	0.0236	0.7296	-	-	-	-	-	-	-
GCh90	0.0369	<0.0001	<0.0001	0.0022	0.4129	0.8804	>0.9999	0.0012	>0.9999	0.9932	0.6021	0.5789	<0.0001	0.0143	0.6099	>0.9999	-	-	-	-	-	-
SCh10	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	>0.9999	<0.0001	<0.0001	<0.0001	<0.0001	-	-	-	-	-
SCh35	0.0309	<0.0001	<0.0001	0.0027	0.4566	0.8499	>0.9999	0.0015	>0.9999	0.9889	0.5556	0.5324	<0.0001	0.0173	0.6559	>0.9999	>0.9999	<0.0001	-	-	-	-
SCh50	<0.0001	<0.0001	<0.0001	0.7642	>0.9999	0.0044	0.4715	0.6330	0.1179	0.0208	0.0010	0.0009	<0.0001	0.9827	>0.9999	0.6785	0.5556	<0.0001	0.6021	-	-	-
SCh75	0.0020	<0.0001	<0.0001	0.0404	0.9605	0.2661	0.9999	0.0236	0.9506	0.6021	0.0984	0.0909	<0.0001	0.1786	0.9937	>0.9999	>0.9999	<0.0001	>0.9999	0.9889	-	-
SCh90	0.0007	<0.0001	<0.0001	0.0886	0.9947	0.1405	0.9970	0.0539	0.8331	0.3908	0.0454	0.0416	<0.0001	0.3254	0.9997	0.9999	0.9991	<0.0001	0.9995	0.9993	>0.9999	-

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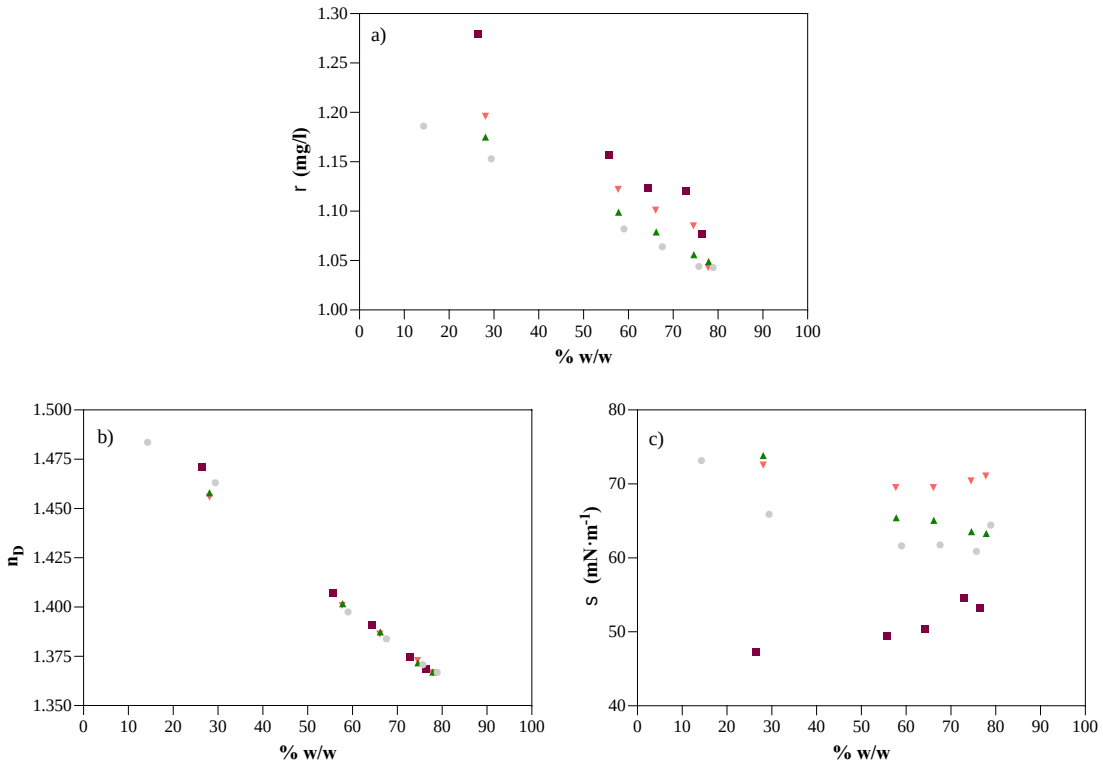

Figure S1. a) Density, b) refractive index, and c) surface tension of studied DESs formed by xylitol (), fructose (), glucose (), and sorbitol () with choline chloride versus % water weight/weight.