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ORIGINAL ARTICLE

Choline chloride-based deep eutectic solvent as an inhibitor of metalloproteases (collagenase and elastase) in cosmetic formulation

Filipe Smith Buarque[1] · **Silas Arandas Monteiro e Silva**[2] · **Bernardo Dias Ribeiro**[1]

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Abstract
Green chemistry and engineering are potential alternatives for achieving higher sustainability and lower generation of hazardous compounds in chemical product design, production, and use. Deep Eutectic Solvents (DES) are characterized as green solvents and have become increasingly attractive due to their characteristic design solvents. In this work, two DES (choline chloride (ChCl)/glycerol and ChCl/Urea), aqueous solutions of the DES-forming components, and green tea extracts obtained with DES were used as anti-ageing active in cosmetic products using in vitro tests to inhibit extracellular matrix metalloproteases (such as collagenase and elastase). Finally, the stability of the formulations with DES as a cosmetic active was also evaluated. The results showed that DES based on ChCl/Urea and ChCl/glycerol exhibited remarkable inhibition values of collagenase (91.1 and 92.7%, respectively) and elastase (49.8 and 45.7%, respectively). However, pure urea displayed better inhibition values (66%) for elastase, possibly due to its direct contribution to intramolecular hydrogen bonds. ChCl/glycerol showed remarkable stability in the average cube diameter values, which may indicate no change in the conformation of the micellar structure of the cosmetic formulation. Moreover, the formulation containing this DES remained stable at room temperature. Given the remarkable results, DES can be applied in cosmetic products for anti-ageing purposes.

Keywords DES · Green solvents · Metalloproteases inhibitors · Formulation skin care

Introduction

The search for new environmentally friendly solvents constitutes one of the most attractive topics in green and analytical chemistry (Płotka-Wasylka et al. 2017). In the last decade, DESs have been reported as alternative solvents with some advantages, such as reducing or eliminating the use of hazardous organic solvents (Abbott et al. 2004; Zhang et al. 2012; Ruesgas-Ramón et al. 2017; Huang et al. 2019). These new solvents are simpler to prepare and do not need complex purification schemes (Abbott et al. 2004; Zhang et al. 2012; Hayyan et al. 2013).

DES is the association of two or more compounds by hydrogen bonds (acceptor and donor) (Abbott et al. 2003; Durand et al. 2016) and is considered environmentally acceptable due to low volatility, low vapor pressure, high thermal stability, non-toxicity, biocompatibility, biodegradability, and nonflammability (Liu et al. 2019; Altunay and Elik 2022a). The term "solvent design" describes how their physical–chemical properties can be adapted by manipulating the chemical structure. Therefore, it is possible to synthesize the DES for a specific application (Martins et al. 2019; Altunay and Elik 2022a). Due to this remarkable property, these compounds can be applied in health-related industries such as pharmaceuticals and cosmetics (Smith et al. 2014; Macário et al. 2019; Altunay and Elik 2022b). In this context, DES could be applied in an action against skin aging. This is the body organ with the most significant exposure to solar radiation, pollution, and microorganisms, accumulating much damage over time. Therefore, several structural

✉ Bernardo Dias Ribeiro
bernardo@eq.ufrj.br

Filipe Smith Buarque
filipesmih@eq.ufrj.br

[1] Biochemical Engineering Department, School of Chemistry, Federal University of Rio de Janeiro, Av. Athos da Silveira Ramos, 149, Ilha do Fundão, Rio de Janeiro 21941-909, Brazil

[2] Faculty of Pharmaceutical Sciences, State University of Campinas, Rua Cândido Portinari, Cidade Universitária, São Paulo 13083871, Brazil

and morphological skin functions deteriorate (Zouboulis and Makrantonaki 2011; Pérez-Sánchez et al. 2018).

Neutrophils are the main ones responsible for elastase production when induced to an inflammatory response by ultraviolet radiation, promoting a higher production of metalloproteases. The metalloproteases of the extracellular matrix of the skin are responsible for the degradation of structural proteins of the skin. These alterations cause wrinkles and flaccidity in the skin (Klein and Bischoff 2011; Sbardella et al. 2012). One way to combat these wrinkle formations is the development of a cosmetic active to inhibit these metalloproteases (Sharangi 2009). Antioxidants (such as polyphenols), collagenase, and elastase inhibitors are widely used in cosmetic products.

Polyphenols are effective inhibitors of metalloproteases; therefore, epigallocatechin gallate (EGCG) was used as a control because a low concentration of the active is required to promote inhibition (Zillich et al. 2015). Collagen is the main protein of the dermis, 75 wt%, composed of 70% type I and 15% type III (Their primary function is to maintain the strength and resilience of the skin, but aging (often associated with solar radiation) can result in a change in the regulation of collagen degradation and synthesis (Lad 2006; Roy et al. 2013). Elastin is an extracellular matrix protein (2 to 4 wt% of the dermis) responsible for the elasticity in connective tissues, forming a dermis–epidermis network with thinner fibers and lower concentration. Elastin synthesis is decreased throughout chronological aging, thus, contributing to skin flaccidity (Zeisel and da Costa 2009; Klein et al. 2013).

Given the above, this work aims to evaluate using DESs as solvents in preparing plant extracts and cosmetic actives to develop sustainable downstream processes. Choline chloride is chosen as hydrogen bond acceptor (HBA) due to its low cost, low toxicity, biodegradability, and biocompatibility (Leite et al. 2021). At the same time, glycerol and urea are selected as hydrogen bonding donors (HBD). For this purpose, the inhibition of two metalloproteases, elastase, and collagenase, was evaluated in pure solvents and green tea (*Camellia sinensis*) extracts, allowing the identification of the active against skin aging. Furthermore, the accelerated stability of a cosmetic formulation in the presence of these actives for up to 60 days using digital image processing was also evaluated.

Materials and methods

Materials

Some reagents were purchased from Merck: glycerol, urea, choline chloride, jojoba oil, octyldodecanol, sodium hydroxide, disodium EDTA, trizma, tricine, sodium chloride, calcium chloride, N-Succinyl-Ala-Ala-Ala-Ala-p-nitroanilide, and N-(3-[2-Furil] acryloyl)-Leu-Gly-Pro-Ala (FALGPA). The enzymes elastase from porcine pancreas (type I, ≥ 4.0 units mg^{-1} protein) and collagenase from *Clostridium histolyticum* (type II, 0.5–5.0 FALGPA units/mg solids) were also acquired from Merck. Cosmetic ingredients such as nikkomulese wo (galena), pemulen tr-1, dimethicone, tocopheryl acetate, chemynol (Fagron), pelemol 6gpr (Midelt), dry flo pure (Sarfam) were obtained from local suppliers. Green tea (Coca-Cola), cyclomethycaine, epigallocatechin gallate (EGCG) (Assessa) were supplied from a donation.

DES preparation

DES were synthesized by adding a hydrogen bond donor (HBD) (glycerol and urea) to a hydrogen bond acceptor (HBA) (choline chloride, ChCl) in a molar ratio of 2:1. The mixture was heated to 80 °C for 1 h. Briefly, HBDs and HBA were added gravimetrically to closed vials and heated in a heat block under 80 °C with constant agitation for 1 h (Zhang et al. 2012). The Karl-Fischer titrator detected a water content of 0.963% for ChCl/Urea and 0.857% for ChCl/Glycerol.

Preparation of cosmetic formulations

The cosmetic formulation chosen for this work was based on an easy-spread BB cream published in Cosmetic and Toiletries International (Rigano 2013) and Cosmetic and Toiletries Brazil (Rigano 2015). Thereby, phase A was added over phase B under stirring at 400 rpm and heated up to 75 °C with the agitation of 400 rpm using an impeller with a centrifugal propeller and kept for 15 min. Then, the solution was slowly cooled down to 30 °C, allowing the addition of phase C under continuous stirring for another 15 min. Finally, at a 25 °C, phases D and E were added, along with some of the water lost in the process. Table 1 provides more details of the phases, components, and concentrations in the formulations.

Analytical methods

Inhibitory potential of DES

To evaluate the inhibitory potential of DES as cosmetic actives, it is necessary to demonstrate some inhibitory potential on collagenase and elastase enzymes. Thus, 100% relative activity was considered in the absence of the cosmetic active. All other activities in the presence of the active would be correlated to this value, allowing the calculation of the percentage of inhibition (Eq. 1).

Table 1 Cosmetic formulations applied with green actives

Phases	Components	wt%		
		Control	ChCl/urea	ChCl/glycerol
A	Nikkomulese WO	5	5	5
	Pemulen TR-1	0.1	0.1	0.1
	Jojoba oil	1	1	1
	Pelemol 6GPR	1	1	1
	Octyldodecanol	3	3	3
B	EDTA 2Na	0.05	0.05	0.05
	Water	qsp	qsp	qsp
	NaOH 20%	0.025	0.025	0.025
	Active	0	10	10
C	Cyclomethycaine	5	5	5
	Dimethicone	5	5	5
	Tocopheryl Acetate	0.2	0.2	0.2
D	Dry Flo	3	3	3
E	Chemynol	0.5	0.5	0.5

$$\%\text{Inibition} = 100 - \frac{100 \times \text{ActiveEnz}_{\text{with active}}}{\text{AtivEnz}_{\text{without active}}}. \quad (1)$$

The concentrations of the active agents studied ranged from 0.5 to 100 g L^{-1}, and EGCG was also included as a control inhibitor. The multifunctionality of DES was also evaluated enabling to verify if these solvents can have a synergistic effect with green tea, when used for extraction of phenolic compounds since green tea is the main source of EGCG. The extraction conditions used a 1:5 ratio of green tea/DES under 50 °C with stirring at 2000 rpm for 12 h in the Thermomixer system.

Enzymatic activity: collagenase

The method evaluates the hydrolysis of FALGPA peptide by collagenase, which releases a greenish-yellow coloration and is detected at a wavelength of 345 nm. A solution of 2.0 mM FALGPA was prepared in ultrapure water at pH 7.5 using 50 mM tricin buffer + 10 mM calcium chloride + 400 mM NaCl. The reaction consisted of adding 90 µL of the FALGPA solution and the activity to be studied at a specified concentration, 6.7 µL of the enzyme solution at 0.8 g L^{-1}. It was evaluated the color generation for 5 min. The blank was composed of the substrate and active. Equation 2 shows the calculation of enzyme activity (Van Wart and and Randall 1981).

$$\frac{\text{FALGPA units}}{\text{mL enzyme}} = \frac{\left(\Delta\text{Abs}_{\text{active}}/\text{min} - \Delta\text{Abs}_{\text{blank}}/\text{min}\right) \times V_T(\text{mL}) \times f}{\varepsilon \times V_{ENZ}(\text{mL})}, \quad (2)$$

where ΔAbs is the absorbance variation obtained in a time interval, V_T is the total volume of the reaction mixture, f is the dilution factor, ε is the molar extinction coefficient of the FALGPA at 345 nm, and V_{ENZ} is the volume of the enzyme solution used.

Enzymatic activity: elastase

The elastase method aims to evaluate the hydrolysis of the peptide Succinyl-Ala-Ala-Ala-p-nitroanilide through the release of the greenish-yellow component (wavelength of 410 nm). The 4.4 mM Succinyl-Ala-Ala-Ala-p-nitroanilide solution was prepared using 100 mM Tris HCl buffer in ultrapure water at pH 8.0. The reaction consisted of adding 180 µL of buffer with the active at a known concentration, 6.7 µL of the substrate solution and the enzyme solution, and the color generation was evaluated for 5 min. The blank used was prepared with the substrate and active. Equation 3 shows the calculation of the enzyme activity.

$$\frac{\text{Units}}{\text{mL enzyme}} = \frac{\left(\Delta\text{Abs}_{\text{active}}/\text{min} - \Delta Abs_{\text{blank}}/\text{min}\right) \times V_T(\text{mL}) \times f}{\varepsilon \times V_{ENZ}(\text{mL})}, \quad (3)$$

where ΔAbs is the absorbance variation obtained in a time interval, V_T is the total volume of the reaction mixture, f is the dilution factor, ε is the molar extinction coefficient of p-nitroaniline at 410 nm, and V_{ENZ} is the volume of the enzyme solution used (Bieth et al. 1974).

Cosmetic emulsion characterization: micelle diameter

The cosmetic formulations were diluted tenfold in 70% ethanol solution. Then, 10 µL were collected and analyzed on glass slides covered with a coverslip in an optical microscope at 1000× magnification using the tools available in the commercial software Image-Pro Plus® 5.0 (Media Cybernetics, Inc.). The images were captured in a 24-bit (approximately 16 million colors) RGB matrix and stored in TIFF format.

Accelerated stability

The formulations were initially centrifugation at 3000 rpm for 30 min. Afterward, the formulation was placed in opaque plastic containers under different conditions: 25 °C, 40 and 50 °C (drying oven), 4–5 °C (freezer), absence (closet) and presence of natural light (window). The stability of cosmetic emulsions was verified by the variation in the size of micelle diameters seen under the optical microscope at the time of preparation and after 30 and 60 days.

Results and discussion

Deep eutectic solvents (DES)

The HBDs and HBA were selected from components that are low-cost, safe, and easily acquired (Jeong et al. 2017). These individual constituents have been reported in the cosmetic area for obtaining important characteristics. Glycerol has hygroscopic properties that justify its main use as a humectant in cosmetics, helping skin hydration in concentrations above 3% (Thau 2002). Urea also influences hydration as one of the constituents of the natural moisturizing factor of the stratum corneum. Still, it may have a proteolytic function in high concentrations, dissolving filaggrin and breaking the protein bonds between corneocytes (Scheinfeld 2020). Choline is part of the B complex of vitamins, known as an essential nutrient for the body's functioning in 1998 by the United States Institute of Medicine (Institute of Medicine 1998). The Institute highlights that choline is necessary for cell membrane signalization and acts in to absorb beta-carotene and its conversion into vitamin A (Zeisel and da Costa 2009). Moreover, choline can be considered a low-cost raw material, biodegradable, and of low toxicity. Klein et al. (2013) evaluated the cytotoxicity of choline salts containing 12- to 18-carbon fatty acids and found it equal to sodium and potassium soaps in SK-Mel-28 (CLS 300337) keratinocytes. Rengstl et al. (2014) proved the results, showing that the influence on cytotoxicity is on the type of anion used, not the choline cation; thus, not penetrating the biphospholipid layer of the model liposome when carboxylate anions had up to 8 carbons. Through the reported results, all choline salts tested could be considered inoffensive.

Some studies have been reporting the toxicity of choline-based DES. For example, Hayyan et al. (2013), Juneidi et al. (2015), and Wen et al. (2015) studied DES composed of ChCl/Urea and ChCl/Glycerol concerning toxicity against model organisms, such as microorganisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*), shrimp larvae (*Artemia salina*), fish (*carp*, *Cyprinus carpio*), rats, and also human cancer cell (prostate, PC3; melanoma, A375; liver, HepG2; colon adenocarcinoma, HT29; breast, MCF-7; oral keratinocyte-derived carcinoma, H413), or normal like oral keratinocytes (OKF6). The authors reported low antimicrobial activity and low toxicity of both DES studied in all studies.

The efficacy of a cosmetic active can be evaluated by methods in vivo, in vitro, or in silico analysis. Usually, the study that proves the effectiveness in vivo must be preceded by evaluations or safety data on the active to avoid damage to the health of human volunteers. In addition, they are more costly financially and time, requiring prior authorization from the research institution and the National Research Ethics Committee. Thus, the development of in silico, especially in vitro, has intensified interesting recent years, enabling faster analyses, lowering associated costs, and reducing the participation of volunteers (Roy et al. 2013). In vitro analyses can use human or animal tissues, differentiated cells (keratinocytes, fibroblasts, as examples), or enzymes, which have essential functions in the skin, such as tyrosinase hyaluronidase, elastase, and collagenase (Lad 2006).

Collagenase

Table 2 shows that the collagenase inhibition was studied using various concentrations of two DES, their pure components, control compounds (EGCG and green tea aqueous extract), green tea extracts + DES, and aqueous solutions of the pure components (ChCl + glycerol or urea). DES based on ChCl/glycerol and ChCl/Urea showed inhibition values above 90% (IC_{50} of 4.59×10^3 and 3.09×10^3 µM, respectively). As seen in Table 2, these values were higher than the pure components' inhibition in which showed % inhibition of 36.6% for glycerol, 50.9% for choline chloride, and 75.4% for urea. However, the values obtained for DES were lower than the control (100% inhibition).

Compared with aqueous solutions of the pure components (ChCl + glycerol or urea), similar results were obtained for the inhibition of collagenase. This can indicate that the synergy between choline chloride and the hydrogen donor (glycerol or urea) is essential for enzyme inhibition. Dai et al. (2013) reported that very dilute DES solutions could lose their properties by weakening the hydrogen bonding interactions between the major components. Thus, it is possible that

Table 2 Evaluation of the inhibitory potential of actives on collagenase

Actives	CI50 (µM; % g L^{-1})	% Inhibition
EGCG	9.45; 0.43×10^{-3} [40]	100
ChCl/urea	4.59×10^3; 0.12	91.1
ChCl/glycerol	3.09×10^3; 0.10	92.7
ChCl	6.75×10^5; 9.42	50.9
Urea	4.83×10^5; 2.89	75.4
Glycerol	ND; ND	36.6
ChCl + urea	ND; < 1.0	90.2
ChCl + glycerol	ND; < 1.0	90.3
Green tea aqueous extract	ND; 0.50	86.1
Extract ChCl/urea + green tea	ND; 0.06	90.4
Extract ChCl/glycerol + green tea	ND; 0.05	87.5

ND not determined

DES, when submitted to a large dilution behaves as aqueous solutions between its constituents, which justifies the similar results between DES (ChCl/Urea and ChCl/Glycerol) and aqueous solutions (ChCl + Urea and ChCl + Glycerol).

As DES proved to be active towards collagenase, these compounds were tested as extracting solvents for phenolic compounds such as catechins and tannins from green tea, which are known to be applied as inhibitors (Holzer et al. 2011). Thus, DES ChCl/Urea had a much higher inhibitory power than water as the extracting solvent. Both DES showed IC_{50} values (Table 2) about ten times lower than the green tea aqueous extract. This behavior can indicate a high synergy between green tea phenolic compounds and DES as inhibitors and shows that these DES can be multifunctional, acting as cosmetic active for inhibition and extracting solvent simultaneously.

Elastase

In general, developing an activity that inhibits the elastase produced by the skin or macrophages could be crucial, because there would be a lower possibility of restructuring the extracellular matrix and, consequently, lower flaccidity. Elastase inhibition was analyzed using various actives (Table 3), as reported in the previous topic 3.2. Some clinical studies have confirmed that plant secondary metabolites may act as elastase inhibitors and protect the skin against aging and structural damage to the extracellular matrix (Azmi et al. 2014; Bose et al. 2017). Inhibition by DES occurs similarly to that observed for collagenase. ChCl and urea components show better results (acting as DES or aqueous solution) than glycerol. However, pure urea showed more significant effects on elastase inhibition.

Monhemi et al. (2014) concluded through molecular dynamic simulation that urea contributes directly to intramolecular hydrogen bonds. However, this compound acts only on the enzyme surface as a constituent of DES. Kouadri-boudjelthia and Wallach (1997) comment that actives with a carbonic chain above 14 carbons and still being anionic may have inhibitory activity on elastase produced by leukocytes, but mainly by the interaction of the active with elastin. Aromatic groups such as flavonoids and hydroxylated terpenoids are also options for actives, but the glycosylated ones have less inhibitory activity (Siedle et al. 2007). Thring et al. (2009) also studied the inhibition of green tea aqueous extract using a 1:20 ratio between tea and water, different from that used in this work (1:5) but obtaining close results using a 100 g L^{-1} green tea solution. The authors observed a 47% inhibition for collagenase and 10% for elastase. In addition, EGCG using 250 µM (or 0.114 g L^{-1}) was also seen to have over 90% inhibition relative to elastase, while only 35% when collagenase was used. However, the authors do not inform whether this control was commercial or isolated by the research group, which creates doubts regarding its purity and possible interferences. The aqueous extract also presents a high inhibitory potential in this work, but it is reduced to half when DES are used. Marijan et al. (2022) showed that DES formed by glycerol + betaine + glucose (molar ratio 20:4:1). It is essential to note that the eutectic was diluted in water (8:2). The authors reported a value of 285.1 µL solvent mL^{-1} for 50% inhibition of elastase activity. The high glycerol concentrations may explain this good activity that the solvent contains. Namely, it has been demonstrated that high glycerol concentration can negatively affect elastase activity (Azmi et al. 2014).

The high performance of this studied is also further supported by other studies previously published. For instance, Shamseddin et al. (2017) studied resveratrol (RES) as a metalloprotease-9 (MMP-9) inhibitor using natural DES (NADES). The NADES 1,2-propanediol:choline-chloride:water (1:1:1 molar ratio) exhibited better results in both a biocompatible solubility and a strong increased MMP-9-inhibitory activity, at least ten-fold higher than the organic solvents dimethyl-sulfoxide (DMSO). Following in vivo validations, some NADES could potentially be considered as the new generation of formulation for druggable compounds. Macario et al. (2019) evaluated the effect of DES on the cytotoxicity of two cell lines used as a skin model for cosmetics (keratinocytes (HaCaT) and tumor melanocytes (MNT-1)). The effect of three HBA ([Ch]Cl, [N1111]Cl, and [N4444]Cl) and three HBD (hexanoic and butanoic acid, ethylene glycol, 1-propanol, and urea) was evaluated. Results were promising since DES based on [Ch]Cl and [N1111]Cl showed good biocompatibility and increased cell viability in the HaCaT cell line for the tested cells. Juszczak et al. (2022) applied NADES in the phenolic compound extraction from *Jasione montana*, where it was observed that NADES based on proline and glycerol (2:5 molar ratio) in an aqueous solution at 50 wt% showed

Table 3 Evaluation of the inhibitory potential of actives on elastase

Actives	CI50 (µM; % g L^{-1})	% Inhibition
EGCG	1.53×10^3; 0.07	100
ChCl/urea	ND; ND	49.8
ChCl/glycerol	ND; ND	45.7
ChCl	ND; ND	49.0
Urea	1.18×10^6; 7.11	66.0
Glycerol	ND; ND	17.4
ChCl + urea	ND; ND	48.8
ChCl + glycerol	ND; ND	33.8
Green tea aqueous extract	ND; 4.68	100
Extract ChCl/urea + green tea	ND; 9.13	55.1
Extract ChCl/glycerol + green tea	ND; 8.54	56.5

ND not determined

better extraction values. Furthermore, the extracts presented as potent inhibitors of collagenase and elastase with IC_{50} values of 4.14 µL extract mL^{-1}. Maidim et al. (2017) studied the inhibition of collagenase and elastase from grape pomace phenolic extracts using ethanol and hot water. The authors reported that the extracts from hot water were more efficient showing IC_{50} values for collagenase and elastase of 1.41 and 2.67 mg L^{-1}. Jin et al. (2019) observed optimal collagenase and elastase inhibitor values using glycerol-xylitol-based DES for phenolic extracts from the three-leaf mixture (*Ginkgo biloba* L., *Cinnamomum camphora* L., and *Cryptomeria japonica*).

Accelerated stability of cosmetic formulations

The cosmetic formulations were centrifuged and then equilibrium to visualize phase separation, in which no change was observed in these formulations. To evaluate the stability of cosmetic emulsions, a simple computational routine was used in MATLAB software for image processing. Then, morphological operations such as erosion (removal of small debris), reconstruction, and filling were applied to form the final binary image, as shown in Fig. 1. Finally, the quantification of objects and calculation of morphological parameters (such as micelle diameter and circularity) were performed (Ribeiro et al. 2013). Circularity is defined as the ratio between the maximum and minimum axis value measured (a micellar standard is confirmed when the value approaches 1.0), where the vertical axis represents the number of objects in percent. The horizontal axis represents the distribution over 40 circularity classes. Each class represents a range of values; for example, a range 10 is values between 1.0 and 1.1 circularity.

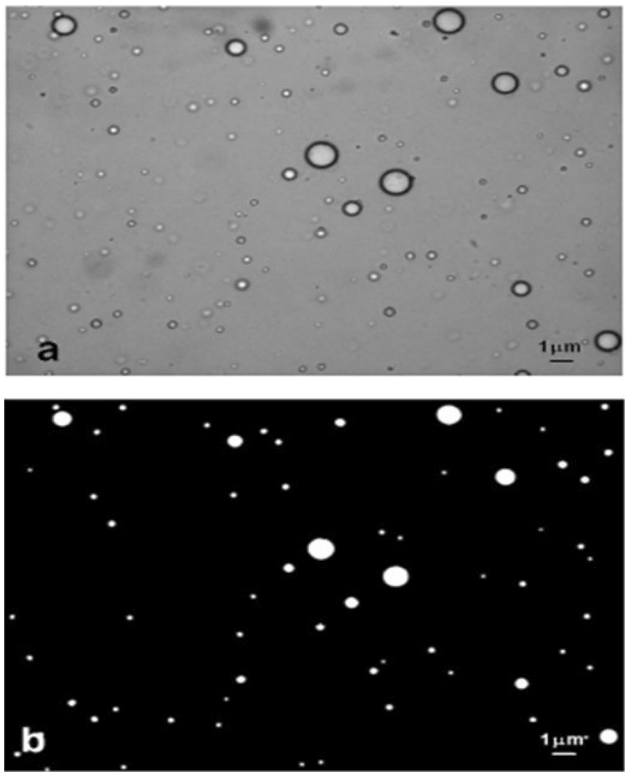

Fig. 1 Emulsion stability image processing using MATLAB software before (**a**) and after (**b**) the binarization process

Figure 2 shows that all samples studied were circular, and values less than or equal to 2.0 were observed. This behavior indicates no external interference in the micelle analysis. The analysis of emulsion stability can be determined by obtaining the average micelle diameter at each analysis point and for each formulation studied. Their variation with storage time can indicate loss of stability, such as flocculation, coalescence, and cream formation. This variation may be associated with the oxidation and hydrolysis of its components. Models' mathematical models such as the Van den Tempel theory that correlates the volume of particles with time and an exponential increase indicates the coalescence of the emulsion can be employed (Santana et al. 2015).

Table 4 shows that the ChCl/glycerol formulation shows remarkable stability compared to the control formulation and ChCl/Urea due to the mean cube diameter values being more constant, despite showing a higher standard deviation. In this case, the deviation represents the dispersion in the Gaussian distribution of diameters. Thus, the sample will be narrower and more homogeneous (Amaral et al. 2006). The decrease in the average diameter value in the ChCl/Urea formulation may indicate a change in the conformation of the micellar structure not captured in this processing. Tables 5 and 6 demonstrate that the control sample (no DES) presents a profile indicating initial emulsion instability by similar coalescences at all conditions; however, this behavior is insignificant at low temperatures. In the presence of the active ChCl/glycerol DES, the emulsions remained stable at all conditions except at low temperatures indicating flocculation, and at 50 °C, which may indicate coalescence. Concerning the compound ChCl/Urea as active, they tended to aggregate in all states.

Conclusion

The pharmaceutical industry initially approached DES as a compound to be avoided due to incompatibility where solid mixtures became liquid. Therefore, these alternative solvents have demonstrated that they can be positively applied to facilitate the permeability of low or no toxicity components. In this work, it can be observed that from DES based on constituents of low or zero toxicity, it is possible to obtain inhibition of metalloproteases, such as collagenase and elastase, and still allow their use in processes of

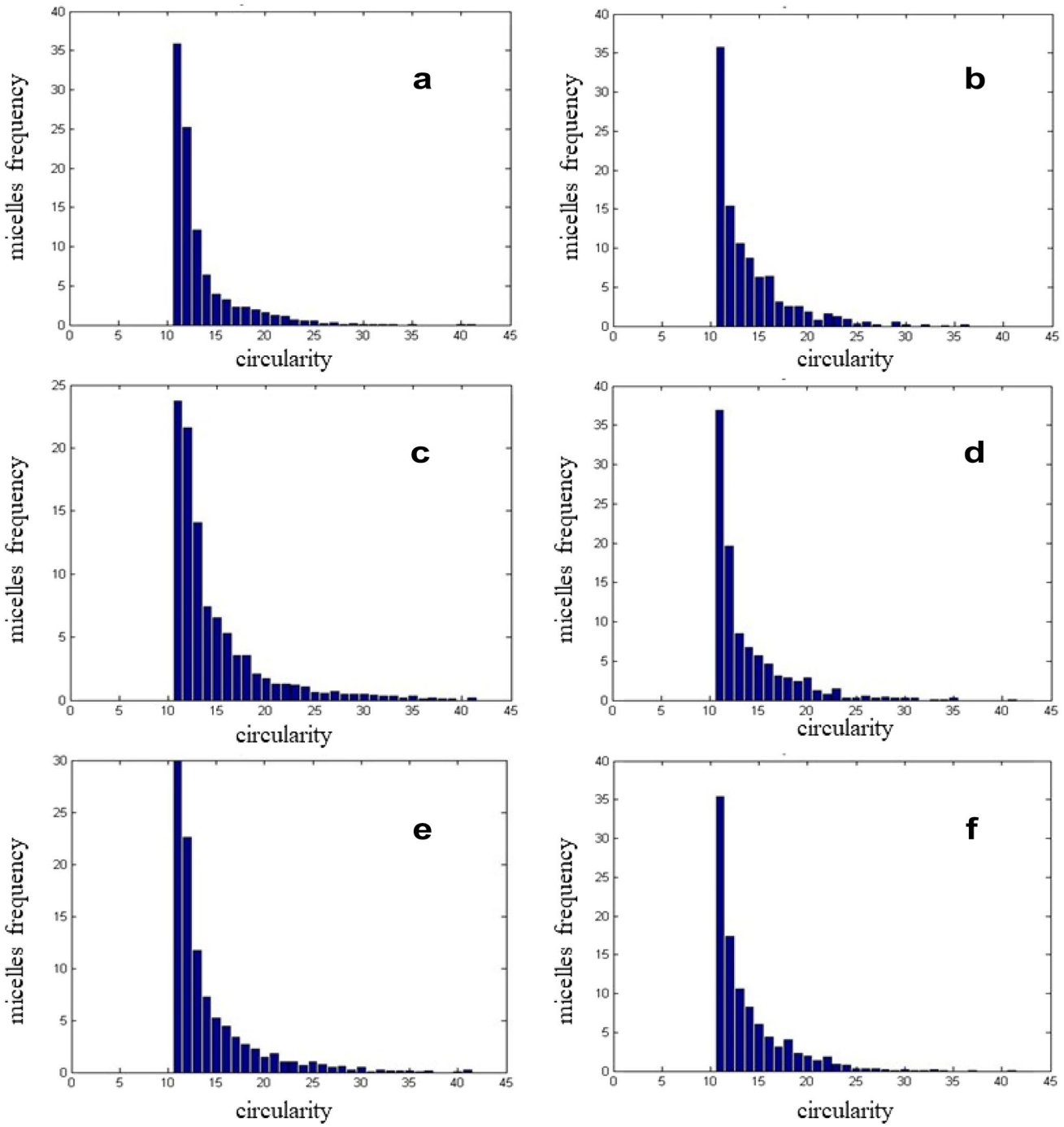

Fig. 2 Evolution of the circularity of cosmetic formulations in 60 days. The vertical axis represents the percentage frequency of micelles with a given circularity and the horizontal axis the ranges of circularity values. Formulations: **A**, **B** control at 0 and 60 days; **C**, **D** with ChCl/Glycerol at 0 and 60 days; **E**, **F** with ChCl/Urea at 0 and 60 days

extraction of phenolic assets, thus promoting synergy and multi-functionality. The addition of DES to the cosmetic formulations showed a marked improvement in the hydration sensation and spread of the product. Therefore, the use of DES as cosmetic actives can be applied in creams for anti-aging purposes.

Table 4 Evolution of the average diameter of cosmetic formulations in 60 days

Formulation	Time (days)	No. objects analyzed	Average diameter (μm)	Cubic mean diameter (μm^3)	Standard deviation
Control	0	2777	1.67	4.68	0.90
Control	30	2084	1.88	6.69	1.31
Control	60	897	2.38	13.41	1.31
ChCl/glycerol	0	1680	2.26	11.50	1.63
ChCl/glycerol	30	1347	2.10	9.26	1.32
ChCl/glycerol	60	1207	2.31	12.26	1.69
ChCl/urea	0	1678	2.41	13.96	1.86
ChCl/urea	30	1200	2.05	8.64	1.49
ChCl/urea	60	1329	1.88	6.69	1.26

Table 5 Evolution of the average diameter of cosmetic formulations at 40ºC in 60 days

Formulation	Time (days)	No. of objects analyzed	Average diameter (μm)	Cubic mean diameter (μm^3)	Standard deviation
Control	0	2777	1.67	4.68	0.90
Control	30	2589	1.78	5.67	1.36
Control	60	1236	2.50	15.59	1.86
ChCl/glycerol	0	1680	2.26	11.50	1.63
ChCl/glycerol	30	1464	2.34	12.87	1.72
ChCl/glycerol	60	1072	2.22	10.92	2.02
ChCl/urea	0	1678	2.41	13.96	1.86
ChCl/urea	30	1598	2.21	10.84	1.61
ChCl/urea	60	867	2.02	8.28	1.11

Table 6 Evolution of the average diameter of cosmetic formulations exposed to other conditions in 60 days

Formulation	Time (days)	No. of objects analyzed	Average diameter (μm)	Cubic mean diameter (μm^3)	Standard deviation
Solar radiation					
Control	0	2777	1.67	4.68	0.90
Control	60	1192	2.33	12.70	1.40
ChCl/glycerol	0	1680	2.26	11.50	1.63
ChCl/glycerol	60	681	2.29	12.06	1.26
ChCl/urea	0	1678	2.41	13.96	1.86
ChCl/urea	60	1375	2.13	9.61	1.41
4 °C					
Control	60	1144	2.23	11.16	1.42
ChCl/glycerol	60	789	2.37	13.25	1.50
ChCl/urea	60	721	2.33	12.65	1.68
50 °C					
Control	60	924	2.30	12.12	1.70
ChCl/glycerol	60	628	2.67	18.97	1.90
ChCl/urea	60	1202	2.45	14.78	1.80
Without sunlight					
Control	60	1335	2.39	13.62	1.58
ChCl/glycerol	60	1058	2.03	8.40	1.20
ChCl/urea	60	1137	2.30	12.20	1.62

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Declarations

Conflict of interest The authors declare no competing interests.

Consent to participate The authors agreed to participate in this work.

Consent for publication All the authors have provided their consent for publication.

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