Enzymatic Treatment of Wool Fabric: Effects of the Surfactants

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ABSTRACT

By use of the commercial protease, the wool fabric has been treated enzymatically. The modified form of the Michaelis-Menten equation has been used and the maximal velocity and the half-saturation constant, V_max and K_m, were calculated. The effects of different types of surfactants (cationic 'CAS', non-ionic 'NNS', anionic 'ANS', amphoteric 'AMS') on the rate of enzymatic reaction were studied. By considering Lineweaver-Burk plot, K_m and V_max values are: 0.37, 2; 0.07, 5.15; 0.10, 5.98; 0.10, 2.89; μmol/min for CAS, NNS, ANS, AMS surfactants, respectively. In the absence of surfactant (WOS), the K_m and V_max values are: 0.059 and 4.52 μmol/min, respectively. The results obtained showed that the amount of released amino acid (raa) in presence of either ANS or NNS surfactant were higher than that in the absence of the named surfactants. In presence of CAS or AMS surfactants, the amount of raa, was decreased. With the use of surfactants results obtained from scanning electron microscopy on the treated fabrics and also from determination of one of the measurable physical properties (tensile strength), indicate that there is a good correlation between amount of raa and loss of the tensile strength of fabric. The measured kinetic parameters (K_m and V_max) were also changed accordingly. AMS showed to act more properly on the uniformity of the wool substrate as compared with that of the other tested surfactants. Moreover, as the catalytic specificity, the ratio of V_max/K_m was determined.

Key Words: wool enzyme, surfactant, Lineweaver-Burk plot, specificity

INTRODUCTION

There have been great interests in recent years toward using different types of enzymes to treat textile fibres and fabrics. Compatibility of enzymes and in fact most of the biological processes with the environment have been considered as a point of strength for these enzymatic digestions (biopolishing) [1–4]. For example, the use of protease on wool fabric finishing corresponds to its anti-felting, anti-shrinkage, bleaching and luster

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Effects [5-10]. Effects of different parameters in relation to the chemical and physical properties of the textile in the enzymatic treatments of the fabrics have been studied: concentration of enzyme, type of the used buffer solution and pH of the reaction medium [3, 4, 7].

Hydrophobicity character of the cell membrane especially in wool textile makes the wool to be highly resistant toward proteolytic attack by the protease [11]. Therefore, in expressing protease activity the mentioned character may lead to some unpredictable results [12]. Moreover, in lipolytic treatments of the wool textiles, effects of using surface-active agents have been studied [12]. The results obtained from application of non-ionic and ionic showed that these agents had significant effects on the deactivation of the lipase [12]: adsorption of the hydrocarbon fraction of the surfactant (non-ionic and also ionic) and transfer to the active site of the lipase having hydrophobic character may lead to inactivation of the enzyme [12]. However in the same work, it has been shown that the use of anionic surfactant along with protease caused 50% reduction in the activity of the protease although when non-ionic surfactant was used, the decrease in the activity of the protease was only 2%. The adsorption of anionic surfactant to the catalytic site of the protease having protonated amine group was considered to be the cause of the partial deactivation of the commercial protease used in that study [12]. Usually the effect of protease on the wool textile is evaluated by measuring the tensile strength and/or weight loss [4-6]. Results obtained by Shen’s research showed that by use of non-ionic surfactant along with protease deactivating effect measured as the weight loss, was observed on the enzyme, although on tensile strength loss no significant effect was obtained [12]. In a separate study with the use of ionic detergents, a reduction in the rate of proteolytic reaction was obtained while the use of non-ionic detergent led to increase digestion of chemically treated or untreated wool textile [11].

From this condensed reviews on the literature it seems that the extent of proteolytic reaction on the wool textile could be controlled more properly by use of surface-active agents.

Therefore, the objective of this study was to evaluate the performance of four different types of surfactants in the proteolytic treatment of wool fabric, using a commercial bacterial protease. In this proteolytic treatment also improvements in the morphology of the wool substrate along with its tensile strength were searched.

**Backgrounds of the Enzyme Kinetics of Fabric Hydrolysis**

In this study kinetic parameters of the protease were measured in the presence or absence of the tested surfactants. There are a lot of discussions in the literature regarding the enzymatic hydrolysis of cellulose by cellulase [13-15]. Hydrolytic reactions of cellulose by cellulase have been considered to be very slow turnovers and scientists do not always select to hydrolyze cellulose at very low enzyme concentrations. In fact in many assays, use of the Michaelis-Menten kinetics may not be applicable. On the basis of research works reported by Wood Ward et al. [16], an alternative approach has been proposed by Baily [16], the following equation:

\[
V = V'_{\text{max}} \frac{[E]}{K_m'} + [E]
\]  

which could be used in the place of the Michaelis-Menten formula:

\[
V = V_{\text{max}} \frac{[S]}{K_m + [S]}
\]

In eqn (1), [E] is concentration of enzyme, \(K_m\) is half saturation constant, \(V'_{\text{max}}\) is analogous of maximal velocity to Michaelis-Menten parameters. In this equation the initial velocity is related to the enzyme concentration consequently the analogous parameters \((V'_{\text{max}}, K'_m)\) can be evaluated. Nature of the cellulose (amorphous and crystalline components) could be one of the several reasons for slow enzymatic digestion of this substrate [16]. Wool fabrics from outside to inside possess several layers somehow different in their nature [17]. One could assume the wool fabric as a hydrated solid and same as the assumption with cellulose as the substrate for cellulase action, the use of eqn (1) could be more realistic approach to this enzymatic hydrolysis. The
EXPERIMENTAL

Material and Methods

Materials

Wool sample: 100% wool fabric obtained from Iran Merinos was used as substrate. The specifications of the fabric are shown in Table 1.

Enzyme: Novolan protease enzyme from Novo-Nordisk; surfactants-anionic: linear alkyl benzene sulphonate (LABS) from Arvin Co; -non-ionic: Marlophen, nonyl fenol from Hiles Company; -cationic: Tinegal MR from Ciba; -amphoteric: Albegal a from Ciba; surfactants were kindly supplied by Ciba, Hiles and Arvin Co.

Methods

Prior to the application of enzyme, its optimum temperature and pH were determined. The experiments were carried out in Linitest dyeing apparatus in a bath (liquor ratio 20:1) with 10 steel disks (could provide extended agitation). The amount of released tyrosine was determined using spectronic 21 Duv Milton Roy spectrophotometer.

A 10.0 g sample was treated with 200 mL of solution of 0.1, 0.2, 0.5, 1, 2 g/L of protease in a phosphate buffer pH 8.5 (0.05 M) at 50 °C, after 30 min the reaction solution was taken out and the concentration of raa (tyrosin), indicating the initial rate of reaction was determined by Folin-Lowry method [18, 19]. The result of each experiment has been reported in the form of average three replicates. Using the amount of raa the kinetic parameters were measured with and without (1 g/L) the surfactant. Action of the surfactant was evaluated using the same technique as suggested for the rate of enzymatic reaction via measuring raa.

Physical Parameters

(a) Micrographs of the wool fabric, using scanning electron microscope (XL 30 Philips) are shown in Figure 2.
(b) Variation in strength was measured on an Instron tester.

RESULTS AND DISCUSSION

Considering the proteineous nature of the wool fabrics, amino acid released upon treatment of the wool with protease has been used as an index for determining the extent of this type of the proteolytic reactions [19]. Effects of different types of the surfactants on the enzymatic treatment of the wool fabrics were evaluated, and the results are given in the form of bar graph in Figure 1. In presence of either ANS or NNS, the action of the protease showed an increase pattern in comparison with that of the proteolytic reaction on the fabric samples in the absence of any of the surfactants (WOS).

Considering the concentrations of the enzyme used (0.1–2.0 g/L), range of increase in the amount of raa (percent) for ANS and NNS is 1.5–25% and 1.5–29%, respectively. Performance of the protease in the presence of CAS and AMS showed a decreasing pattern as compared to that obtained with the reaction with no surfactant present (Figure 1). Range of the change in the amount of raa for CAS and AMS is 49–54% and 37–64%, respectively. The results obtained for the percentage of tensile strength loss are presented in Table 2.

The pattern of the change in strength loss is shown to be similar with the protease behaviour on the wool substrate in the presence of the tested surfactants: loss of the tensile strength for the fabrics increased when either ANS or NNS was used as compared to that of the reaction in the absence of the surfactants (Table 2). While the strength loss of the

<table>
<thead>
<tr>
<th>Weight/unit area (g/m²)</th>
<th>Yarn twist (T.P.M)</th>
<th>Yarn count</th>
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</thead>
<tbody>
<tr>
<td>265</td>
<td>249, 275</td>
<td>40/2</td>
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Table 1. Specification of wool fabric.
Enzymatic Treatment of Wool Fabric: Effects of the Surfactants

Figure 1. Extent of enzymatic hydrolysis of the wool fabric in presence of the surfactants at different concentrations of the enzyme.

The tensile strength of the wool fabric decreased when CAS was used along with the protease, the result obtained for the tested AMS showed that the loss of the strength did not change significantly over that obtained with the proteolytic reaction when no surfactant was present (Table 2). The results of tensile strength measurement therefore, give an indication for the increase in sensitivity of the wool fabrics to the protease in the presence of either ANS or NNS. While trend of the change in the tensile strength in presence of the AMS although shows a declining pattern as compared to that of the above named surfactants but it is very similar to that obtained when no surfactant was used.

The micrographs obtained from SEM, shows that surfaces of the fabric samples became rather uneven in the enzyme treated wool (Figure 2). With the use of the AMS, the extent of the evenness of the surface of the fabrics shows to be higher as compared to that of the enzyme treated wool in the presence of either ANS or NNS (Figure 2). It seems that increase in unevenness could be traced to occurrence of some kinds of structural damages on the surfaces of the fabrics which have been observed when the wool is treated enzymatically in the presence of ANS and NNS. The results obtained from the measurement of the tensile strength are in good agreement with the micrographs presented in Figure 2.

As it has been mentioned in the literature, the reaction rate as a function of the substrate concentration such as cellulose could be determined and evaluated rather an the unrealistic approach when the reaction takes place on hydrated cellulose, because it is impossible to change the concentration of substrate sites effectively [16]. In this study we used the modified form of the classical Michaelis-Menten equation to measure the kinetic parameters of the commercial protease during the enzyme treatment of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tensile strength loss (%)</th>
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<tbody>
<tr>
<td>WOS</td>
<td>10</td>
</tr>
<tr>
<td>CAS</td>
<td>4</td>
</tr>
<tr>
<td>AMS</td>
<td>16</td>
</tr>
<tr>
<td>ANS</td>
<td>26</td>
</tr>
<tr>
<td>NNS</td>
<td>30</td>
</tr>
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</table>

Table 2. Effect of different types of surfactants on the tensile strength loss of the wool fabric samples treated enzymatically (protease).
Figure 2. Micrographs obtained from (A): wool fabrics when no enzyme was used; (B): enzymatically treated wool fabrics in the presence of different types of the surfactants: B-1: CAS, B-2: NNS, B-3: ANS, B-4: AMS, and in the absence of any surfactants (B-5).
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Table 3. Calculated kinetic parameters for different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( V'_\text{m} (\text{pmol/min}) )</th>
<th>( K'_\text{m} (\text{pmol}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOS</td>
<td>4.52</td>
<td>0.056</td>
</tr>
<tr>
<td>CAS</td>
<td>2.0</td>
<td>0.037</td>
</tr>
<tr>
<td>AMS</td>
<td>2.88</td>
<td>0.10</td>
</tr>
<tr>
<td>ANS</td>
<td>2.98</td>
<td>0.10</td>
</tr>
<tr>
<td>NNS</td>
<td>5.15</td>
<td>0.07</td>
</tr>
</tbody>
</table>

wool fabrics. Using the linear form of the eqn (1):

\[
\frac{1}{V} = \frac{K'_\text{m}}{V'_{\text{max}}} \left( \frac{1}{[E]} \right) + \frac{1}{V'_{\text{max}}}
\]

The related double reciprocal plots are drawn as in Figure 3. In the presence of either ANS or NNS, the maximal velocity (Table 3) of protease action on wool fabrics was increased as compared to that when no surfactant was used: values of \( V'_{\text{max}} \) are 5.98, 5.15 and 4.52 (pmol/min), respectively. Although when CAS or AMS were used the maximal velocity for the reaction decreased in comparison with that of the reference reaction (pmol/min): 2 for CAS and 2.88 for AMS (Figure 3 and Table 3).

Interaction(s) between any component of the enzyme reaction mixture and the enzyme itself would have a definite role in the expression of the enzyme activity. It is possible for example, to interpret the results obtained in terms of active site of the enzyme carrying an amino acid containing R side chain group with a negative charge (i.e., dissociated carboxyl group). The CAS having positive charge may interact favorably with the catalytic site of the enzyme and the active site would not be available for the substrate acceptance. By using the CAS in the proteolytic reaction the amount of the raa diminished and maximal velocity was decreased.

Although the enzyme carrying the negative charge on its active site could express a kind of repulsive force toward the ANS having negative charge, in this case, the maximal velocity has been increased by 15% with corresponding increase in the amount of raa (Figure 1). NNS may express a kind of hydrophobic characteristics, which means that this type of surfactant in the reaction mixture may stay away from the catalytic site of the enzyme with its hydrophilic character, as the acceptance of wool fabric molecules by the active site of the enzyme would be increased.
The results obtained from measuring the amount of raa as well as the calculated value of the maximal velocity, both of these are in agreement with this interpretation. Design of the molecular structure of the wool fabrics is in a way that it seems two charges are available on this substrate [17]. The AMS also carries two charges [20]. Decreasing trend of the amount of raa along with the calculated value of the $V'_{\text{max}}$ in this reaction indicate that the dominant character of this amphoteric surfactant toward the commercial protease has been determined by the structural position carrying the positive charge, although the amount of raa for the AMS treatment is higher than that for CAS treatment. Therefore, one may interpret that negative charge on the AMS also has a role in the enzymatic reaction on the wool fabrics (Figure 1).

The calculated kinetic parameters, $V'_{\text{max}}$ and $K'_{m}$, are given in Table 3. By considering cellulase to treat cotton, Cavaco-Paulo and Almedia used the saturation constant as an apparent dissociation constant for the enzyme [14]. Higher $K'_{m}$ can be explained by the fact that the wool fabrics as the substrate have more accessibility to the protease [14]. Therefore the use of either AMS or ANS in the reaction mixture may cause the substrate to become greater accessible for the enzyme is ready for catalysis.

As the significance of the $V'_{\text{max}}$ has been mentioned above, one may notice that the highest values for $K'_{m}$ belong to ANS and AMS treatments (Table 3), although $K'_{m}$ for the action of the enzyme in presence of either AMS or ANS is the same. But, considering $V'_{\text{max}}$ and $K'_{m}$ values, the amount of raa and also the results obtained from both tensile strength measurement and SEM, along with the discussion given above, one may conclude that enzyme in the presence of ANS is more flexible as compared to the behaviour of the enzyme in the reaction mixture containing AMS. In other words, less conformational change for the protease when AMS is present in the mixture led to lower conversion rate and the specificity of the enzyme which is calculated in the form of a ratio of $V'_{\text{max}} / K'_{m}$ is also lower (Figure 4). In the research conducted by Cavaco-Paulo and Almedia, the catalytic term has been obtained by dividing $V'_{\text{max}}$ by $K'_{m}$ [14].

In our study the calculated values of enzymatic specificities for the treatments have been presented in the form of bar graph in the Figure 4. By considering all the results obtained from physical and chemical measurements one may conclude that the use of AMS along with the enzyme is preferred as compared to the action of other surfactants in the enzymatic reaction mixture.

![Figure 4](image-url)  
**Figure 4.** Catalytic specificity for the wool fabric as a substrate for protease action, with or without the surfactants in the reaction mixture.
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CONCLUSION

The effects of different four types of surfactants were studied by measuring kinetic parameters of protease enzymatic treatment on wool fabrics and using Lineweaver-Burk plot for determining the rate of reaction. Measuring the rate during enzymatic reaction showed that the presence of ANS and NNS increase the rate of reaction and hence increase the damages on treated fabrics. In presence of CAS the reaction rate is not changed very much. By considering the ratio of $V_{\text{max}}/K_m$ as an index for the catalytic specificity, results showed that the enzyme is less flexible and it also has lower specificity in presence of AMS. Although, considering the results of the physical and chemical studies, specially SEM, one may conclude that the uniformity of enzymatically treated wool appeared to be more acceptable, and its preferred performance in relation to the roles of other tested surfactant has been discussed.

REFERENCES