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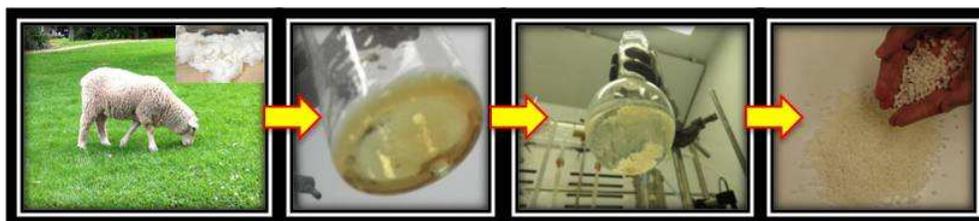


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## Dissolution and regeneration of wool keratin in ionic liquids

Azila Idris, R. Vijayaraghavan, Usman Ali Rana, A. F. Patti and D. R. MacFarlane

Substantial dissolution of wool was obtained in [AMIM][dca] and [choline][thioglycolate] ionic liquids as well as deep eutectic solvent mixtures under a variety of conditions including the use of a reducing agent.



## Dissolution and regeneration of wool keratin in ionic liquids

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### ABSTRACT

Wool keratin, a natural biopolymer, is potentially an important *renewable* source of raw materials for the polyamide plastics industry. Large quantities of non-spinnable and short fibers of wool are discarded globally and hence are available as low value waste materials. In this study, we have investigated different solvents, including ionic liquids and deep eutectic mixtures, for the dissolution and processing of wool. The results show that substantial dissolution of wool (up to 475 mg wool per gram of solvent) can be obtained in 1-allyl-3-methylimidazolium dicyanamide [AMIM][dca] ionic liquid at 130 °C. Our studies also indicated enhanced dissolution (an additional 50-100 mg/g) of wool upon the addition of a reducing agent to the

ionic liquids. Water insoluble fractions (20-40%) were obtained on the addition of water to the dissolved wool. This regenerated fraction was characterized for structural and chemical changes and found to contain a larger fraction of  $\beta$ -sheet and random coil than the starting material. The water soluble fraction was characterised and the results indicated the presence of fragments of low molecular weight polypeptide chains.

## 1. INTRODUCTION

Biopolymers have gained significant attention as new alternatives to petroleum based materials. Although recent research focuses more on cellulosic fiber, it is also possible to consider biopolymer based protein fibers as a potential renewable source of polyamides.<sup>1</sup> Keratin is one of the most abundant biopolymers in this class and is available from a variety of sources. Wool keratin is one of these sources and large quantities of non-spinnable and short wool fibers are available globally as waste from the textile industry. These are currently discarded and, as a result, alternative uses of these materials is attracting the attention of researchers.<sup>2,3</sup>

Wool is a fibrous protein (approximately 95 wt. % pure keratin<sup>4</sup>) that consists of about 11-17 % cysteine.<sup>5-7</sup> This protein is insoluble in water, organic solvents, dilute acids, alkalis and shows resistance to degradation in common solvents.<sup>8</sup> This is due to the tight packing of the  $\alpha$ -helix and  $\beta$ -sheets present in the polypeptide structure of wool keratin. In addition, the presence of inter- and intramolecular bonding of polar and nonpolar amino acids and strong disulfide bonds in wool keratin provides conformational constraints to the polypeptide backbone and stability to the

protein structures.<sup>9, 10</sup> The diameter of the fibers varies significantly among different sheep breeds,<sup>11, 12</sup> between 11.5 and 47  $\mu\text{m}$ .<sup>13</sup>

Solubilisation of wool, which requires partial disruption of the keratin structure, is difficult but can be achieved in a number of ways, including reduction,<sup>1, 14, 15</sup> oxidation<sup>1, 15, 16</sup> and sulfitolysis of the disulfide bonds<sup>1, 17, 18</sup>. The reagents used in these reactions are, however, often toxic and difficult to recycle. These limitations have inspired researchers to develop new dissolution techniques, including the use of ionic liquids (ILs). Much has been published on the application of ILs as solvents for the dissolution of biopolymers such as cellulose, silk, starch, lignin and saccharides.<sup>19-26</sup> Studies of keratin dissolution have been more limited<sup>2, 3, 27, 28</sup> and mostly focused on feather keratin<sup>27, 29-31</sup> because of the greater availability of feather raw material. One of the significant differences between these types of keratin is the difference in cysteine content; feather and wool keratin contain ~ 7% and 11-17% cysteine units in their amino acid sequences, respectively.<sup>5-7</sup> The higher cysteine content in wool makes this biomass more difficult to dissolve.<sup>32, 33</sup> On the other hand, the higher cysteine content offers the potential of higher glass transition ( $T_g$ ) materials if the S-S bonding is preserved or reformed in the regenerated material.

Here we investigate a number of ionic liquids and deep eutectic solvents for the dissolution and regeneration of wool keratin with the ultimate aim of providing the basis for a process which could utilize waste wool fibres to produce useful materials. Deep eutectic solvents were also studied since these exhibit many remarkable solvent properties including for biopolymers,<sup>34, 35</sup> and are easy to synthesize from inexpensive starting materials.<sup>34, 36, 37</sup> There are several additional variables yet to be explored to improve the solubilisation of wool, including the influence of a reducing agent for cleavage of the disulphide bonds; the dissolution experiments here were

conducted with and without a reducing agent. Although many reagents are capable of reducing the disulphide bond, only a few such as thiols<sup>38</sup> and ammonium bisulfite have the required ability and reactivity to simultaneously preserve and maintain the peptide bonds of the protein structure. In this study, 2-mercaptoethanol was investigated for this purpose.<sup>1, 39</sup> Structural elucidation of the materials obtained was carried out by <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectroscopy (electron spray ionisation) along with XRD, ATR-FTIR, thermal analysis and gel electrophoresis.

## 2. EXPERIMENTAL

### 2.1 Materials

The medium Merino wool (approximately 23  $\mu\text{m}$  diameter) used in these experiments was provided by Australian Wool Innovation Limited (AWI). It was cleaned and defatted using a 1:1 v/v of hexane and dichloromethane solvent mixture in a Soxhlet extractor for 48 hours. The cleaned wool sample was dried in a vacuum oven at 70  $^{\circ}\text{C}$  for 48 hours. The remaining, “bound” water fraction was quantified by TGA at approximately 10%. Since this bound water is difficult to remove prior to dissolution, our process design needed to recognize that residual water was always present. This is in distinct contrast to lignocellulose dissolution processes in ILs<sup>26, 40</sup> in which low water content is an important aspect.

1-butyl-3-methylimidazolium chloride ([BMIM]Cl, 98% purity) was purchased from Sigma Aldrich. 1-allyl-3-methylimidazolium chloride, [AMIM]Cl and 1-allyl-3-methylimidazolium dicyanamide, [AMIM][dca] and choline thioglycolate were synthesized according to literature<sup>27, 41</sup> procedures. For the preparation of these ILs, allylchloride (98%), *N*-methylimidazole (99%), choline hydroxide (20 wt% in H<sub>2</sub>O), thioglycolic acid (98%), silver

nitrate (99%) and sodium dicyanamide (96%) were obtained from Sigma Aldrich. For NMR analysis, deuterated chloroform ( $\text{CDCl}_3$ ) and deuterated methanol ( $\text{CD}_3\text{OD}$ ) purchased from Merck was used. Unless otherwise stated, all organic solvents and reagents were used as received from commercial suppliers. Water contents, as indicated by mass loss around 100 °C in the TGA data (Figure S1), were below 1% for the chloride ILs, and between 1% and 5% for the thioglycolate and dca ILs, both of which are difficult to completely dry. However, it is important to note that since wool is also contains difficult to remove bound water, our goal was to study a process that could be carried out under practical conditions and typical water contents.

## 2.2 Dissolution of wool

The solubility of wool fibre was determined at 130 °C for 10 hours in glass vials which were placed into a heating block under a  $\text{N}_2$  atmosphere. In order to quantify the solubility, 50 mg quantities of the wool fibers were sequentially added to the ionic liquid (2 g) and continually stirred with a magnetic stirrer until the fiber was observed visually to have completely dissolved (at 130 °C the solution was sufficiently fluid for this to be practical). In addition, a laser beam was used to identify the presence of small particles, through observation of any light scattering from the solutions. In some cases, particularly at high wool contents, the rate of wool dissolution appeared to decrease markedly, probably due to the increasing viscosity of the solution; for this reason we describe these ultimate observations as “limiting solubility” to indicate that these are kinetically limited values rather than thermodynamic solubilities.

The regeneration of the dissolved wool keratin was achieved through the addition of water (30 ml) to the IL solutions. The precipitate was separated using a centrifuge at 5000 rpm for 25 minutes. The regenerated keratin obtained was washed 3x with water (40 ml) via centrifugation

in order to remove any ionic liquid that may be retained in the regenerated material. Then, it was dried *in vacuo* for 3 days at 45 °C. The regenerated solid keratin obtained was a light yellowish brown color. The water - ionic liquid solution from the washing of the precipitate was then evaporated by rotary evaporator to leave the water soluble keratin fraction and the ionic liquid. Thus far, we have not been able to separate the soluble fraction of keratin from the IL. However we have been able to identify the soluble proteins using SDS-PAGE analysis, as described below.

The powder X-ray diffraction (PXRD) patterns were obtained at  $22 \pm 2$  °C using a Sietronics powder diffractometer. For each XRD experiment, approximately, 1-2 g of the finely ground sample was placed randomly on a locally designed flat brass sample holder fitted with an o-ring sealed Mylar sheet cover, providing an airtight atmosphere.  $\text{CuK}\alpha 1$  radiation ( $\lambda = 1.540$  Å) was produced at 40 kV and 25 mA. The data were collected in the Bragg-Brentano ( $\theta/2\theta$ ) horizontal geometry using a  $2\theta$ -range of 5 to 50.0° ( $2\theta$ ) range with a step size of 0.02° and an accompanying scan rate of 0.5 ° / min.

Fourier transform infrared spectra were obtained using a Bruker IFS Equinox FTIR system coupled with a Golden Gate single bounce diamond micro-Attenuated total reflectance crystal and a liquid nitrogen cooled Mercury/Cadmium Telluride detector. The FTIR was performed in the wavenumber range of 600  $\text{cm}^{-1}$  to 4000  $\text{cm}^{-1}$ . The spectra were recorded with a resolution of 4  $\text{cm}^{-1}$  with 50 scans. Spectra were baseline corrected.

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 400 MHz on a Bruker DPX-400 spectrometer. All samples were measured as solutions in deuterated chloroform and deuterated methanol. Chemical shifts are reported in ppm on the  $\delta$  scale, and the coupling constant is given in Hz. Chemical shifts were calibrated on the solvent peak unless otherwise specified. Solid state 1D  $^1\text{H}$  static NMR spectra of neat and regenerated samples were acquired at a Larmor frequency of 300 MHz on a Bruker AV-300 spectrometer. The  $^{13}\text{C}$  CP MAS NMR spectra of these samples were acquired using a 4 mm rotor with Kel-f cap at 10 kHz spinning rate. The contact time in the CP MAS experiments was 2.4 msec with a recycle delay of 1 sec and cw decoupling. The number of scans was  $\sim 90,000$  to  $100,000$ . In the case of 1D  $^1\text{H}$  static NMR experiments, the pulse length was  $\sim 3.6$   $\mu\text{sec}$  with a recycle delay of 5 sec and 16 to 20 scans.

Differential scanning calorimetry (DSC) was conducted on a DSC Q100 series instrument from TA Instruments with 5-10 mg of sample in closed aluminium pans, at a ramp rate of  $10$   $^\circ\text{C}$  per minute. All samples were cooled to  $-150$   $^\circ\text{C}$ , held for 5 minutes and heated to  $200$   $^\circ\text{C}$ . Thermal scans below room temperature were calibrated via the cyclohexane solid-solid transition and melting point at  $-87.0$   $^\circ\text{C}$  and  $6.5$   $^\circ\text{C}$  respectively. Thermal scans above room temperature were calibrated using indium, tin and zinc with melting points at  $156.6$   $^\circ\text{C}$ ,  $231.9$   $^\circ\text{C}$  and  $419.5$   $^\circ\text{C}$  respectively. Transition temperatures are reported using the peak maximum of the thermal transition.

The thermal stability of the neat wool and regenerated materials was investigated by TGA using a Pyris 1 in a flowing dry Argon atmosphere between  $25$   $^\circ\text{C}$  and  $500$   $^\circ\text{C}$ , at a heating scan rate of  $10$   $^\circ\text{C}$   $\text{min}^{-1}$ . The samples were first dried under vacuum in an oven at a temperature of  $70$

°C. These samples were then loaded into aluminum crucibles and equilibrated for 15 minutes at the starting temperature of 25 °C before running each experiment.

Electrospray ionisation mass spectra were recorded on a Micromass Platform II API QMS Electrospray Mass Spectrometer. Samples dissolved in methanol were subjected to a suitable cone voltage, usually 25 V to 35 V. Measurements were made in both the positive and negative modes.

Protein samples in the water soluble fraction were diluted in 4 x NuPAGE® loading buffer (Life Technologies) and electrophoresed using the Hoefer miniVE vertical electrophoresis system (Amersham Biosciences) in 4-12% Bis-Tris NuPAGE® gradient gels (Life Technologies). Proteins were stained and visualised by silver staining.

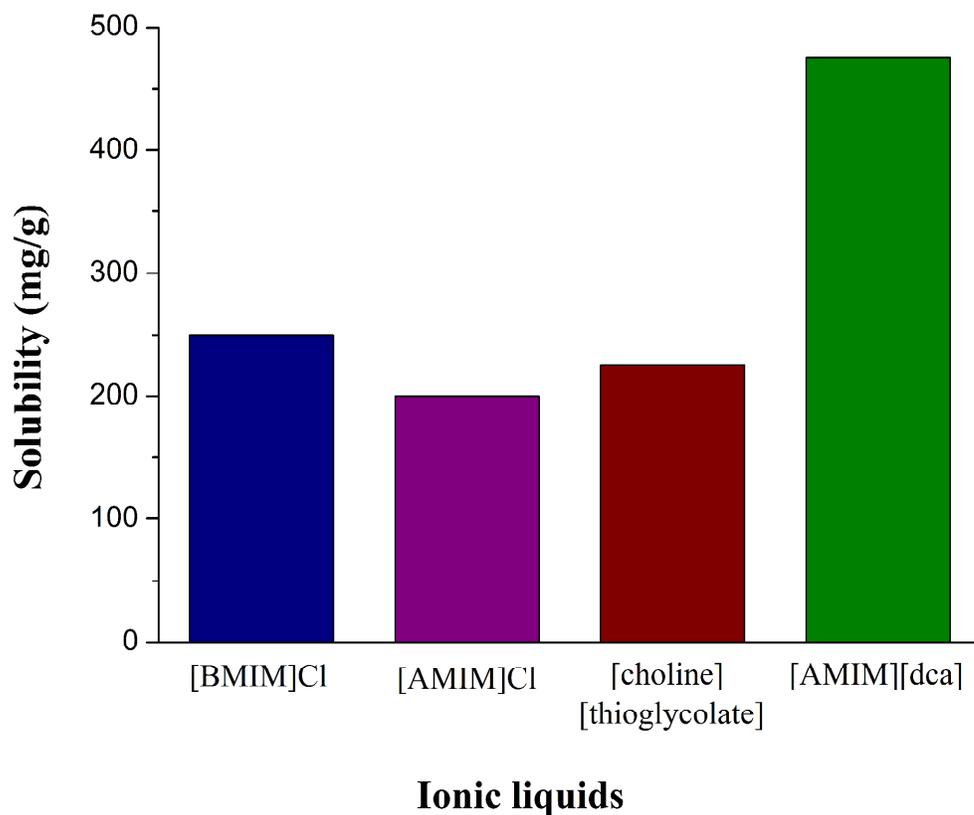
### 3. RESULTS AND DISCUSSION

#### *Dissolution and regeneration of wool keratin in ionic liquids*

Prior to starting the dissolution process, the stability of all ionic liquids used in this work was studied. The TGA data of all of the ILs has been included in the supplementary information (Figure S1) and the ILs showed small weight loss between 100 °C and 130 °C due to water loss. Thereafter, they were stable until decomposition above 200 °C. This temperature was chosen for the dissolution studies since better solubility was observed at this temperature in literature reports of wool dissolution by other methods.<sup>2</sup>

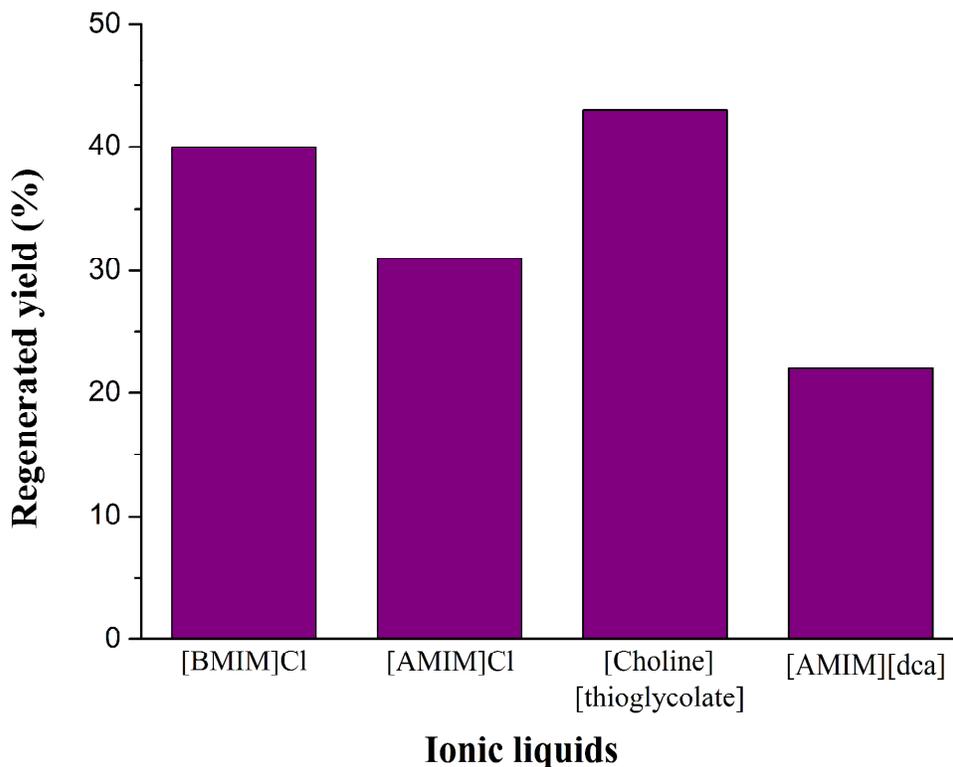
The apparent limiting solubility results for wool fibre in [BMIM]Cl, [AMIM]Cl, [choline][thioglycolate], and [AMIM][dca] are shown in Figure 1. As the solutions become viscous, the dissolution rate slows considerably, hence it is likely that the true solubility limit is even higher than these results. The highest solubility was found to be 475 mg(wool)/g(IL) in [AMIM][dca]. Solubility in [choline][thioglycolate] was 225 mg/g. The results for [BMIM]Cl and [AMIM]Cl are in approximate agreement with the work of Li *et al.*<sup>42</sup> who observed 250 mg/g and 200 mg/g dissolution respectively. Xie *et al.*<sup>2</sup> observed significantly lower solubility for the chloride salts, however neither paper identifies the type of wool and fiber diameter involved and it is likely that this factor will have some effect on the apparent rate and extent of solubility. From this it appears that the [dca] anion plays a significant role in effecting solubility compared to the other ionic liquids. It is notable that the viscosity of the solutions are lower in the dca case.<sup>43</sup> In the literature,<sup>44</sup> it was also reported that the high solubility of carbohydrates (glucose and xylose) in [BMIM][SCN] is due the strong [SCN] hydrogen bond interactions; similar effects might be expected of the dca anion and hence the high solubility for keratin observed here.

It is interesting to compare the treatment conditions developed here with those that have emerged in the dissolution of lignocellulose materials. Despite the distinct difference in the chemical nature of the biopolymers involved (polypeptide in the case of keratin as opposed to polysaccharide and poly aromatic ether in the cases of cellulose and lignin respectively) the dissolution conditions are quite similar. The conditions required in the lignocellulose case have been reviewed recently by Da Costa Lopes *et al.*<sup>26</sup> and broadly involve temperatures above 100°C for multiple hours of treatment, similar to the times and temperatures that appear to be necessary for keratin dissolution.<sup>2, 27</sup>



**Figure 1.** Solubility of wool in ionic liquids.

Figure 2 shows the fraction of aqueous-insoluble material regenerated by precipitation from water in each case. It has been reported that wool keratin consists of approximately 40 % hydrophilic and 60 % hydrophobic groups in the amino acid sequence.<sup>6</sup> Therefore, if some degree of cleavage of the protein occurs then it can be expected that a fraction of the material will become soluble in water, as observed. The nature of this water soluble fraction that is generated is characterised further below. The fraction of regenerated material that precipitated from the water-[AMIM][dca] mixture was found to be lower than other ILs, perhaps due to the ability of the dicyanamide anion to disrupt the hydrogen-bonding in the protein aggregates and this H-bonding is not easily reformed in some cases to regenerate the insoluble material.



**Figure 2.** Regenerated yield of aqueous insoluble wool keratin from ionic liquids.

#### Dissolution of wool in deep eutectic solvents

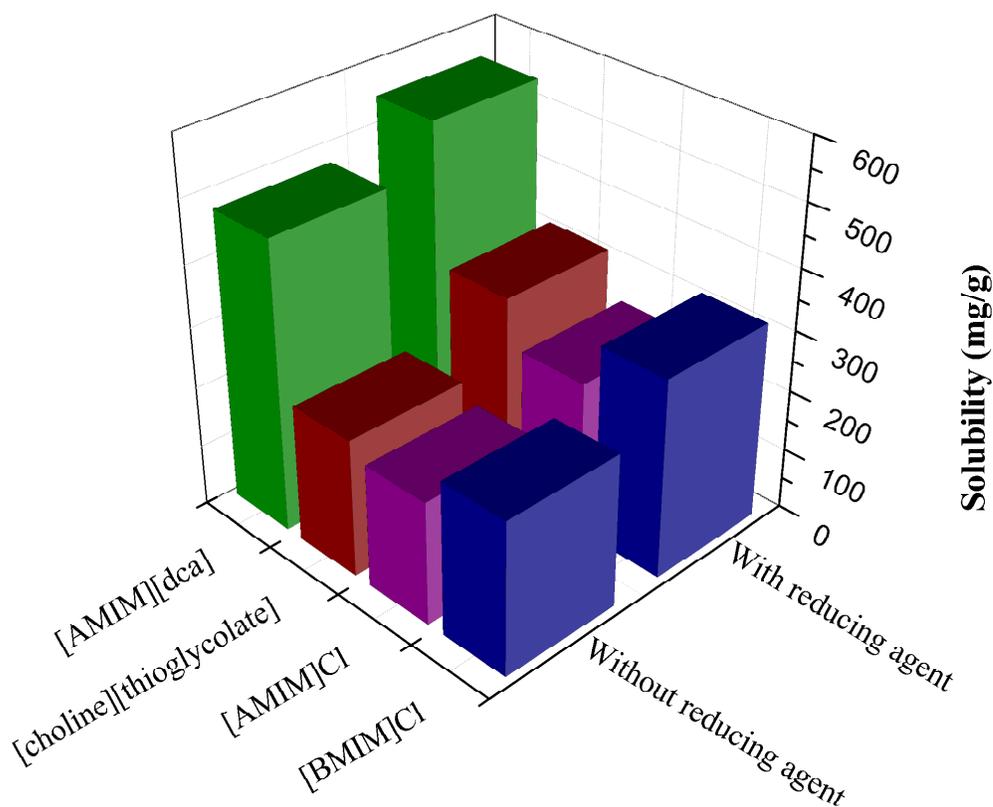
Deep eutectic mixtures were also studied as inexpensive alternatives to ionic liquids. Compositions and solubility characteristics of wool in the deep eutectic solvent mixtures are shown in Table 1. It can be seen that wool dissolved to a small extent in mixtures of choline chloride/urea and choline chloride/oxalic acid in a 1:2 mole ratio. No wool solubilisation was noted for calcium chloride/urea. Thus, although Biswas *et al.*<sup>45</sup> reported that mixtures of choline chloride/zinc chloride and choline chloride/oxalic acid are excellent solvents for dissolution of starch<sup>45</sup> and cellulose,<sup>46</sup> it appears that these solvents are not effective for keratin dissolution.

**Table 1.** Solubility of wool in deep eutectic solvent mixtures.

Component A	Component B	Molar ratio (A:B)	Solubility (mg/g $\pm$ 12.5)
choline chloride	urea	1:2	120
choline chloride	oxalic acid	1:2	30
calcium chloride	urea	1:2	insoluble

### Influence of reducing agents

Figure 3 shows the solubility of wool in ionic liquids with and without a reducing agent. In each case, the inclusion of a reducing agent resulted in an increase in limiting solubility. This action is thought to be due to the cleaving of the inter- and intra- molecular disulphide bonds via a reduction reaction.<sup>39, 47</sup> The breaking of disulphide bonds disrupts the tertiary and quaternary structures of the wool protein, as well as allowing the breakdown of the wool keratin into smaller fragments.<sup>39</sup>

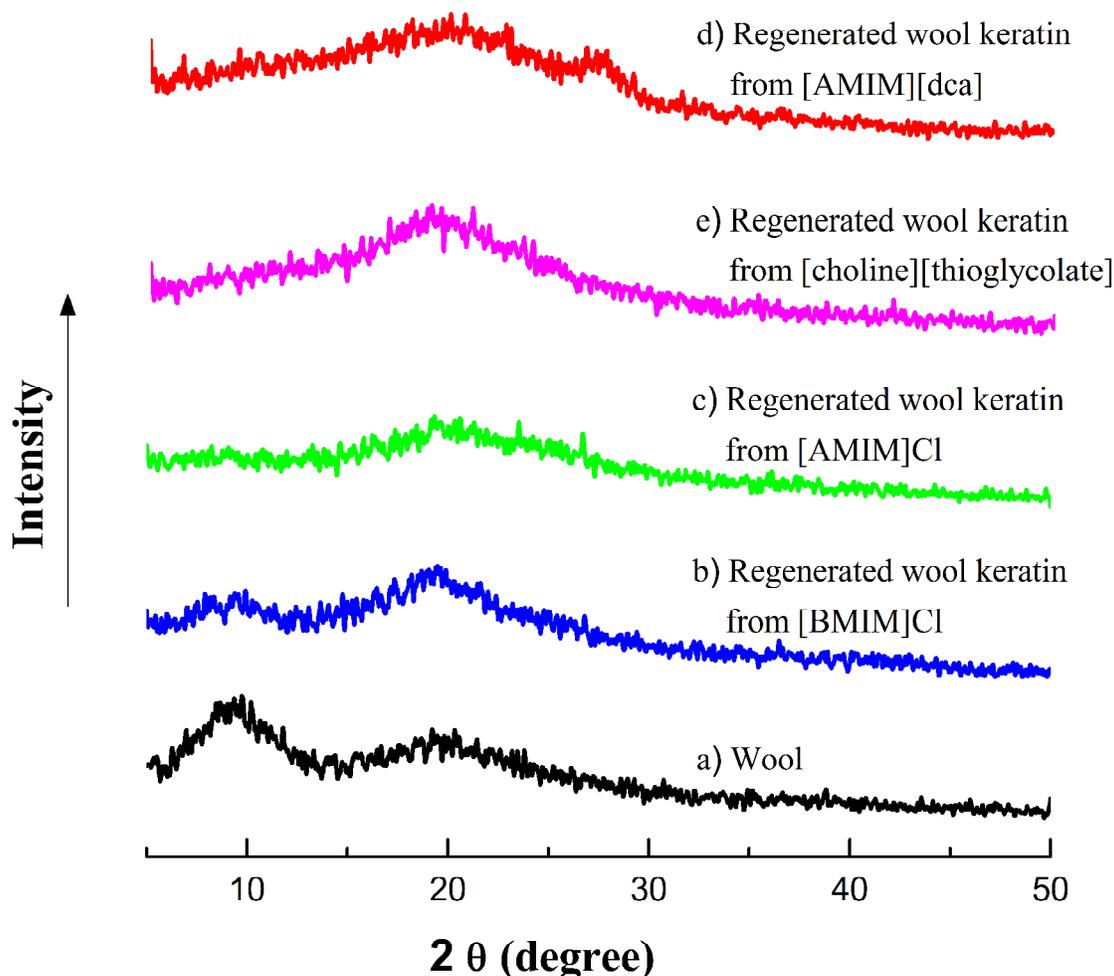


**Figure 3.** Solubility of wool in ionic liquids, with and without a reducing agent.

#### Characterisation of regenerated wool keratin

The X-ray diffraction (XRD) spectra of the raw wool and regenerated wool keratin materials are presented in Figure 4. Two crystal structures are typically observed for raw wool;<sup>48, 49</sup> an  $\alpha$ -helix structure is manifest in peaks appearing at  $2\theta = 9^\circ$  (0.98 nm) and  $17.8^\circ$  (0.51 nm) whereas the  $\beta$ -sheet structure shows peaks appearing at  $2\theta = 9^\circ$  (0.98 nm) and  $19^\circ$  (0.47 nm). Compared to raw wool, the regenerated wool keratin samples (Figure 4(c)-(e)), the XRD study clearly showed the disappearance of the peak at about  $9^\circ$ . This indicates that the crystallinity of these regenerated samples is significantly reduced by the dissolution and regeneration process. In the

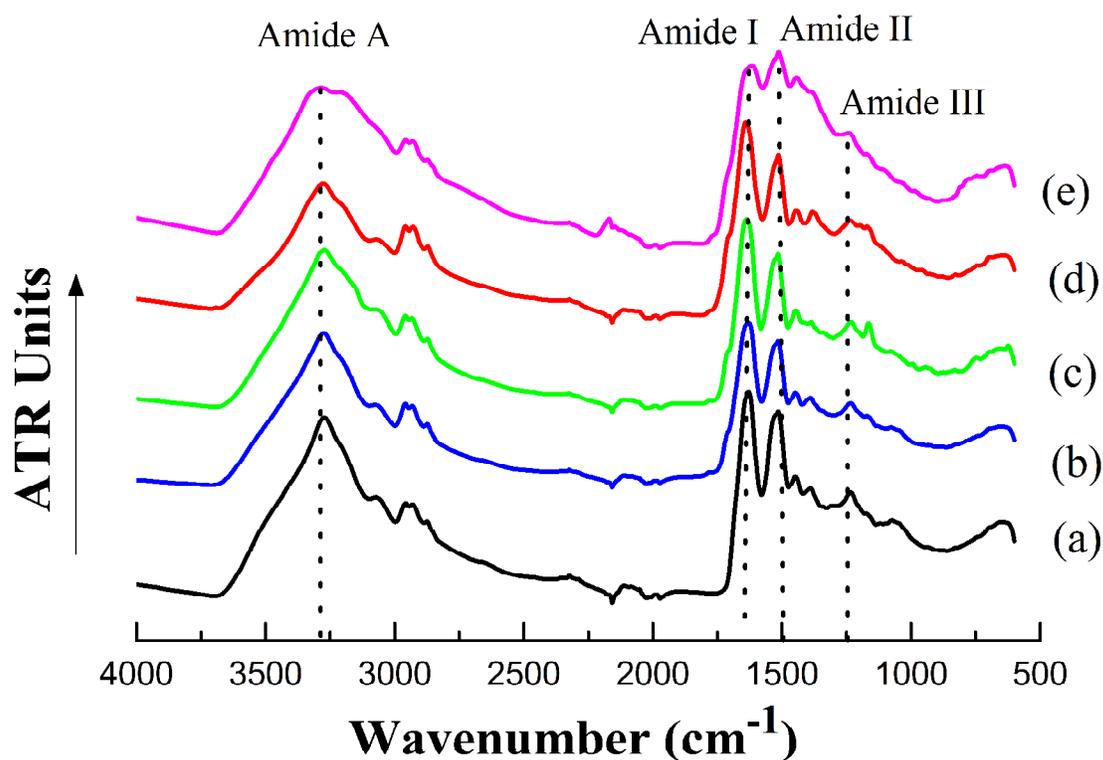
case of the regenerated material from [AMIM][dca] (Figure 4(d)), an additional broad diffraction peak appeared, centered around  $2\theta = 27^\circ$ . This observation suggests the formation of a disordered/amorphous material.<sup>50</sup>



**Figure 4.** XRD patterns of wool and regenerated wool keratin.

In Figure 5, the ATR-FTIR spectra of raw wool and regenerated keratin are compared. The spectra showed characteristic absorption bands ascribed predominantly to the peptide bonds (-CONH) and these have been labeled as Amide A, Amide I, Amide II and Amide III bands. A

medium absorption band in the range of 3283-3273  $\text{cm}^{-1}$  is attributed to N-H stretching (Amide A), while Amide I showed a strong absorption band that occurred in the range of 1640-1614  $\text{cm}^{-1}$ . A strong band was observed in the range of 1516-1513  $\text{cm}^{-1}$  and assigned to C-N stretching and N-H bending vibrations (Amide II). Another weak band was recorded in the range of 1234-1242  $\text{cm}^{-1}$  indicating the C-N, C-O stretching, N-H and O=C-N bending vibrations (Amide III).<sup>32, 51, 52</sup> No additional bands were seen in the ATR-FTIR of the regenerated samples. However, the  $^{13}\text{C}$  NMR results indicate that some chemical changes have occurred and these are described below.



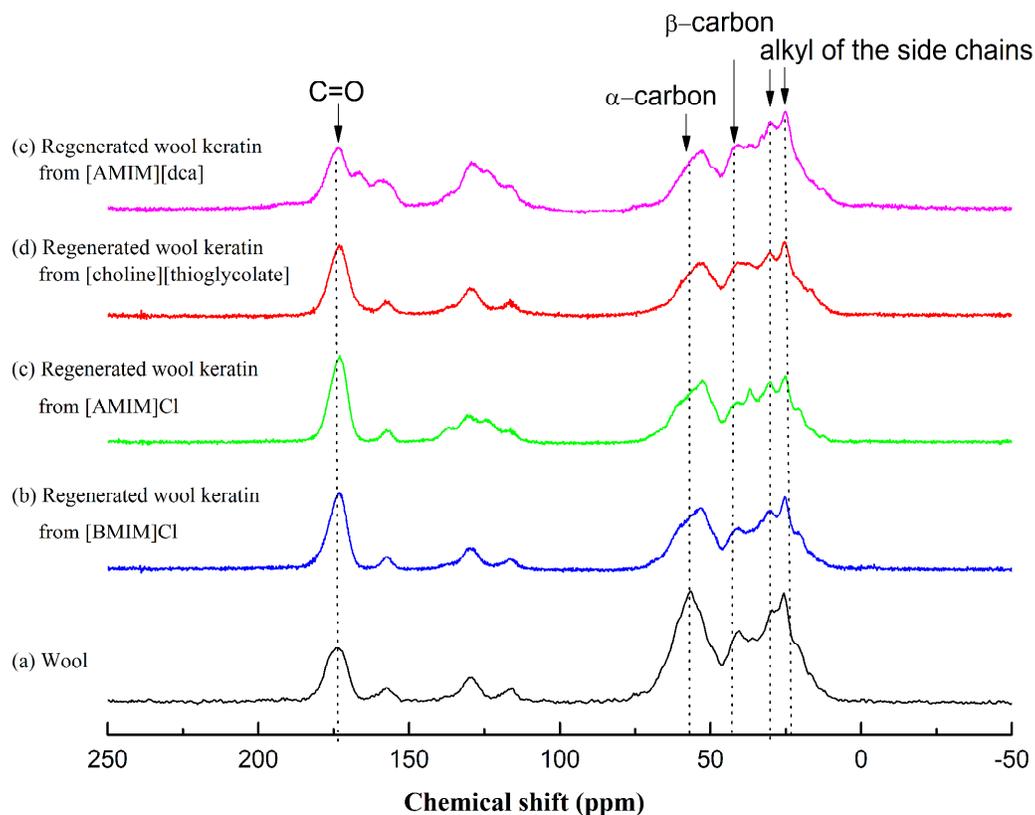
**Figure 5.** ATR-FTIR of (a) wool and regenerated wool keratin from (b) [BMIM]Cl, (c) [AMIM]Cl, (d) [choline][thioglycolate], (e) [AMIM][dca].

$^{13}\text{C}$  CP MAS spectra of the raw and regenerated keratin materials are shown in Figure 6. The spectra show an asymmetric peak with a maximum between 172 ppm to 174 ppm. This peak is attributed to the amide carbonyl carbons of the keratin protein. The peak at 175 ppm is assigned to the  $\alpha$ -helix of keratin, while that at 172 ppm is attributed to the  $\beta$ -sheet molecular and random coil conformations.<sup>53-55</sup> The peak at around 130 ppm indicates the presence of aromatic group containing amino acids in the keratin.<sup>53</sup> The  $\alpha$ -carbons were recorded between 52 ppm and 56 ppm, while the  $\beta$ -carbons (present in leucine and cross-linked cysteine residues) were observed at 40 ppm.<sup>54</sup> The carbon peaks recorded between 30 ppm and 40 ppm indicated the presence of proline, glutamic acid and glutamine residues. The NMR signal at low chemical shifts is associated with the alkyl groups of the side chains.<sup>54</sup> Cysteine groups were difficult to observe (25 ppm to 29 ppm) in the NMR spectra as they overlap with the chemical shift of the alkyl groups.

The  $\alpha$ -carbon peak between 52 ppm to 56 ppm was broadened and formed a shoulder with its maximum moving to lower frequency in the regenerated keratin materials (Figure 6(b)-(e)). The broadening of peaks in the regenerated keratin samples may be due to the ability of the ionic liquids to disrupt hydrogen bonding of the original keratin raw material, leading to the unfolding of the polypeptide chains. This would result in the formation of a greater fraction of  $\beta$ -sheet and random coil structures, in agreement with the XRD data discussed above.

Figure 6(e) shows that the peaks from the carbonyl carbon in the material regenerated from [AMIM][dca] in the region of 170 - 180 ppm were significantly different from the raw wool and the other regenerated keratin materials. The additional peak around 172 ppm from random coil and  $\beta$ -sheet structures seems to suggest a greater degree of disruption of the protein during

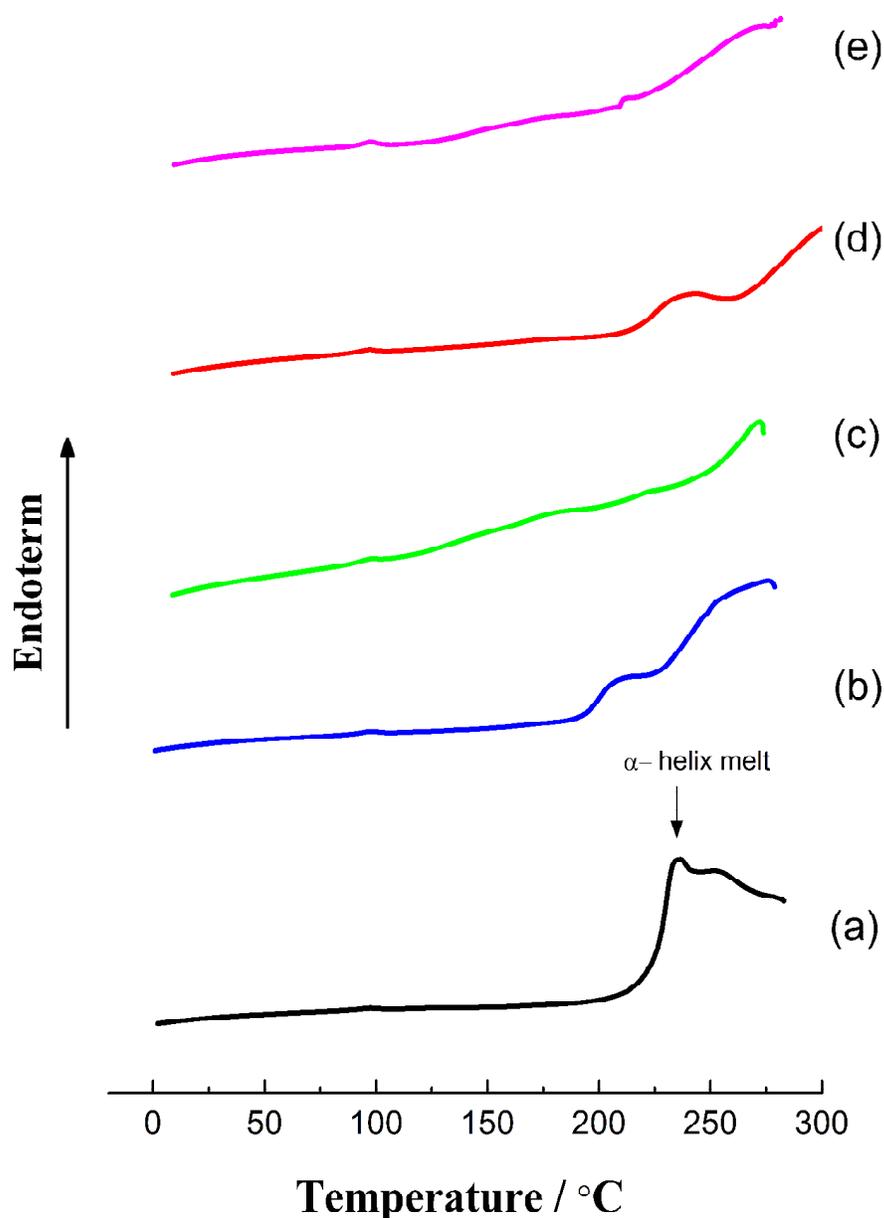
dissolution in this case. The same observations are reported by Ando *et. al*<sup>56</sup> in their solid state NMR study of wool keratin.



**Figure 6.** The  $^{13}\text{C}$  CP MAS NMR spectra of (a) wool and regenerated wool keratin from (b) [BMIM]Cl, (c) [AMIM]Cl, (d) [choline][thioglycolate], (e) [AMIM][dca].

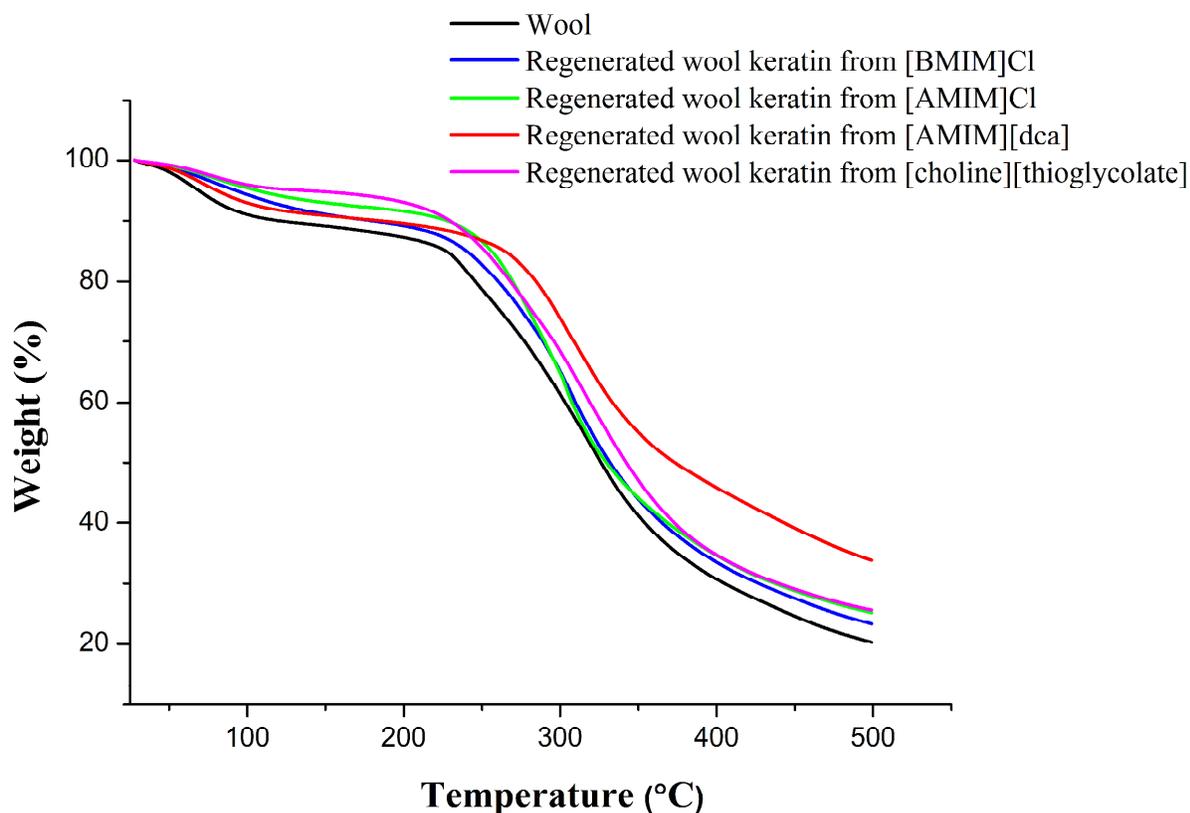
The phase behaviour of the raw wool and regenerated keratin materials was studied using differential scanning calorimetry (DSC), Figure 7. The samples were subjected to three consecutive heating and cooling cycles in order to obtain reproducible results and the third cycles are reported. The DSC trace of the raw material (Figure 7(a)) shows a peak around 230 °C which corresponds to  $\alpha$ -helix disordering and decomposition,<sup>57</sup> (some literature also describes this

transition as a "melt"<sup>58</sup>). In the case of the regenerated keratin materials (Figure 7(b)-(e)), no sharp peak was observed; this is consistent with the loss of crystallinity that was observed in the XRD data. Broad endothermic steps observed between 150 °C and 200 °C may indicate the glass transition in an amorphous fraction of the material.



**Figure 7.** DSC thermograms of (a) wool and regenerated wool keratin from (b) [BMIM]Cl, (c) [AMIM]Cl, (d) [choline][thioglycolate], (e) [AMIM][dca].

The thermal stability of the materials was investigated by thermogravimetric analysis (TGA), (Figure 8), indicating that the thermal stability of the original wool keratin has been retained. A two-step decomposition process was observed in all cases. The TGA curves show a small weight loss at 100 °C, which is due to the evaporation of bound water present in the keratin materials.<sup>42, 59, 60</sup> The decomposition between 250 °C and 400 °C could be initiated by the denaturation and degradation of the wool protein molecules.<sup>42, 59, 60</sup> It was also reported that volatile compounds including hydrogen sulphide and sulphur dioxide are released from wool due to the cleavage of the disulphide bonds that occurred between 230 °C and 250 °C.<sup>60</sup>



**Figure 8.** TGA plot of wool and regenerated wool keratin.

### Characterisation of water soluble fraction

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis with a standard marker was employed to characterize the residual water soluble fraction remaining after the regeneration of the **insoluble** keratin material. The protein band was visualized by silver staining. A typical gel pattern is shown in Figure S2 in the supporting information. The bands that appear on the gel confirm the presence of water soluble protein fractions in the solution. Their molecular weights range from as low as 15kDa to >120kDa. In the electrophoresis separation patterns obtained by Tonin *et al.*<sup>61</sup> with their steam exploded wool, two main keratin fractions were obtained, described as low sulfur proteins and high sulfur proteins in the molecular weight range between 67 kDa-43 kDa and 28 kDa-11 kDa, respectively. In the case of the [BMIM]Cl, [AMIM]Cl and [AMIM][dca] samples here, this distinction is not clear, a broad range of molecular weights being present. In the [choline][thioglycolate] case, the low sulfur protein fraction appears to have been reduced in molecular weight. Since the continuous bands observed in gel electrophoresis were in the low molecular weight range, it was concluded that the ionic liquids had cleaved the protein into smaller polypeptide chains as also observed by Tonin *et al.*<sup>61</sup>

## CONCLUSIONS

In summary, we have investigated a number of ionic liquids and deep eutectic mixtures as solvents for the dissolution of wool. The dissolution in deep eutectic solvents is poor compared to the ionic liquids and therefore it appears that the ionic medium and the nature of some of the ions must play a major role in the dissolution process. In particular, the highest limiting solubility

(475 mg/g) was achieved in [AMIM][dca]. The addition of a reducing agent such as mercaptoethanol caused increased dissolution of the wool by 50-100 mg/g. The regenerated wool keratin retained the protein backbone, as observed by ATR measurements, while the crystallinity was substantially lost, as determined by XRD methods. The dissolution also produced a breakdown of the polypeptide chains into lower molecular weight fragments, some of which became water soluble as observed by gel electrophoresis. Further work relating to the processing of the regenerated materials into fibers and films as well as the recycling of the ILs is currently underway.

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