

Ionic Liquids and Deep Eutectic Solvents and Their Use for Dissolving Animal Hair

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Abstract

In recent years, one of the priority areas of research in chemistry has become the processes carried out in an environment of liquid organic salts, the so-called ionic liquids (ILs), which are assessed as environmentally friendly or “green” alternatives to conventional organic solvents. ILs are non-volatile, highly polar solvents that dissolve many organic, inorganic, and organometallic compounds. Since they have no detectable vapor pressure, ILs are considered as potential substitutes for volatile organic compounds traditionally used as solvents. So-called deep eutectic solvents (DES) is a group of ILs that are liquid mixtures of a number of organic and (or) inorganic components taken in a certain ratio (eutectic or close to eutectic). DES deserve a special attention due to their negligible saturated vapor pressure, availability, low cost, as well as ability to dissolve at relatively high concentration of metal salts, metal oxides and various polymers. Particularly DES based on a mixture of choline chloride with urea (DES-1) or a mixture of choline chloride and adduct of urea with hydrogen peroxide (DES-2) give eutectics that are liquid at ambient temperature and have unusual solvent properties, including an ability to dissolve an animal hair in the presence of low concentration of sodium sulfide or ammonium thioglycolate. It was found that depending on the ratio between DES-1 and DES-2 in the mixture of two Deep Eutectic Solvents and the nature of sulfur-containing additive, the solubility of rabbit hair under used conditions, varies from 51% to 79%.

Keywords

Deep Eutectic Solvents, Ionic Liquids, Polymer Solubility, Keratin, Hydrogen Bonds, Animal Hair

1. Introduction

1.1. Basics of Ionic Liquids (ILs)

ILs are non-volatile, highly polar solvents that dissolve many organic, inorganic, and organometallic compounds. Since they have no detectable vapor pressure, ILs are considered as potential substitutes for volatile organic compounds traditionally used as solvents. That is why ILs are among the most promising solvents in terms of minimal environmental impact, and they provide the development of environmentally friendly technologies for chemical processes. The term “ionic liquids” means substances that are liquids at temperatures below 100°C and their properties are determined by the structure of ions, in particular, the symmetry or asymmetry of the cation, the nature of the functional groups in the cation, the size of the cation and anion [1]-[6].

Typical ions in the composition of ILs are: cations of ammonium, phosphonium, pyridinium, 1,3-dialkylimidazolium; N-alkylpyridinium, tetraalkylammonium, tetraalkylphosphonium, trialkylsulfonium and various anions which include chlorides, iodides, bromides, sulfates, hexafluorophosphates, etc.

The nature of the anion has a great influence on the properties of ILs—melting point, thermal and electrochemical stability and viscosity. The polarity as well as the hydrophilicity or hydrophobicity of ILs can be optimized by appropriate selection of the cation/anion pair, and each new anion and cation provides additional opportunities for varying the properties of ILs.

The increased attention to ILs is due to the presence of the following specific properties: 1) A wide range of liquid state ($> 300^{\circ}\text{C}$) and low melting points ($T_m < 100^{\circ}\text{C}$). 2) High electrical conductivity. 3) Good solubility in relation to a variety of inorganic, organometallic and organic compounds and polymers [1] of natural and synthetic origin. 4) Catalytic activity, which leads to an increase in the selectivity [2] of organic reactions and the yield of the target product. 5) Non-volatility, reusability. 6) Non-combustibility, lack of explosiveness, non-toxicity and consequent absence of harmful effects on the environment. 7) Unlimited possibilities in the directed synthesis of ILs with desired properties. Qualities 3 and 4 make ionic solvents especially attractive in the synthesis of polymers.

The advantages of ILs in compiling with other classes of substances determine their use as reaction means for synthesis [7], solvents [8], electrolytes in electric batteries [9], plasticizers [10], catalyst [11] [12], etc.

1.2. Low-Temperature Eutectic Solvents: Versatile Alternatives to Ionic Liquids

Low-temperature eutectic solvents or deep eutectic solvents (DES) [9] [13] [14] [15] are liquid mixtures of a number of organic and (or) inorganic components taken in a certain ratio (eutectic or close to eutectic). DES are considered as a new and very promising class of ionic liquids, which can be used in various fields of industry, including for dissolving various materials [7] [16].

The main advantages of DES, determining the prospects of their use in technology, include such properties as high solubility of metal salts, negligible saturated vapor pressure, availability, low cost and environmental safety [15] [17] [18] [19].

DES can be formed between various quaternary ammonium salts and carboxylic acids [8] [9]. Physical properties largely depend on the structure of the carboxylic acid, but the phase behavior of the mixtures can simply be modeled taking into account the molar fraction of the carboxylic acid in the mixture.

Physical properties, such as viscosity, conductivity, and surface tension of these DES, are similar to ionic liquids at ambient temperature, and understanding the reason for these properties is achieved using hole theory. It was shown that the conductivity and viscosity of these liquids are controlled by the mobility of ions and the presence of voids of suitable sizes, which is consistent with the fluidity of other ionic liquids and molten salts.

An example of DES is a mixture of substituted quaternary ammonium salts, such as hydroxyethyl trimethylammonium chloride (choline chloride), with urea, which gives eutectics that are liquid at ambient temperature and have unusual solvent properties [13] [20].

Eutectic occurs when the molar ratio of urea to choline chloride is 2. The freezing point of the eutectic mixture is 12°C, which is much lower than the freezing temperature of both components (melting point of choline chloride = 302°C and urea melting point = 133°C). This property allows the use of DES as a solvent at ambient temperature. This significant depression of the freezing point should result from the interaction of urea molecules with a chloride ion. In addition to urea, several other derivatives, such as thiourea, 1-methylurea, 1,3-dimethylurea, 1,1-dimethylurea, tetramethylurea, acetamide and benzamide, were tested as components of eutectic mixtures.

Using amides of various structures and choline chloride as an example, it was found that only those compounds that are capable of forming hydrogen bonds with chloride ions exhibit the formation of a homogeneous liquid with a significant decrease in the freezing temperature compared to pure amide. It seems that amides with the greatest ability to form hydrogen bonds (*i.e.* urea and thiourea) exhibit the greatest decrease in freezing point when mixed with choline chloride. According to [21], choline chloride was also assigned a provitamin status (vitamin B4).

However, other sources indicate that choline chloride is not a vitamin in the sense that it cannot be produced by the human body.

It is important that DES are capable of dissolving proteins and amino acids, sugar and polysaccharides. Some DES, such as the eutectic mixture of glycerol and choline chloride sold by Scionix under the trade name Glyceline, are harmless, completely biodegradable and can be used where other solvents cannot, for example, in food and cosmetics.

It is obvious that IL and DES have some unique characteristics that distin-

guish them from conventional organic solvents, such as the lack of an effective vapor pressure, the wide temperature range of their aggregation state, such as liquids, high polarity and charge density, hydrophobic or hydrophilic characteristics and unique solvating properties.

The British company Scionix Ltd. [21] produces various types of DES based on choline chloride, which is a completely non-toxic product that is produced on a large scale as a feed additive for chickens (Table 1).

1.3. Dissolution of Keratin, as the Main Components of Animal Hair, in ILs and DES

Keratin belongs to a family of fibrous structural proteins. Such materials as hair, feathers, nails, hooves, claws and horns are made of keratin [17] [18] [22] [23] [24] [25] [26]. It is known for its extremely low solubility in water and organic solvents. According to [22] [23] preparation of keratin solution will allow to explore many new applications including manufacturing textile fibers, additives for animal feed, neatening reagents for leather and wool or leather, and many others. It should be noted that many tons of non-spin wool fibers are dumped during wool weaving every year. It means that developing processes for using and reusing the resources are important with an economic and environmental point of view.

Some progress with dissolving keratin by ILs was reported in publications [17] [24] [25]. The authors of study [17] investigated the ability to extract keratin from turkey feather using traditional ILs such as 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), as well as novel IL [choline][thioglycolate]. It was expected that the presence of a thiol functional group in the latter will provide disulfide bridge reducing, and enhancing the keratin solubility. In was found that the process performed within 10 hours at 130°C under nitrogen atmosphere leads to a significant percentage of solubility (up to 45%).

According to presented experimental data, the chloride ion in ILs appears to be as effective as the thioglycolate ion, and no advantages of [choline][thioglycolate] compared to [BMIM]Cl and [AMIM]Cl in dissolving the keratin was found. In the opinion of the authors of paper [17], it indicates that that disulfide cleavage is not a significant factor in the keratin's dissolution. It was established that around 50 wt% of feather material in all three types of ILs solutions became too

Table 1. Composition of the five DES proposed by Scionix Ltd. [21].

Product Description	Starting compounds (Molar ratio)
Glyceline	choline chloride:glycerin (1:2)
Ethaline	choline chloride:ethylene glycol (1:2)
Maline	choline chloride:malonic acid (1:1)
Oxaline	choline chloride:oxalic acid (1:1)
Reline	choline chloride:urea (1:2)

viscous for effective dissolution at 130°C. It was found that these ILs have an ability to disrupt hydrogen bonds in keratin that enhances its solubility. The authors of studies [24] [25] came to the same conclusion using [BMIM]Cl as the solvent for keratin.

According to paper [18] a number of DES based on choline chloride and hydrogen bond donors such as urea, ethylene glycol, citric acid and oxalic acid were tested as solvents for keratin as main component of rabbit hair. It was found that the solubility of rabbit hair evaluated at 98°C within 2 hours increased in the raw choline chloride-ethylene glycol < choline chloride-urea < choline chloride-citric acid < choline chloride-oxalic acid from 15% - 20%, 19% - 22%, 20% - 27% and 36% - 71%, respectively. As a result, the DES based of choline chloride and oxalic acid (molar ratio 1:1, 1:2 and 1:3) was chosen for more detailed study which showed that solubility of rabbit hair can reach 89% after stirring for 2 hrs at temperature of 120°C.

In this study, we report the dissolution of keratin from the rabbit hair in DES based on a mixture of choline chloride with urea (DES-1) or a mixture of choline chloride and adduct of urea with hydrogen peroxide (DES-2) in the presence of low concentration of sodium sulfide or ammonium thioglycolate at temperature of 30°C.

2. Experimental Part

Chemicals

Choline chloride, Cat #C7,9700; Urea, Cat #20,8884; Urea-Hydrogen Peroxide adduct, Cat #289132; Ammonium hydroxide, Cat #211228; Thioglycolic Acid, Cat #T3758; Oxalic Acid, Cat #75688 and Sodium Sulfide, Cat #208043 were purchased from Sigma-Aldrich and used as received. The white rabbit hair (breed White Giant) used for solubility studies was purchased from CREATIVE-EXPORTS LTD (Kharkov, Ukraine).

Synthesis of DES-1 and DES-2 has been performed following published procedure [13]. Synthesis of DES-3 has been performed according to publication [9].

Procedure for making DES-1 based on choline chloride and urea (1:2 mol).

141.030 grams (1.0 Mol, 99.0% purity) of choline chloride is added to a glass beaker containing 122.57 g (2.0 Mol, 98% purity) of urea. The temperature of the beaker maintained at 50°C for 30 min. The beaker is agitated with a glass rod. A noticeably viscous visually clear solution of the DES-1 with freezing point about 12°C is obtained.

Procedure for making DES-2 based on choline chloride and Urea-Hydrogen Peroxide adduct (1:2 mol).

141.030 grams (1.0 Mol, 99.0% purity) of choline chloride is added to a glass beaker containing 193.96 g (2.0 Mol, 97% purity) of Urea-Hydrogen Peroxide adduct. The temperature of the beaker maintained at 35°C for 30 min. The beaker is agitated with a glass rod. A noticeably viscous visually clear liquid of

the DES-HP with freezing point between 10°C and 15°C is obtained.

Synthesis of DES-3 (freezing point around 34°C) based on choline chloride and Oxalic acid (1:1 mol) has been performed according to published procedure [9].

Spectrophotometric method for evaluation of rabbit hair's solubility in experiments with different compositions of DES.

The proposed spectrophotometric method includes the following steps.

- 1) Washing rabbit hair with acetone and drying it overnight at 60°C.
- 2) Preparing 4 solutions dissolving 5, 10, 15 and 20 mg of washed rabbit hair in 5 g of DES-3 prepared from choline chloride and oxalic acid (1:1 mol) under conditions described in paper [27], *i.e.* by stirring with magnetic bar for 2 hrs at temperature of 120°C.
- 3) Measuring the absorbance spectra of prepared solutions in the spectral region of 250 - 400 nm using Agilent Cary 60 UV-Vis spectrophotometer (**Figure 1**).
- 4) Assuming (based on data [18]) that the solubility of the rabbit hair at loading 20 mg/5 g DES-3 is ~89%, create the calibration graph which determines the linear relationship between the solubility of hair (%) and intensity of absorbance of investigated solution at wavelength of 277 nm.

3. Results and Discussion

The main difference between the technology of dissolving hair described in patent [28] and procedure implemented in this study is that instead of using high concentration of strong oxidizers, such as 50% solution of hydrogen peroxide or 32% solution of peracetic acid, we use the mixture of two DES. One of them is DES-1 prepared from choline chloride and urea at molar ratio of 1:2. The second one is DES-2 prepared from choline chloride and urea-hydrogen peroxide adduct (UHP). It should be noted that the UHP is stable, inexpensive and an easily

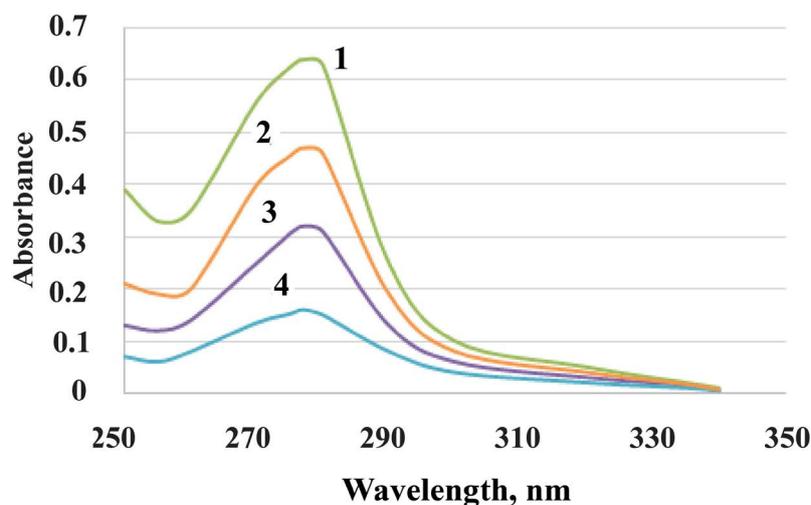


Figure 1. Absorbance spectra of rabbit hair in DES-3 at concentrations: 0.4% (1); 0.3% (2); 0.2% (3) and 0.1% (4).

handled reagent. UHP is a non-toxic, odorless crystalline solid. This adduct, which contains 35% of H_2O_2 , sets the hydrogen peroxide free during its application. UHP is used in an efficient solid state oxidation of different organic molecules: hydroxylated aldehydes and ketones (to hydroxylated phenols), sulfides (to sulfoxides and sulfones), nitriles (to amides) and nitrogen heterocycles (to *N*-oxides) [29].

UHP is commonly encountered in cosmetic dentistry, where it is used to “bleach” teeth. The active ingredient is hydrogen peroxide, which acts to oxidize interprismatic extrinsic staining within tooth enamel. There are several methods of applying the peroxide gel to the tooth ranging from night-guard application at home or in-surgery application. The bleaching obtained is proportional to the length of time the peroxide is applied to the tooth, and the concentration used. Concentrations of UHP used for tooth whitening purposes range between 10% and 35%. Higher concentrations carry a higher risk of side effects such as chemical burns. 10% is widely regarded as safe. A 10% solution of UHP in glycerol is used to treat ulcers and other lesions in the mouth. A 6.5% concentration solution is used to loosen and remove earwax [30].

Table 2 summarizes data on composition of mixtures of DES-1 and DES-2 (4 different ratios) used for partial oxidation of rabbit hair which was followed by addition of ammonium thioglycolate or sodium sulfide. In all cases concentration

Table 2. Results on solubility of rabbit hair in eight different compositions consisting of DES-1 and DES-2 with addition of ammonium thioglycolate or sodium sulfide (pH ~12 - 13).

#	Composition (Ratio DES-1: DES-2, w/w) (Total Amount—5 g)	Concentration of hydrogen peroxide in mixture, %*	Thioglycolic Acid, g**	Ammonium Hydroxide (aqueous 28%), g**	Sodium Sulfide** $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$, g	Solubility of Hair*** %
1	1:2	13.46	0.082	0.118	N/A	79
2	1:2	13.46	N/A	0.046	0.154	73
3	1:1	10.05	0.082	0.118	N/A	75
4	1:1	10.05	N/A	0.046	0.154	69
5	2:1	6.72	0.082	0.118	N/A	76
6	2:1	6.72	N/A	0.046	0.154	68
7	3:1	5.02	0.082	0.118	N/A	58
8	3:1	5.02	N/A	0.046	0.154	51

*) Estimated with assumption that starting concentration of H_2O_2 in UHP is 35% and DES-1 contains 1.348 parts of UHP (M.w. 94.1) for 1 part of choline chloride (M.w.139.6) which leads to concentration of H_2O_2 in non-diluted DES-2 as high as 20.1%; **) Due to reaction of 0.082 g of Thioglycolic Acid with 0.118 g of 28% aqueous solution of ammonium hydroxide about 0.097 g (1.94% from amount of DESs) is produced; 0.154 g of $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$ contains about 0.05 g of pure Na_2S (1.0% from amount of DESs); ***) Estimated by spectrophotometric method (absorbance at 277 nm) using a calibration graph presented in **Figure 2**. **Note:** Diluted solutions of rabbit hair in mixture of DES-1:DES-2 have a strong absorbance with a maximum at wavelength between 272 and 280 nm which is typical for keratin's solutions, as well as for solid keratin [31]. It is obvious that the origin of this maximum is related to the absorption of the aromatic amino acids, such as tyrosine and tryptophan.

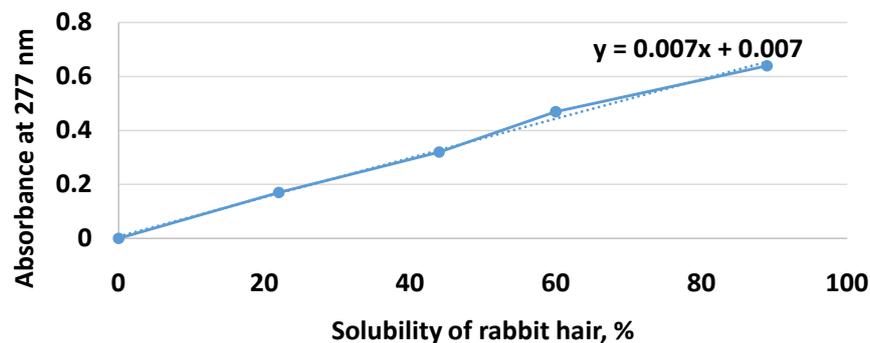


Figure 2. Calibration graph used evaluation of rabbit hair's solubility in DES-based compositions (**Table 2**).

of ammonium thioglycolate and Na_2S was 1.94% and 1% respectively from the amount of DES-1 and DES-HP compositions.

It is obvious that the molar concentration of sulfur in compounds used in existing dehairing process varies in relatively broad range depending on the structure of these compounds. For instance, in molecule of NaHS (M.w. 56) the molar content of sulfur is $(32/56) \times 100 = 57.1\%$. However, in molecule of ammonium thioglycolate $\text{C}_2\text{H}_7\text{O}_2\text{NS}$ (M.w. 109) which is example of another compounds used for dehairing process, the molar content of sulfur is much less, *i.e.* $(32/109) \times 100\% = 29.4\%$.

It should be noted that the reducing of NaHS content from 2% to 1% would be important for practical application. In this case the overall content of sulfur would be changed from 57.1% to 28.55%.

In case of using ammonium thioglycolate the overall 28.55% content of sulfur would correspond to $2 \times (28.55/29.4) = 1.94\%$ of ammonium thioglycolate.

The following conclusions can be made from the data presented in **Table 2**:

1) Depending on the ratio between DES-1 and DES-2 and the nature of sulfur-containing additive the solubility of rabbit hair under used conditions, varies from 51% to 79%.

2) An addition of 1.94% of ammonium thioglycolate (as product of reaction between thioglycolic acid and ammonium hydroxide) to the mixture of DES-1 and DES-2 provides slightly improved solubility of the rabbit hair compared to the addition of 1% sodium sulfide. In former case depending on the DES-2 content in the mixture of DES the solubility varies from 58 to 79%, in later case—from 51% to 73%. **Note:** In two experiments performed at 1:3 (w/w) ratio of DES-1:DES-2 (the results are not shown in **Table 2**) and the same concentrations of two other components (thioglycolic acid and aqueous solution of ammonium hydroxide), as indicated in **Table 2** for experiment #1, the solubility of rabbit hair was estimated within 77% - 78%.

3) In general, increasing the content of DES-2 in the mixture of two DES enhances solubility of the rabbit hair in case of addition and ammonium thioglycolate and sodium sulfide.

According to data presented in **Table 2**, sodium sulfide addition is not dra-

matically different in performance compared to the ammonium thioglycolate addition. Considering that cost is a very important issue for the practical application, it would be reasonable to emphasize that the DES-based composition with lower content of DES-HP would be more affordable compared to the composition with a high content of this component.

Figure 3 illustrates the preparation of the keratin film made of solution of rabbit hair in the mixture of DES-1 and DES-2 2:1 (w/w) by “precipitation” technique which includes immersion of keratin’s solution (composition #6 in **Table 2**) placed on glass slide into excess of DI water.

In summary, we investigated a new method for the dissolution of animal hair in the mixture of two deep eutectic solvents. Dissolution in existing technologies [32] [33] is typically performed in aqueous media with the use of sulfur reducing products (sulfides, sulfhydrylate or thioglycolate) and strong oxidizers. The sulfur reducing products and oxidizers used at high concentration are very reactive and dangerous to use. Therefore, any attempt to decrease amount of these reagents for regulatory and safety concerns could be of interest for manufacturers involved in dehairing of animal hides. Currently scale-up of proposed method is underway.

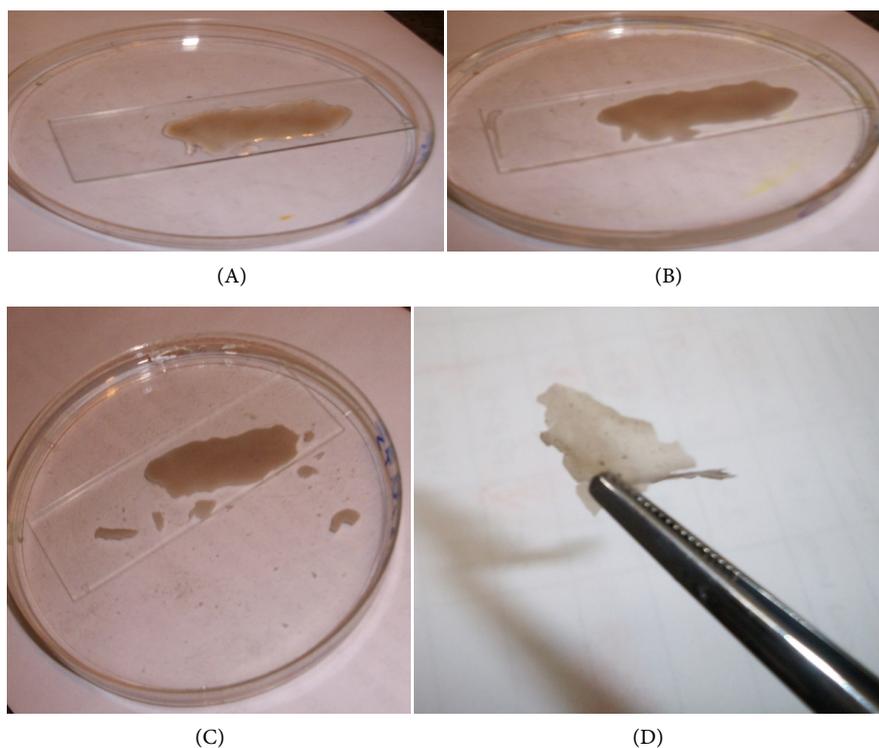


Figure 3. Formation of film from supernatant prepared from composition #6 (**Table 2**) by “precipitation” technique: (A) Appearance of supernatant on the surface of glass slide; (B) Glass slide in the petri dish immediately after adding DI water; (C) the same after 3 hrs at room temperature; (D) Appearance of film after taking it off from the glass slide and drying for 5 hrs at 50°C. **Note:** The composition #6 (**Table 2**) without addition of rabbit hair was placed on the glass slide and subjected to the treatment with DI water under the same conditions as it is shown in (A)-(C). Complete dissolution of the mixture in water was observed.

4. Conclusions

1) We propose a novel method of dissolving animal hair that is using reduced amount of sulfur-containing component and oxidizer compared to known methods. After further investigation and scale-up proposed procedure could be used for developing industrial method of removing animal hair and epidermis and it includes the following steps:

a) Oxidation of animal hair performed by using the mixture of two deep eutectic solvents (DES) one of which is based on choline chloride and urea and another one is based on choline chloride and adduct of urea and hydrogen peroxide. The content of oxidizer (hydrogen peroxide) in proposed method several times is lower than in known processes which are based on using relatively high content of oxidizers, such as peracetic acid (32% aqueous solution) or 50% aqueous solution of hydrogen peroxide.

b) Treatment of hair subjected to oxidation procedure with a reduced amount of sulfur-containing products, such as ammonium thioglycolate (1.94%) of sodium sulfide (1.0% of pure Na_2S or 2.96% of $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$) with addition of minor amount of ammonium hydroxide (below 1%).

2) All proposed materials are biodegradable, non-toxic, don't contain Volatile Organic Compounds and comply with MRSL [34], Chem IQ, [35], REACH [36] and TSCA [37] regulations.

3) The pH of the reaction is between 12 and 13, the reaction time is 10 hours (or below), the process temperature doesn't exceed 30°C.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Koschella, A. and Heinze, T. (2005) Solvents Applied in the Field of Cellulose Chemistry—A Mini Review. *Polímeros: Ciência e Tecnologia*, **15**, 84-90. <https://doi.org/10.1590/S0104-14282005000200005>
- [2] Daly, D. and Rogers, R.D. (2009) Method of Preparing High Orientation Nanoparticle-Containing Sheets or Films Using Ionic Liquids, and the Sheets or Films Produced Thereby. U.S. Patent No. 7550520.
- [3] Fan, L.L., Li, H.J. and Chen, Q.H. (2014) Applications and Mechanisms of Ionic Liquids in Whole-Cell Biotransformation. *International Journal of Molecular Sciences*, **15**, 12196-12216. <https://doi.org/10.3390/ijms150712196>
- [4] Mehrkesh, A. and Karunanithi, A.T. (2016) Predicting Melting point and Viscosity of Ionic Liquids Using New Quantum Chemistry Descriptors. *Fluid Phase Equilibria*, **427**, 498-503. <https://doi.org/10.1016/j.fluid.2016.07.006>
- [5] Xiao, J., Chen, G. and Li, N. (2018) Ionic Liquid Solutions as a Green Tool for the Extraction and Isolation of Natural Products. *Molecules*, **23**, 1765. <https://doi.org/10.3390/molecules23071765>
- [6] Angell, C.A., Ansari, Y. and Zhao, Z. (2012) Ionic Liquids: Past, Present and Future. *Faraday Discussions-Royal Society of Chemistry*, **154**, 9-27.

- <https://doi.org/10.1039/C1FD00112D>
- [7] Price, K.N., Hartshorn, R.T., Rohrbaugh, R.H., Scheper, W.M., Showell, M.S., Baker, K.H., Sivik, M.R., Scheibel, J.J., Gardner, R.R., Reddy, P.K., Aiken, J.D. and Addison, M.C. (2004) Ionic Liquid Based Products and Method of Using the Same. U.S. Patent Application 20040077519.
- [8] Abbott, A.P., Davies, D.L., Capper, G., Rasheed, R.K. and Tambyrajah, V. (2004) Ionic Liquids and Their Use as Solvents. U.S. Patent Application 20040097755.
- [9] Abbott, A.P., Boothby, D., Capper, G., Davies, D.L. and Rasheed, R.K. (2004) Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society*, **126**, 9142-9147. <https://doi.org/10.1021/ja048266j>
- [10] Wasserscheid, P. and Welton, T. (2007) Ionic Liquids in Synthesis. 2nd Edition, Wiley-VCH Verlag, Weinheim, Germany. <https://doi.org/10.1002/9783527621194>
- [11] Wasserscheid, P. and Keim, W. (2000) Ionic Liquids-New "Solutions" for Transition Metal Catalysis. *Angewandte Chemie International Edition*, **39**, 3772-3789. [https://doi.org/10.1002/1521-3773\(20001103\)39:21<3772::AID-ANIE3772>3.0.CO;2-5](https://doi.org/10.1002/1521-3773(20001103)39:21<3772::AID-ANIE3772>3.0.CO;2-5)
- [12] Endres, F. (2012) Interfaces of Ionic Liquids. *Physical Chemistry Chemical Physics*, **14**, 5008-5009. <https://doi.org/10.1039/c2cp90031a>
- [13] Abbott, A.P., Capper, G., Davies, D.L., Rasheed, R.K. and Tambyrajah, V. (2003) Novel Solvent Properties of Choline Chloride/Urea Mixtures. *Chemical Communications*, No. 1, 70-71. <https://doi.org/10.1039/b210714g>
- [14] Abbott, A.P., Barron, J.C., Ryder, K.S. and Wilson, D. (2007) Eutectic Based Ionic Liquids with Metal Containing Anions and Cations. *Chemistry—A European Journal*, **13**, 6495-6501. <https://doi.org/10.1002/chem.200601738>
- [15] Abbott, A.P., Capper, G., Davies, D.L., Munro, H.L., Rasheed, R.K. and Tambyrajah, V. (2001) Preparation of Novel, Moisture-Stable, Lewis-Acidic Ionic Liquids Containing Quaternary Ammonium Salts with Functional Side Chains. *Chemical Communications*, No. 19, 2010-2011. <https://doi.org/10.1039/b106357j>
- [16] Adetunji, C.O., Makanjuola, O.R., Arowora, K.A., Afolayan, S.S. and Adetunji, J.B. (2012) Production and Application of Keratin-Based Organic Fertilizer from Microbially Hydrolyzed Feathers to Cowpea (*Vigna unguiculata*). *International Journal of Scientific & Engineering Research*, **3**, 164-172.
- [17] Idris, A., Vijayaraghavan, R., Rana, U.A., Patti, A.F. and MacFarlane, D.R. (2014) Dissolution and Regeneration of Wool Keratin in Ionic Liquids. *Green Chemistry*, **16**, 2857-2864. <https://doi.org/10.1039/C4GC00213J>
- [18] Wang, D., Yang, X.H., Tang, R.C. and Yao, F. (2018) Extraction of Keratin from Rabbit Hair by a Deep Eutectic Solvent and Its Characterization. *Polymers*, **10**, 993. <https://doi.org/10.3390/polym10090993>
- [19] Chen, J., Vongsanga, K., Wang, X. and Byrne, N. (2014) What Happens during Natural Protein Fibre Dissolution in Ionic Liquids. *Materials*, **7**, 6158-6168. <https://doi.org/10.3390/ma7096158>
- [20] Abbott, A.P., Alaysuy, O., Antunes, A.P.M., Douglas, A.C., Guthrie-Strachan, J. and Wise, W.R. (2015) Processing of Leather Using Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering*, **3**, 1241-1247. <https://doi.org/10.1021/acssuschemeng.5b00226>
- [21] <http://scionix.co.uk/service/performance-auditing>
- [22] Xie, H., Li, S. and Zhang, S. (2005) Ionic Liquids as Novel Solvents for the Dissolu-

- tion and Blending of Wool Keratin Fibers. *Green Chemistry*, **7**, 606-608.
<https://doi.org/10.1039/b502547h>
- [23] Hameed, N. and Guo, Q. (2010) Blend Films of Natural Wool and Cellulose Prepared from an Ionic Liquid. *Cellulose*, **17**, 803-813.
<https://doi.org/10.1007/s10570-010-9411-0>
- [24] Forsyth, S.A., MacFarlane, D.R., Thomson, R.J. and von Itzstein, M. (2002) Rapid, Clean, and Mild O-Acetylation of Alcohols and Carbohydrates in an Ionic Liquid. *Chemical Communications*, 714-715. <https://doi.org/10.1039/b200306f>
- [25] Phillips, D.M., Drummy, L.F., Conrady, D.G., Fox, D.M., Naik, R.R., Stone, M.O., Trulove, P.C., De Long, H.C. and Mantz, R.A. (2004) Dissolution and Regeneration of Bombyx Mori Silk Fibroin Using Ionic Liquids. *Journal of the American Chemical Society*, **126**, 14350-14351. <https://doi.org/10.1021/ja046079f>
- [26] Bai, C., Wei, Q. and Ren, X. (2017) Selective Extraction of Collagen Peptides with High Purity from Cod Skins by Deep Eutectic Solvents. *ACS Sustainable Chemistry & Engineering*, **5**, 7220-7227. <https://doi.org/10.1021/acssuschemeng.7b01439>
- [27] Wang, D., Yang, X.H., Tang, R.C. and Yao, F. (2018) Extraction of Keratin from Rabbit Hair by a Deep Eutectic Solvent and Its Characterization. *Polymers*, **10**, 993-911. <https://doi.org/10.3390/polym10090993>
- [28] Timmons, S.F., Blanchard, C.R. and Smith, R.A. (2000) Porous and Bulk Keratin Bio-Polymers. U.S. Patent No. 6159495.
- [29] Fritchie Jr., C.J. and McMullan, R.K. (1981) Neutron Diffraction Study of the 1:1 Urea: Hydrogen Peroxide Complex at 81 K. *Acta Crystallographica Section B*, **37**, 1086-1091. <https://doi.org/10.1107/S0567740881005116>
- [30] Damavandi, J.A., Bahador Karami, B. and Mohammad Ali Zolfigol, M.A. (2002) Selective Oxidation of N-Alkyl Imines to Oxaziridines using UHP/Maleic Anhydride System. *Synlett*, **6**, 933-934. <https://doi.org/10.1055/s-2002-31903>
- [31] Bendit, E.G. (1960) The Ultraviolet Absorption Spectrum of Solid Keratin. *Journal of the Textile Institute Transactions*, **51**, T544-T561.
<https://doi.org/10.1080/19447026008662584>
- [32] Shavandi, A., Silva, T.H., Bekhit, A.A. and Bekhit, A.E.A. (2017) Keratin: Dissolution, Extraction and Biomedical Application. *Biomaterials Science*, **5**, 1699-1735.
<https://doi.org/10.1039/C7BM00411G>
- [33] Rouse, J.G. and Van Dyke, M.E. (2010) A Review of Keratin-Based Biomaterials for Biomedical Applications. *Materials*, **3**, 999-1014.
<https://doi.org/10.3390/ma3020999>
- [34] https://www.roadmaptozero.com/mrsl_online/
- [35] https://s3.amazonaws.com/content.stockpr.com/vfcsustainability/files/pages/resources/policies-standards/VF+CHEM-IQ+Program_2019_Appendix.pdf
- [36] http://ec.europa.eu/-environment/chemicals/reach/reach_en.htm
- [37] <https://www.epa.gov/compliance/toxic-substances-control-act-tsca-compliance-monitoring>