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Investigation of Novel Deep Eutectic Systems for the Reduction of Water Usage in Leather Manufacture

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Abstract

Water in the wet processes of leather manufacture acts as a solvent, transportation medium, support system and is an integral component of the collagen matrix stability at the macro and molecular levels. There have been proposals as to what extent water can be substituted with non-aqueous media, partly due to its role in collagen and processing. As the water content increases from bulk to float water, the system acts as a transport medium for chemical as well as a support system in processing vessels. Here, Deep Eutectic Solvents (DES) are investigated as an alternative solvent system to conventional tanning systems. The novel solvent is non-aqueous, has a high solubility for metal salts, low toxicity, is readily biodegradable and operates at variable temperatures without the release of harmful vapours. These properties make the DES system a potential alternative in leather processing, improving reagent uptake with the potential of lowering effluent discharge. The research focuses on the role of the solvent associated with stabilising collagen, such as the influence of inter- and intra-fibrillar, as well as bulk water, using type 2 DES systems. Thermal, mechanical analysis and electron microscopy were undertaken to determine the effects of the solvent system. The studies indicated that the solvent used, as well as the choice of counter ion, have an important effect on the thermal and mechanical stability of collagen.

Keywords: Non-aqueous, deep eutectic solvent (DES), ionic liquid, solvent, collagen, thermal stability.

1 – Introduction

In the century and a half of the history of chromium tanning, the industrial process has been conducted in water. Conventionally, leather production needs an average of 40 m³ of water per tonne of raw material during processing (Rydin, Black, *et al.* 2013, Manteiro de Aquim *et al.* 2009). The water in the wet processes of leather manufacture acts as a solvent, transport medium, support system and is an integral part of the collagen (Bella, Brodsky, *et al.* 1995). There are three types of water that can be identified in the collagenic structure; i) intramolecularly bound water, ii) intra- and interfibrillary bound water, which forms a sheath between fibrils and fibres by hydrogen bonding (Lees 2003, Reich 2007, Steiner 2002) and iii) bulk water which acts as a solvent for the chemical modification of native collagen to be transformed into leather (Orgel, Miller, *et al.* 2001). Furthermore, the system acts as a transport medium for chemicals as well as a support system for the material in processing vessels.

Over the years, several attempts have been made to modify the solvent system, either by mixing water with miscible organic solvents or by substituting water by an immiscible organic solvent (Covington 2011). In this research, an entirely different approach is adopted wherein the reagent to be fixed in the tanning reaction is actually part of the solvent system, which is referred to as an ionic liquid or, more strictly, a deep eutectic solvent. Clearly, one of the benefits of this new way of leather processing is the savings which can be made in the requirements for fresh water (Abbott, Alaysuy, *et al.* 2015).

There are four types of deep eutectic solvent and can be generally represented below:

- Type 1: Metal salt + organic salt
- Type 2: Metal salt hydrate + organic salt
- Type 3: Organic salt + hydrogen bond donor
- Type 4: Metal salt (hydrate) + hydrogen bond donor

A deep eutectic solvent (DES) is a type of ionic solvent composed of a mixture of two or more different chemical compounds, held together in a defined spatial arrangement by chemical bonds to have a melting point much lower than either of the individual components involved in the chemical reaction (). The two solid compounds (A and B) shown in Figure 1 are mixed together to obtain an ionic liquid at the eutectic point.

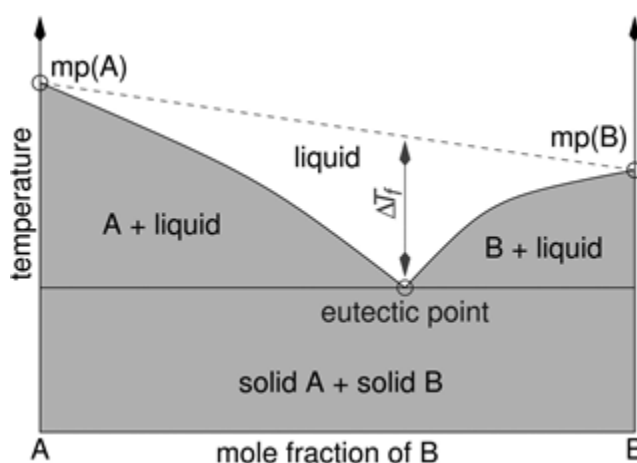


Figure 1: Schematic representation of a eutectic point on a two component phase diagram (Smith, Abbott, *et al.* 2014).

The melting point of the mixture is dependent upon the interaction between the components at a defined pressure. Ionic compounds generally have a high melting point, but the ionic liquid melting point is lower due to the weaker electrostatic forces between oppositely charged ions (Smith, Abbott, *et al.* 2014, Abbott, Capper, *et al.* 2003, Abbott, Boothby, *et al.* 2004). If the eutectic-based systems of known chemical composition are dissolved in water, they revert into their constituent components (Abbott, Capper, *et al.* 2004).

Compared to the currently used solvents, ionic liquids are distinctively beneficial for operating at variable temperatures without the release of harmful vapours (Liu, Zhao, *et al.* 2013). Ionic liquids allow a high solubility of metal salts, low toxicity and are readily biodegradable. These properties make ionic liquids the most promising solvents for the reduction of water in leather processing, improving uptake and possibly lowering the discharge to effluent treatment (Abbott, Alaysuy, *et al.* 2015).

This preliminary study focuses on type 2 (DES), in which the metal salt is chromium (III) chloride hydrate and the organic salt is choline chloride (although other quaternary ammonium salts can be

used) (Abbott, Capper, *et al.* 2004). Here, results are reported from experiments which begin to define the impact of the new reactions and the limits to applicability in the leather industry.

2 – Materials

The materials used for the experiments were wet salted goat skins preserved in accordance to the European standard (EN 16055:2012) and sourced from the Institute of Creative Leather Technologies, University of Northampton, UK. The DES were synthesised with analytical grade chemicals from Sigma-Aldrich, in collaboration with University of Leicester (UK). The distilled water used for processes complied with the International Organisation for Standardisation (ISO 3696:1995).

3 – Methods

Type 2 Deep Eutectic Solvents (DES) were prepared by heating an organic salt and a metal salt hydrate at 70 °C (molar ratio 2:1 of choline chloride: chromium chloride hexahydrate respectively). Initial stirring using a glass rod, shortly after liquid begins to form a magnetic stirrer was utilised to maintain efficient combination of components.

The goat skins were sampled in accordance with the Chemical, Physical and Fastness Test Sampling Location method (ISO 2418:2002). The pieces were processed using a standard recipe (up to and including the delimiting step) to remove unnecessary components such as hair, fats, proteoglycans and globular protein with a final adjustment to a range of pH values: 2, 4, 6 and 8.

An acetone dehydration process was implemented to extract residual water from the goat skin. The goat skin samples were immersed in a bath of acetone which was replaced periodically until there was no observable change in the specific gravity of the solvent in a 24 hour period. Skins were then removed from the acetone bath and dried in a 40 °C oven overnight. The fully dried skin was cut into 1 cm² pieces and ground in a cutting mill (Laarmann, CM100 model, Netherlands) in accordance to the Preparation of Chemical Test Samples (ISO 4044:2008).

All samples were conditioned for 24 hours at 23 °C in conditioning cabinets (Sanplatec, Type A, Japan) each with a fixed relative humidity (0%, 33%, 50%, 76% and 98%) controlled by saturated salt solution (Table 1). The pH of the conditioned samples was measured in accordance with the Determination of pH (ISO 4045:2008).

Table 1: The cabinet conditions of leather samples prior to testing showing the saturated salt used to ensure that atmosphere and the relative humidity of that atmosphere.

Relative Humidity of Atmosphere (%RH)	Solution used
0	P ₂ O ₅ (not in solution)
33	MgCl ₂ ·6H ₂ O
50	RH of conditioned room
76	NaCl
98	CuSO ₄

The goat skin powder was processed with DES in vials with varying conditions. All the experimental trials were carried out in triplicate in a water bath (Grant, OLS 200 model, United Kingdom). The parameters investigated were moisture, water content, pH, temperature, concentration of the chromium in DES and time.

Water and moisture contents were calculated based on conditioned goat skin powder weight and provided as percentages. The pH of the skin powder was altered (2, 4, 6 and 8) during the final process

prior to drying. The temperature of each reaction was varied by altering the temperature of the water bath to the specified reaction temperature, allowing equilibration of the DES temperature before addition of goat skin powder. Concentration was calculated based on chromium content and is expressed as grams per litre.

At the conclusion of each experiment, samples were rinsed with deionised water, vacuum filtered over a Buchner funnel with a Whatman number 541 filter paper. Consequently, the filtered samples were hydrated with distilled water for 24 hours and kept in vials for thermal analysis.

The blotted samples weighing between 2 mg and 8 mg were placed in 30 μ l gold plated high pressure crucibles for analysis using the Differential Scanning Calorimetry (DSC). Measurement was undertaken at a heating rate of 5 $^{\circ}$ C/min for the temperature range from 25 $^{\circ}$ C to 120 $^{\circ}$ C for all experiments.

4 – Results and Discussion

4.1. Effect of moisture content on type 2 DES processed goatskin powder.

The thermal analysis of goatskin powder samples processed at different moisture contents (3 to 24%) showed they did not acquire additional stability when compared with the control T_s 60.4 $^{\circ}$ C \pm 0.8 $^{\circ}$ C ($\rho \geq 0.05$, Kruskal-Wallis test). The molecular water dominates the structure when the skin samples were fully hydrated.

4.2. Effect of water content on type 2 DES processed goatskin powder.

The thermal analysis results in Figure 2 illustrate the necessity for water to act as a transport medium for the DES to interact with the active sites of the collagen triple helix molecule. They show that the shrinkage temperature is dependent on the water content of the starting material, because there is a significant difference ($\rho \leq 0.05$) in shrinkage temperature as the water is increased; however there is no significant difference in the water content region 200% to 500% ($\rho \geq 0.05$). The shrinkage temperature increases compared to the control sample from 60.4 $^{\circ}$ C to 74.2 $^{\circ}$ C at 100% water content because of the requirement of the higher water content in the collagenous structure to facilitate the ionic mechanism of fixation.

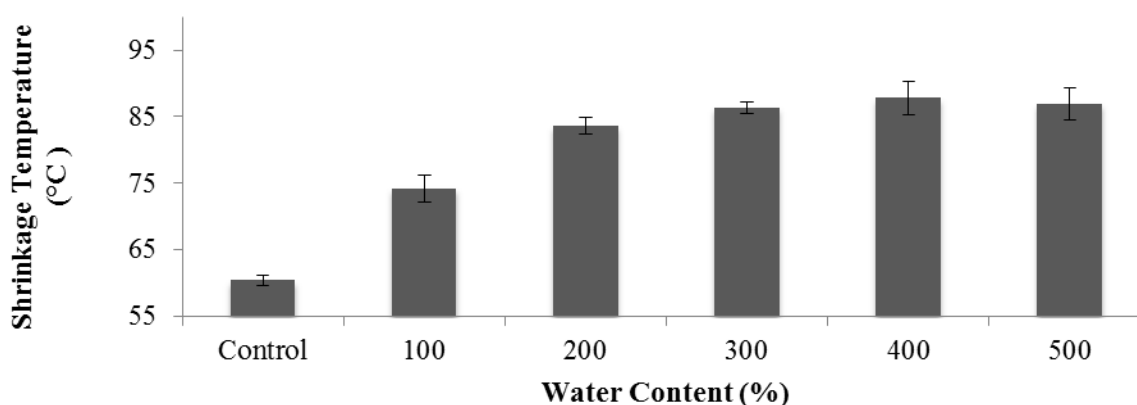


Figure 2: The effect of substrate water content on the hydrothermal stability of chromium tanned goatskin powder processed with the type 2 DES system. Water content was varied by 100% w/w, 200% w/w, 300% w/w, 400% w/w and 500% w/w. Constant parameters: DES concentration of 5 g/l, temperature of 45 $^{\circ}$ C, pH 8 and processed for 24 hours. Control is the native collagen.

The moderate shrinkage temperatures observed are consistent with the view that chromium (III) molecular ions are capable of conferring only moderate hydrothermal stability in the absence of a

second agent for locking the ions together within the supramolecular matrix around the triple helices (Covington 2011).

4.3. Effect of pH on type 2 DES processed goatskin powder.

The thermal analysis results in Figure 3 illustrate that at pH 2, below the isoelectric point when the collagen is positively charged, the type 2 DES species interacted less with the collagen compared with pH 4 and above, more negatively charged carboxyl groups are available. There is a significant difference ($p \leq 0.05$) when the pH increases from 2, however there is no significant difference in shrinkage temperature above pH 4 ($p \geq 0.05$), although the observed trend in diminishing shrinkage temperature may be due to chromium species precipitating and interfering with the fixation reaction.

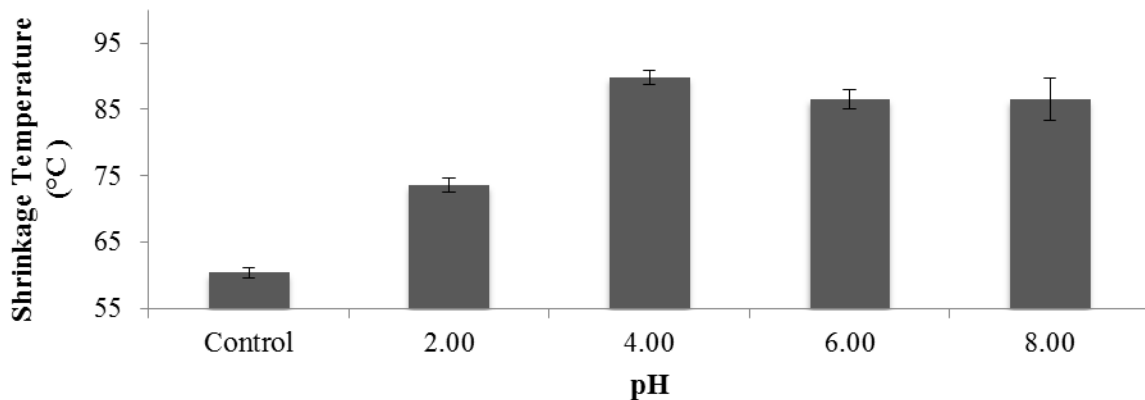


Figure 3: The effect of pH on the hydrothermal stability of goatskin powder processed with the type 2 DES system, pH was varied by 2, 4, 6 and 8. Constant parameters: DES concentration of 5 g/l, temperature of 45 °C, water content of 300% w/w and processed for 24 hours. Control is the native collagen.

4.4. Effect of temperature on type 2 DES processed goatskin powder.

The thermal analysis results in Figure 4 illustrate that temperature from 25 °C to 55 °C does not significantly change the interaction of the type 2 DES species with the collagen ($p \geq 0.05$). The rate of reaction at which the type 2 DES species interact with the collagen appears not to be dependent on the temperature. However, as referred to above, the reaction has reached its maximum effect at the lowest temperature. This means that the process may be more rapid than the conventional aqueous reaction, which is highly dependent on temperature.

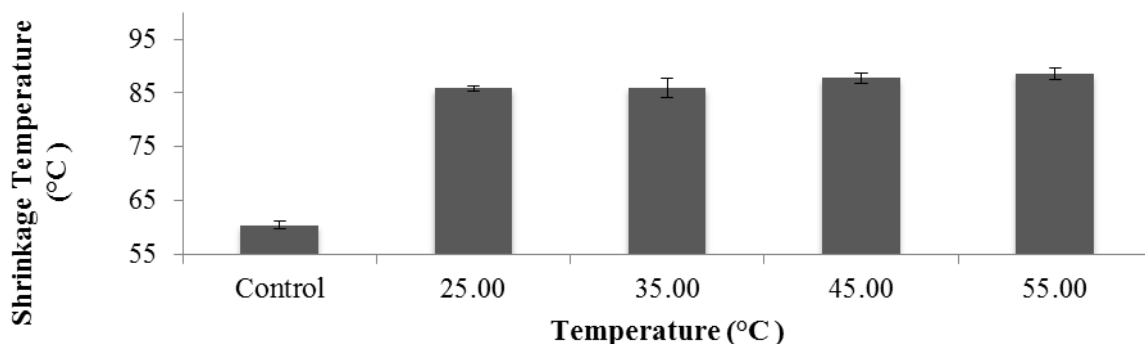


Figure 4: The effect of temperature on the hydrothermal stability of goatskin powder chromium tanned with the type 2 DES system. Temperature was 25 °C, 35 °C, 45 °C and 55 °C. Constant parameters: DES concentration of 5 g/l, water content of 300% w/w, pH 8 and processed for 24 hours. Control is native collagen.

4.5. Effect of type 2 DES concentration on processed goatskin powder.

The higher the concentration of a reactant, the more chance the molecules in the type 2 DES have in colliding with the active sites of the collagen, as indicated by the Figure 5, so the shrinkage temperature increases with increasing concentration from 1.25 g/l to 5 g/l. There is a significant difference ($p \leq 0.05$) when the concentration increases from 1.25 g/l to 5 g/l, but further increase in the concentration does not show a significant difference ($p \geq 0.05$).

As already discussed, these values are approaching the maximum possible value of shrinkage temperature with the concentration of 5 g/l under these experimental conditions.

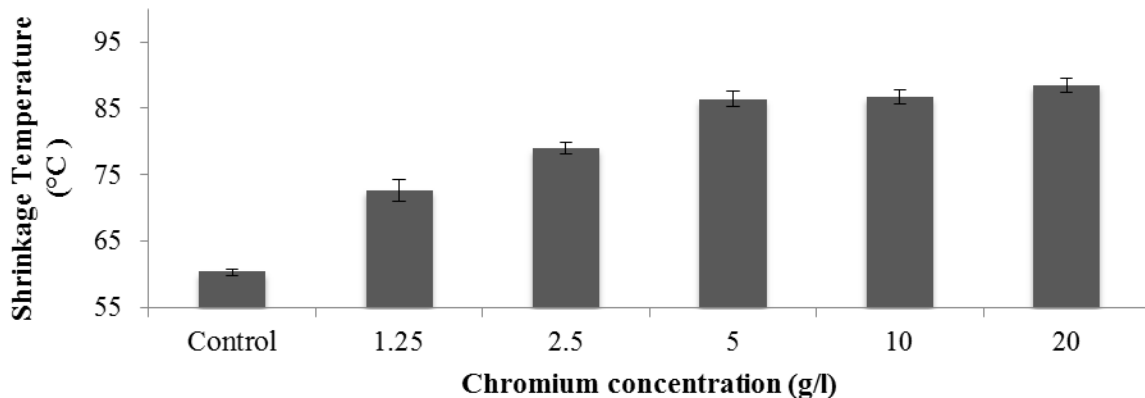


Figure 5: The effect of chromium concentration on hydrothermal stability of goatskin powder processed with the type 2 DES system. Concentration was varied: 1.25 g/l, 2.5 g/l, 5 g/l, 10 g/l and 20 g/l. Constant parameters: water content of 300% w/w, temperature of 45 °C, pH 8, and processed for 24 hours. Control is the native collagen.

4.6. Effect of incubation period on type 2 DES processed goatskin powder.

The thermal analysis of samples processed for different times illustrates that reaction period does not significantly change the interaction of the type 2 DES species with the collagen ($p \geq 0.05$), so, under the given experimental conditions, maximum shrinkage temperature was achieved within two hours. This indicates that the fixation reaction is rapid and may be faster than the conventional process.

4.7. Effect of different counter-ions on type 2 DES processed goatskin powder

The thermal analysis results in Figure 6 demonstrate the effect of washing the type 2 DES chromium tanned goatskin powder with a solution of sodium sulfate. This resulted in an elevated shrinkage temperature.

The rationale of the experiment relates to the link-lock theory of tanning (Covington 2010). Moderate shrinkage temperature is achieved by chromium (III) molecular ions acting individually in the supramolecular matrix, the 'polymer in the box' (Miles, Avery, *et al.* 2005), merely interfering with the shrinking mechanism. The presence of ions such as sulfate, which can interact powerfully with the aquo ligands of the chromium (III) molecular ions via hydrogen bonding, creates a new matrix which resists shrinking strongly and is observed as high shrinkage temperature.

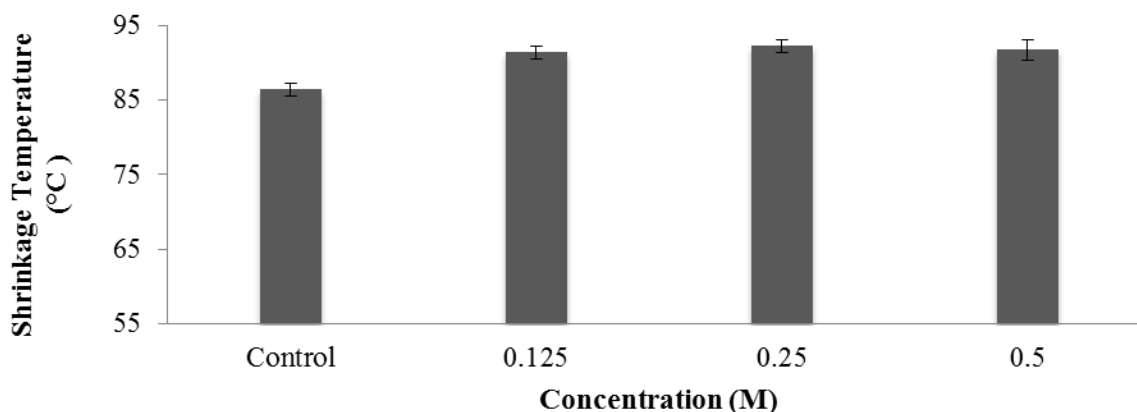


Figure 6: Effect of the sulfate counterion on the hydrothermal stability of the type 2 DES processed goatskin powder. The concentration of sodium sulfate was 0.125 M, 0.25 M and 0.5 M. Control is the DES tanned goat skin T_s $86.4\text{ }^\circ\text{C} \pm 0.8\text{ }^\circ\text{C}$.

The resulting shrinkage temperatures are elevated in comparison with the untreated control, but there is no apparent effect due to sulfate offer, nor are the values as high as conventionally chromium tanned leather. However, this may merely be the influence of the experimental conditions and the nature of the substrate. But, the principle is demonstrated.

The results obtained in this preliminary study are consistent with what is already known about the conventional chromium tanning process. This might be considered surprising, because the chromium species in the ionic liquid are in a very different chemical state compared to solute chromium sulfate tanning salt in aqueous solution. Nevertheless, it has been demonstrated that chromium fixation can occur and the reaction appears to be faster and less reliant on specific conditions than the water-based process.

However, the understanding of the new reaction must be tempered by the recognition that moderate shrinkage temperature is easy to achieve. What is less easy is the attaining of high hydrothermal stability, which is only possible by satisfying the requirements of the link-lock sequence of events. It is those elements of the mechanism which have to be incorporated into the new ionic liquid process if it is to rival conventional operations.

5 – Conclusion

The application of ionic liquids in the heterogeneous technology of leather making is a paradigm shift. The delivery of chromium (III) molecular ions into the collagen matrix is fundamentally different from conventional processing and the results reflect the difference. However, it is clear that the mechanism of chromium fixation via the structural water around the collagen triple helix may not be significantly adversely affected. Therefore, it can be assumed that there is value in pursuing these studies, in particular to investigate the novel chemistry options offered by the myriad variations of ionic solvents possible. This is important in extending the properties and performance of leather and other biomaterials.

6 – References

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