Effect of Alkali Metals and Alkaline Earth Metals Hydroxides on the Structure of Wool Fibres

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Abstract Graphics (For Table of Contents only)
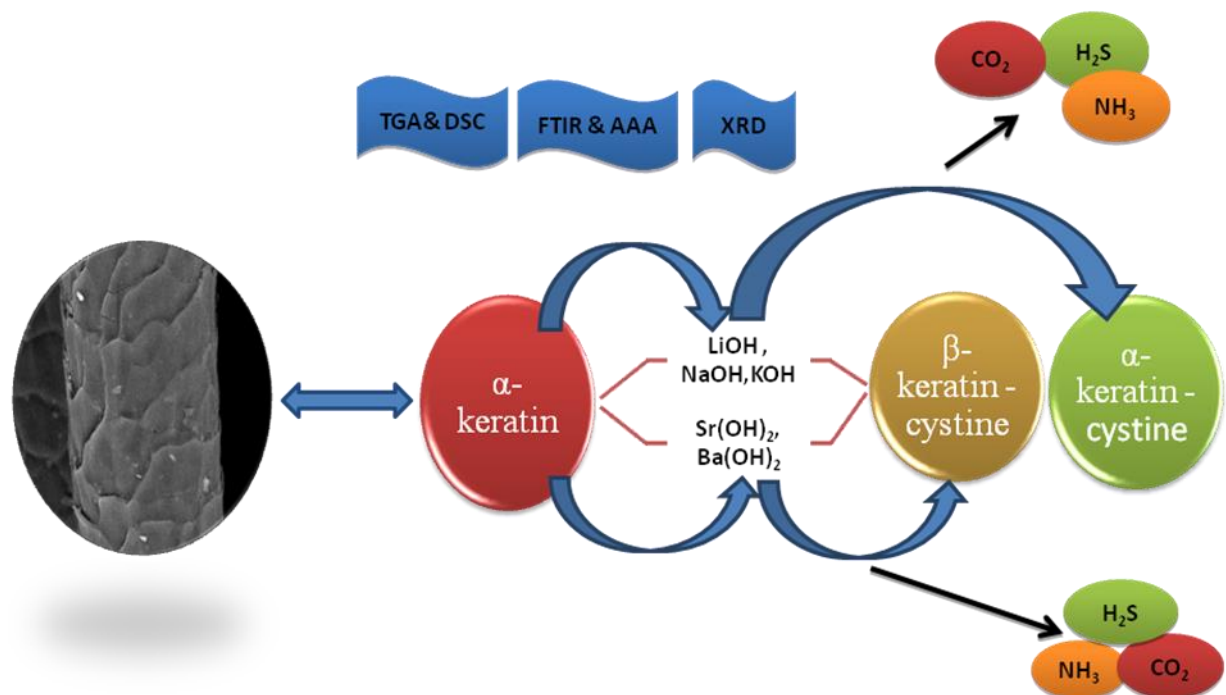
ABSTRACT

Wool is sensitive towards the effect of alkaline solutions which are usually used in the dissolution of wool fibers to regenerate keratin. In this investigation, the effect of different alkalis on the chemical composition and the secondary structure of wool was studied. These were carried out by treatment of wool with equivalent amounts of alkali metal hydroxides (lithium, sodium, and potassium hydroxides) as well as alkaline earth metal hydroxides (strontium and barium hydroxides). The effect of these alkalis on wool was monitored using amino acid analysis, elemental analysis, carboxylic content, acid, and base combining capacity, urea-bisulphite solubility, and Fourier Transform Infrared spectroscopy. Further structure elucidation was conducted by thermo-gravimetric analysis, differential scanning calorimetry, and X-ray diffraction pattern. The results of this investigation prove that the effect of the used alkalis on wool is not quite similar. Cystine, glycine, and the basic amino acids are the most deteriorated species in the alkali-treated wool. Proline is the only amino acid that was increased under the influence of alkalis on wool. Some elimination reactions were involved during alkaline treatment of wool; namely decarboxylation, desulphydration, and deamination. The secondary structure of wool treated with $Sr(OH)_2$ and $Ba(OH)_2$ was changed from the α-helical structure into the β-sheet form.

Keywords: Wool; keratin; macromolecule; fibers; alkali; polymer degradation, chemical composition.

1. INTRODUCTION

Keratins are fibrous proteins, which are the main constituents of wool fibers. Keratin has high stability, distinctive physical properties, and resistance to chemical attacks

due to the presence of inters- and intermolecular disulfide bonds of cystine amino acids in the wool structure [1].

Wool fibers can be partially or fully dissolved by disruption of the keratin structure, and this can be achieved in several ways, including reduction [2], oxidation [3], and sulfitolysis of the disulfide bonds [4].

Treatment of wool with strong alkalis, such as sodium and potassium hydroxides results in breakage of disulphide, peptide, and side-chain amide bonds. In mild alkaline treatment, degradation of cystine leads to the formation of lanthionine, which decreases the solubility of wool in alkali [5]. In a different study, alkaline hydrolysis of wool waste using KOH and NaOH was carried out at 120 °C for 20 minutes. The results showed that the wool hydrolyzates improved soil characteristics and could be used as an alternative bio-fertilizer [6, 7]. Polypeptides extracted from wool treated with NaOH 0.5 N at 95°C for 2 hours were used as reinforcement for polymer composite fibers [8, 9].

Cystine residues along keratin macromolecules have been reported as the most sensitive niches that were attacked upon the treatment of wool fibers with alkalis [10]. Two new crosslinks; in a form of the amino acids lanthionine and lysino-alanine are formed [11]. These new crosslinks are not affected by reducing agents, and therefore the solubility of alkali-treated wool in urea-bisulphite solution is diminished. Alkali treatment of wool results in a loss in mechanical properties together with yellowing of wool [12 - 14].

Keratin is usually extracted from wool using a strong acid, alkali, high concentration of salt solutions or enzymes [15 - 17]. Recent methods use ionic liquids for keratin extraction from wool, feathers, and hair with improved yield [18].

This investigation aimed to study the effect of the treatment of wool with some alkali metals as well as alkaline earth metal hydroxides on its chemical composition, secondary structure, and some of its physical and chemical characteristics. This would enable us to utilize the residues of wool fibers remained after alkaline extraction of keratin from wool.

2. EXPERIMENTAL

2.1. Material

Crossbred wool fibers with main fiber diameter 23 μm, was purchased from the local market. Lithium hydroxide, sodium hydroxide, potassium hydroxide, strontium hydroxide, and barium hydroxide were supplied by ACROS, Geel, Belgium. The reagents used were of laboratory grade.

2.2. Methods

About 5 g wool fibers were treated in 0.2 N aqueous solution of LiOH, NaOH, KOH, $Sr(OH)_2$ or $Ba(OH)_2$ at 70 ℃ for 20 min, and the liquor ratio (L.R.) was 1:20. The sample was rinsed thoroughly with running tap water to remove any residual alkali on the fiber surface until the collected rinsing water has pH 7. The alkali-treated sample was left to dry overnight at ambient temperature.

2.3. Analyses

2.3.1. Weight loss

The loss in weight as a result of the alkaline treatment of wool was calculated from the following equation:

$$\text{Loss in weight (\%)} = \frac{W_1 - W_2}{W_1} X 100$$

Where W_1 is the dry weight of fabric before treatment and W_2 is the dry weight of the same fabric after the treatment.

2.3.2. Amino acid analysis

Amino acid composition of both untreated and alkali-treated wool fibers was determined by HPLC analysis using an Agilent 1260 series [19]. The separation was carried out using ZORBAX Eclipse Amino Acid Analysis (AAA) C18 column (4.6 mm x 150 mm, 5 μm). About 1 g sample was mixed with 10 mL of 6 M HCl and heated at 100°C for 24 h and then filtered before analysis.

2.3.3. Alkali and urea-bisulphite solubility

The alkali solubility of wool samples treated with the said alkalis as well as the untreated wool samples was determined according to (IWTO)-4-66 (D) test method. Urea-bisulphite solubility of the tested samples was determined according to the IWTO-11-65 (E) test method.

2.3.4. Elemental analysis

The amounts of carbon, nitrogen, hydrogen, and sulphur, in the untreated and alkali-treated wool samples, were assessed using Elementary CHNS Analyser, Model Vario EL III Germany.

2.3.5. Base Combining Capacity (Carboxylic content)

The base combining capacity of wool was determined by measuring the quantity of alkali combined with wool according to the method of McPhee [20]. The sample was soaked in 2% HCl for 3-4 h with infrequent shaking. The sample was then filtered and rinsed several times with ethanol/water mixture (60/40) until chloride ions are free detected by silver nitrate solution. Then the sample was filtered and dried.

The dry sample (about 0.5 g) was accurately weighed and inserted in 250 ml Erlenmeyer flask, followed by 50 ml 0.1 N NaOH solution containing 5% sodium chloride. The flask was quick-fitted and allowed to stand overnight with regular slow shaking. The content of the flask was back-titrated with 0.05 N hydrochloric acid using phenolphthalein as an indicator. Blank titration was carried out on an untreated wool sample, and the carboxyl content of the sample was determined as follows:

$$\text{Carboxylic Content} = \frac{(X - Y) \times N_A}{W} \quad \text{meq/100 g fibre}$$

Where "X": volume of HCl solution used in a blank titration, "Y": volume of HCl solution used in a back titration, "N_A": normality of HCl solution, and "W": the weight of the sample in grams.

2.3.6. Acid Combining Capacity

The acid combining capacity of wool fiber was determined according to the method described by Farnworth [21]. The wool sample was immersed in 0.001 N HCl for 24 h. The sample was then rinsed several times with distilled water until the washings showed a pH of 5.2. About 1 g sample of rinsed wool was immersed in 100 ml of 0.1 N HCl and allowed to stand at 21°C for 3 days, with occasional shaking. Portions of supernatant (25 ml each) were then withdrawn from this solution and titrated with 0.1 N NaOH. The amount of acid combined was calculated and expressed as ml of N HCl combined/100 g of dry wool.

2.3.7. Fourier transforms infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) of untreated as well as treated wool fibers were recorded by using an FTIR spectrometer (Nexus 670 Fourier transform

infrared spectrometer; Nicolet, USA) in the region of 4000–400 cm^{-1} with spectra resolution of 4.0 cm^{-1}.

2.3.8. Thermal properties

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were measured using SDT Q2000 Tzero DSC from TA instruments with a rate of heating 10 °C/min.

2.3.9. X-ray diffraction pattern.

The X-ray diffraction analysis was performed at room temperature on raw wool fibers as well as the alkali-treated ones, on a Bruker D8 Avance using Cu Kα as the target with a secondary monochromator to operate at 40 KV and 40 mA. The scans were performed within the range of 4° $<2\theta<60°$ with scanning step 0.02° in reflection geometry.

The crystallinity index (CI), indicating the relative crystallinity degree of fibers, was calculated using the following empirical equation: [22]

$$CI = \frac{I_c - I_a}{I_c} \times 100$$

where CI is the crystallinity index; I_c is the maximum intensity of crystal lattice diffraction and I_a is the minimum diffraction intensity. In general, the higher the CI value indicates higher crystallinity of the fiber sample.

3. RESULTS & DISCUSSION

Many researchers have adopted alkalis for the dissolution of wool fibers to obtain keratin [23–25]. Moreover, some wet processes of wool are carried out in alkaline medium; such as scouring and bleaching [26, 27]. The effect of alkalis on keratin

depends on the type of alkali, temperature, duration of reaction, pH, and the added salts and/or auxiliaries [5]. The effect of some alkali metal hydroxides and alkaline earth metal hydroxide on the chemical composition and the secondary structure of wool will be outlined hereafter underneath.

3.1. Loss in weight

The loss in weight of wool fabric treated with equivalent amounts of LiOH, NaOH, KOH, $Sr(OH)_2$, and $Ba(OH)_2$ at the same treatment temperature and time was recorded in Table **1**.

It is obvious from the data of Table 1 that the loss in weight increases in the following order: $Sr(OH)_2 < NaOH < Ba(OH)_2 < KOH < LiOH$. These results can be explained in terms of two counteracting factors; namely the ionization energy (IE) of the respective metal and the ionic radii (IR) of the corresponding cation of these alkalis. As the IE of the metal decreases, the alkali gets stronger. It is well-known that the IE decreases down the groups and increases across the period in the periodic table. The IE increases in the order $Sr < Li < Ba < Na < K$; Therefore the basicity of the corresponding alkalis increases in the order $Sr(OH)_2 < LiOH < Ba(OH)_2 < NaOH < KOH$ [29]. On the other hand, the ionic radii of the used cations increase in the order: Li^+(90 pm)$< Na^+$(116 pm)$< Sr^{+2}$(132 pm)$< Ba^{+2}$(149 pm)$< K^+$(152 pm) [28]. Consequently, the treatment of wool with LiOH, in which Li^+ ion has the smallest radius, led to the highest loss in weight among the other used alkalis. The relatively small ionic radius of Li^+ ions enhanced its diffusion towards the fiber interior and resulted in an enhanced loss in weight.

Treatment of wool with the strongest alkali among the used ones; namely KOH, resulted in the second most degradative action on wool (after LiOH). This would imply that the cationic radius within the used alkali is more effective that the strength

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of the base regarding the degradation of keratin macromolecules. Treatment of wool with strontium hydroxide which is the weakest alkali among the used ones led to the minimum weight loss.

Table 1: Losses in weight of wool fabrics treated with equivalent amounts of alkali metals and alkali metal hydroxides (5 g wool was dissolved in 0.2 N alkali at 70 ºC, for 20 min, with L.R. 1:20)

Alkali	Loss in weight (%)[a, b]
Lithium hydroxide	62.0
Sodium hydroxide	35.0
Potassium hydroxide	57.2
Strontium hydroxide	12.0
Barium hydroxide	40.0

- **a: average of three measurements**
- **b: values were corrected using a control sample to calculate the humidity percent.**

In conclusion, the values of loss in weight shown in Table **1** can be properly rationalized with neither the ionic radii alone nor the IE values of the used alkalis. The effect of the used alkalis on wool depends on some properties of the used alkalis; among them is the strength of the user bases as well as the respective ionic radii.

3.2. Amino Acid Analysis (AAA)

Some amino acids along keratin macromolecules are sensitive towards alkalis [30]. Therefore, the change in the amino acid composition of wool fibers after being treated with an aqueous solution of 0.2 N LiOH, NaOH, KOH, $Sr(OH)_2$, or $Ba(OH)_2$ was determined. The results of this investigation are summarized in Table **2**.

Table 2: Change in amino acid composition (%) of wool after treatment with equivalent amounts (0.2 N) of alkali metal and alkali earth metal hydroxides

Amino acid	LiOH[(a,b)]	NaOH[(a,b)]	KOH[(a,b)]	Sr(OH)$_2$[(a,b)]	Ba(OH)$_2$[(a,b)]
Glycine	51.2↓	55.4↓	57.6↓	50.9↓	59.8↓
Alanine	5.0↓	9.5↓	6.7↓	19.3↓	20.2↓
Phenyl alanine	25.7↓	28.5↓	30.5↓	22.3↓	29.8↓
Valine	9.4↓	14.7↓	15.1↓	12.2↓	15.0↓
Leucine	22.4↓	24.1↓	25.2↓	27.6↓	28.9↓
Isoleucine	20.1↓	20.9↓	25.6↓	24.1↓	24.2↓
Serine	28.3↓	35.9↓	40.3↓	39.0↓	41.5↓
Threonine	27.1↓	33.8↓	40.9↓	38.5↓	40.1↓
Tyrosine	16.4↓	25.5↓	26.2↓	26.5↓	28.1↓
Aspartic acid	1.3↓	1.0↑	2.2↓	Nil	5.3↓
Glutamic acid	Nil	1.7 ↓	2.3↓	6.4↓	6.9↓
Histidine	57.9↓	62.8↓	63.5↓	52.7↓	52.2↓
Arginine	47.0↓	54.5↓	59.1↓	52.0↓	56.8↓
Lysine	53.5↓	59.4↓	63.5↓	56.5↓	54.4↓
Cystine	62.9↓	100↓	100↓	72.2↓	100
Methionine	16.6↓	24.5↓	27.1↓	14.0↓	27.1↓
Tryptophan	5.5↓	5.4↓	6.1↓	5.9↓	5.9↓

| Proline | 15.9↑ | 16.2↑ | 19.4↑ | 19.7↓ | 14.0↑ |

(a): relative to the untreated wool sample
(b) Each value is an average of three measurements

It is obvious from the data in Table **2** that the proportions of most of the amino acids in alkali-treated wool were highly affected. The brightest result is the consumption of cystine, which showed the greatest decrease in the amino acid composition of wool treated with each of the used alkalis. It has been reported that cystine residues in wool are destroyed upon alkaline hydrolysis in a 5% solution of NaOH at 100 $^{\circ}$C even for a very short time (see scheme **1** below) [31].

Cyctine residue   Cysteine residue   Sulphenic acid residue
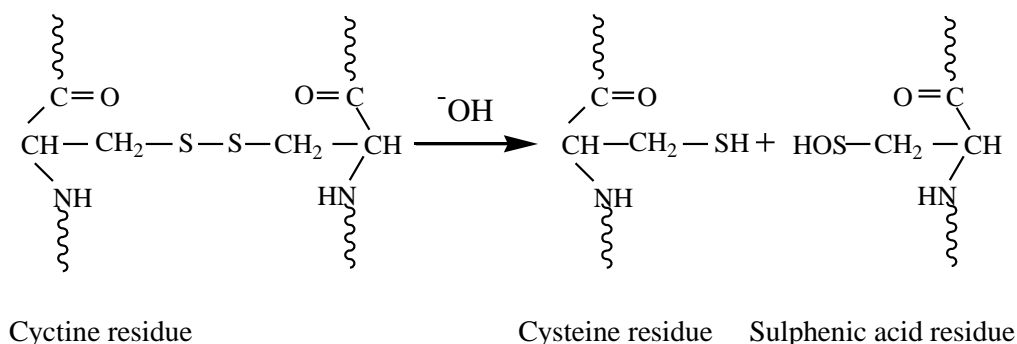
Scheme 1: Cystine dissociation in alkaline medium

Additionally, at pH values between 9 and 12, some of the cystine residues are converted into lanthionine via the formation of dehydroalanine (schemes **2** and **3**) [10].


Sulphenic acid residue   Dehydroalanine residue
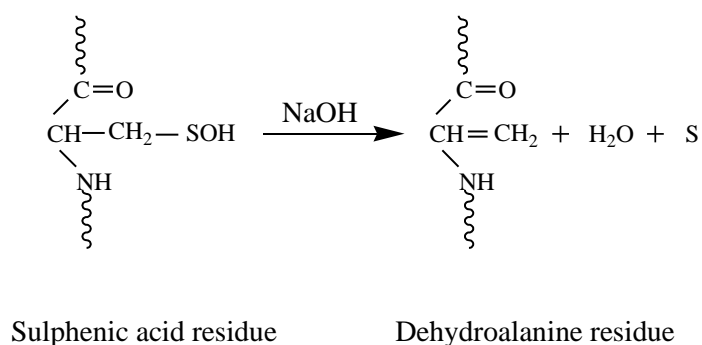
Scheme 2: Formation of dehydroalanine residue

Dehydroalanine   cysteine residue   Lanthionine
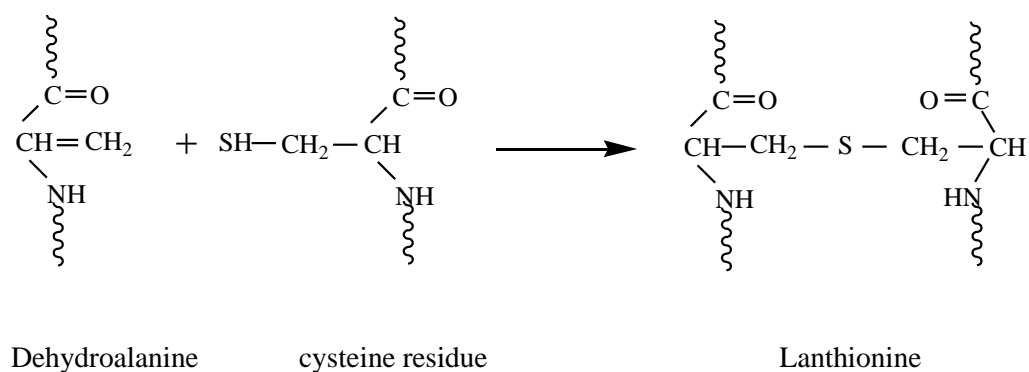
Scheme 3: Formation of lanthionine

A unique and ambiguous point to be addressed here is the increase in proline concentration in wool after being treated with the used alkalis except for $Sr(OH)_2$. This might be attributed to the elimination of the ammonia molecule from some of the lysine residues according to the following scheme **4**:
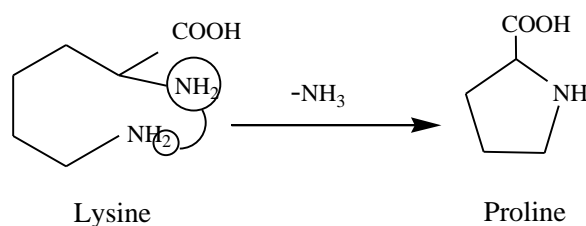
Lysine   Proline

Scheme 4: Proposed mechanism for the conversion of lysine into proline

The above mechanism is merely a hypothesis supported by a coherent decrease in the lysine concentration within the alkali-treated wool. More effort is required to approve or deny this hypothesis.

Meticulous inspection of the data in Table 2 reveals the following:

- Apart from cystine, the concentration of the amino acids, glycine as well as histidine, arginine, and lysine (basic amino acids) in wool keratin decreased sharply upon treatment with all the used alkalis. The decrease in their concentrations ranges between 63.5 % and 47.0 % depending on the nature of amino acid and strength of the used alkali.

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- Amino acids with polar side chains; namely tyrosine, serine, and threonine are less affected by alkaline treatment of wool keratin. Similar results were obtained regarding phenyl alanine, leucine, and isoleucine (with hydrophobic side chains), methionine (sulphur-containing side chain), and tryptophan (heterocyclic side chain). Cornfield and Robson reported that tryptophan is not destroyed in alkaline solution, and the analysis of alkaline hydrolysates forms the basis of one method for the quantitative determination of this amino acid in keratin fibers [31].

- The least affected amino acids are alanine and valine as well as aspartic and glutamic acids (acidic side chains).

3.3. Alkali and urea-bisulphite solubility

The alkali solubility of wool is taken as a measure of the number of disulphide crosslinks along keratin macromolecules. On the other hand, the solubility of wool in urea-bisulphite solution is an indication to the extents of crosslinking, other than cystine linkages, along with keratin macromolecules. The alkali and urea-bisulphite solubility of the alkali-treated as well as untreated wool samples are summarized in Table **3**.

It is easy to conclude from the data of Table **3** that the solubility of the alkali-treated samples in alkali was enhanced due to the rupture of most of the crosslinks between the polypeptide chains along with keratin macromolecules. The shortening of the polypeptide chains in the alkali-treated wool would offer a synergetic effect towards enhanced alkali solubility. On the other hand, the solubility of the alkali-treated wool in urea-bisulphite solution decreased due to the formation of new crosslinks with the fiber structures, such as lanthionine and lysinoalanine amino acid residues.

Table 3: Solubility of alkali-treated as well as untreated wool in alkali and urea-bisulphite solution

Wool sample	Alkali solubility (%)	Urea-bisulphite solubility (%)
Untreated	18.2	36.6
Treated with LiOH	51.2	25.0
Treated with NaOH	63.6	29.1
Treated with KOH	69.4	27.1
Treated with Sr(OH)$_2$	55.3	31.4
Treated with Ba(OH)$_2$	64.8	28.7

The findings of this investigation lead to the hypothesis that the treatment of wool with alkali might lead to the transformation of the α-helical structure of wool keratin into the more crystalline β-conformation. This assumption will be supported by the results of the XRD analysis in section 3.8 below.

3.4. Elemental analysis

The elemental analysis of native wool as well as that of samples treated with different alkalis was used to assess the change in the chemical composition of wool as a result of alkaline treatments. Results of this investigation, summarized in Table **4**, illustrate that there is a limited decrease in the carbon content (4.7–7.5 %) of wool samples treated with all alkalis except Ba(OH)$_2$. Barium hydroxide is a well-known reagent for decarboxylation of amino acids [32]. The decrease in hydrogen contents of wool after being treated with different alkalis ranges between 14.5 – 17.7 %. Similar results were obtained for the nitrogen content of the alkali-treated wool samples. It is noteworthy to mention here that the nitrogen content of wool samples treated with alkaline earth

metal hydroxides is significantly lower than that of those samples treated with alkali metal hydroxides.

On the other hand, the sulphur content of alkali-treated wool decreased below to 70.6 % relative to untreated wool. This could be explained in terms of the liberation of hydrogen sulphide during alkaline treatment of wool which led to a sharp decrease in its sulphur contents [33].

Table 4: Elemental analysis of native as well as alkali-treated wool samples

| Wool sample	C %	H %	S %	N %
Untreated	49.81	11.94	3.10	15.60
Treated with LiOH	47.22	9.90	1.97	14.43
Treated with NaOH	47.45	9.94	1.15	13.31
Treated with KOH	47.28	9.82	0.91	13.23
Treated with Sr(OH)$_2$	46.04	10.21	1.27	11.46
Treated with Ba(OH)$_2$	38.66	10.17	1.20	11.47

3.5. Acid and base combining capacity

The acid combining capacity (ACC) is a measure of the number of basic groups (mainly amino groups) along with keratin macromolecules. The base combining capacity (BCC) of wool indicates the number of acidic groups (carboxylic groups) in its polypeptide chains. In Table **5**, we summarize the values of acid and base combining the capacity of alkali-treated as well as untreated wool samples.

Table 5: Acid and base combining capacity of wool

Wool sample	ACC(ml of 1.0 N HCl /100 g dry wool)	BCC (carboxylic content meq/100 g dry wool)
Untreated wool	81.4	70.1
Treated with LiOH	87.8	74.6
Treated with NaOH	88.6	77.2
Treated with KOH	89.0	79.8
Treated with Sr(OH)$_2$	88.1	78.6
Treated with Ba(OH)$_2$	88.5	83.7

Data of this table clarify that alkaline hydrolysis of wool keratin resulted in a significant increase in the ACC and BCC. It has been reported that the treatment of wool with alkalis involves the attack of the amide group by the strongly nucleophilic hydroxyl ion together with the rupture of the peptide bond and formation of carboxylic and amino groups (scheme **5**).
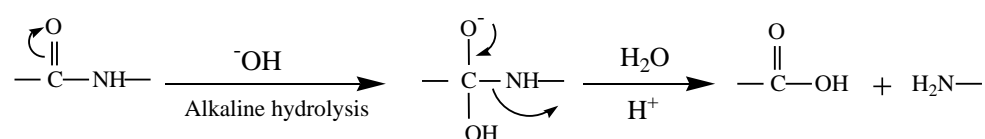
Scheme 5: Alkaline hydrolysis of peptide bonds in wool keratin into carboxylic and amino end groups [34]

3.6. FTIR analysis

The FTIR spectra of alkali-treated as well as untreated wool fibers are shown in Figure **1**. The FTIR spectrum of untreated wool fabric shows typical peaks of amide I, amide II, and amide III. The amide I appear at 1630 cm^{-1} which is associated with the

stretching vibrations of the carbonyl group. The amide II at 1519 cm^{-1} corresponds to the bending deformation of N–H bonds and stretching vibrations of C–N bonds. The amide III at 1235 cm^{-1} belongs to C–N bond stretching and N–H in-plane bending vibrations [35, 36]. The FTIR spectra of wool fibers treated with alkali metal hydroxides are almost similar to that of the untreated wool. On the other hand, the FTIR spectra of wool fibers treated with alkaline earth metal hydroxides disagree with that of the untreated sample. The amide II and amide III peaks at 1519 cm^{-1} and 1235 cm^{-1}, respectively are absent in barium hydroxide-treated wool. Moreover, the amide II peak is not found in the strontium hydroxide-treated wool. These would imply that the alkaline earth metal hydroxide causes the deamination of wool fibers and/or conversion of the amino group into other nitrogen-containing residues together with the transformation of the α-helix structure of keratin into the ß-sheet structure [37]. The latter hypothesis seems to be more appropriate as long as the results of elemental analysis shown in Table **3** prove the presence of nitrogen in wool samples treated with alkaline earth metal hydroxides.

The further detailed spectroscopic analysis will be carried out to estimate the contents of the secondary structure of alkali-treated keratin by fitting the amide I and II peak with Gaussian and Lorentzian components.
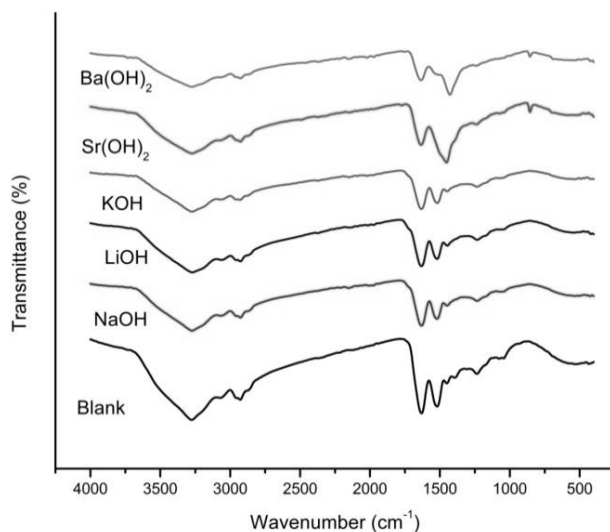
Figure 1: FTIR spectra of alkali-treated as well as untreated wool fibers

3.7. Thermal analyses

The thermal characteristics of alkali-treated as well as untreated wool were monitored using diffraction scanning calorimetry (DSC) and thermal gravimetric analysis(TGA). The results of these investigations are summarized in Table **6**.

The Initial Decomposition Temperatures (IDT) of untreated wool is 222.3 °C at which α-helix disordering takes place and cleavage of the crystal structure of wool fibers begins [38]. Melting of the morphological components of the highly complex hierarchical structure of wool is also taken place at this temperature [39].

Surprisingly, the IDT of alkali metal hydroxide-treated wool is close to that of untreated wool (lowering of the IDA does not exceed 9.58 %, relative to untreated wool). The only wool sample treated with $Ba(OH)_2$ has an IDT lower than that of untreated wool by a factor of ca. 14 %. On the other hand, the IDT of $Sr(OH)_2$-treated wool increased by ca. 23 % relative to that of the untreated wool. These results are clues for the hypothesis that the said alkali affects only the amorphous part of wool

under the used reaction conditions. This assumption will be supported by the findings of the XRD analysis shown in section 3.8 below.

Table 6: Initial decomposition temperatures (IDT), the maximum rate of decomposition, and glass transition temperature (Tg) of alkali-treated and untreated wool.

Treatment	IDT (°C)	Max rate of decomposition			Tg (°C)	Residual weight (%) at 550 °C
		Temperature (°C)	Weight loss %	Rate of decomposition (wt. %/ min)		
Untreated	222.3	316.29	70.5 0	2.23	49.5	14.50
LiOH	218.0	294.00	67.26	2.28	81.6	14.00
NaOH	201.0	293.45	69.78	2.38	80.5	18.00
KOH	208.3	298.49	75.33	2.50	72.1	12.00
Sr(OH)$_2$	272.6	306.16	36.82	1.20	75.0	30.00
Ba(OH)$_2$	191.4	313.97	60.99	1.94	81.9	27.36

The maximum rate of decomposition of all alkali-treated wool samples occurs at a temperature nearby that of untreated wool. It has been reported that at this temperature, denaturation of degradation of keratin macromolecules takes place and volatile gaseous sulphurated products (H_2S and SO_2) are released [37].

The values of weight loss and rate of decomposition at the temperature of maximum decomposition of the alkali-treated wool are not so far from that of the untreated wool. Only the weight loss and the rate of decomposition in the case of Sr(OH)$_2$-treated wool

is ca. 50 % lower than that of the untreated wool; results which are in harmony with the IDT data.

The glass transition temperature (Tg) of all tested samples increased by two folds of the untreated one. The Tg of wool is related to the viscoelastic properties of wool fibers [40].

Data in table **6** reveal also that the residual weight of wool samples treated with $Sr(OH)_2$ and $Ba(OH)_2$ are higher than the other examined samples. This indicates that these samples have a higher degree of crystalinity than the other samples. This assumption will be supported by the findings of the XRD analysis shown in section 3.8 below.

3.8. X-ray diffraction pattern

Figure **2** and Table **7** show summarizes the main findings of the XRD analysis of the untreated wool fibers as well as those of alkali-treated ones. In the alkali-treated samples, there is a prominent 2θ peak around 20° and several minor peaks that can be correlated to the antiparallel ß-sheet structure compared with the natural wool fibers [41]. The disappearance of the deviation of the peak around 9° in the alkali-treated wool proves that the α-helix structure of wool is destroyed by alkalis during the treatment process. Another clue for this transformation is the decrease in the value of d-spacing at both 9.7 and 4.4 Å upon the treatment of wool with the said alkalis [42].
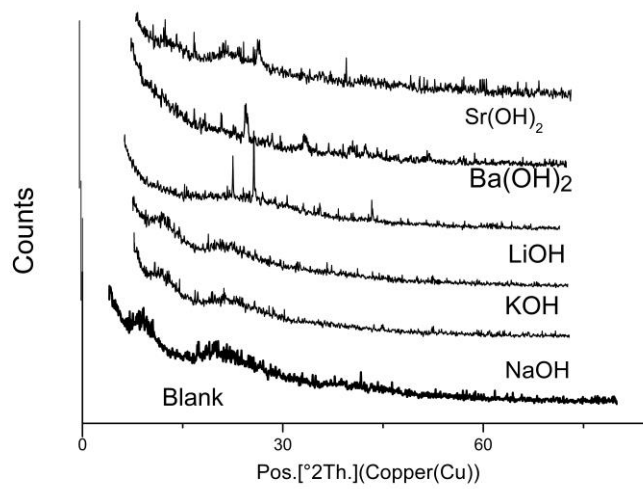
Fig. 2: XRD patterns of untreated as well as alkali-treated wool fibers

It can be also noticed that the crystallinity index of wool fibers treated with alkalis increased compared to the untreated sample, especially in case of treatment with $Ba(OH)_2$ and $Sr(OH)_2$. This assumption is in harmony with the conclusions of the infrared spectra which revealed that the α-helix structure in alkali-treated wool was destroyed.

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Table 7: Effect of treatment of different alkalis on the crystal structure of wool fibers

Sample	2θ	d-spacing (Å)	The crystalinity index (%)
Blank	9.08	9.736	40
	20	4.429	
NaOH-treated	10.03	8.81	42
	21.45	4.13	
KOH-treated	9.27	9.528	45
	21.3	4.161	
LiOH-treated	26.589	3.34	43
	22.929	3.875	
Ba(OH)$_2$-treated	23.97	3.7	55.5
	24.32	3.65	
Sr(OH)$_2$-treated	14.143	6.25	71.1
	21.82	4.07	

Swelling of wool keratin under the influence of the used alkalis would result in the rupture of almost all the intermolecular bonds between the polypeptide chains. This would result in the transformation of the folded α-helix to unfolded β-keratin. It has been reported that the chemical interactions that stabilize the folded α-helical structure of wool include disulphide (covalent) bonds and the weak (non-covalent) interactions such as hydrogen bonds and hydrophobic and ionic interactions [31].

Wlodawer *et al.* [43] concluded that not all polypeptides can form a stable α-helix. Each amino acid residue in a polypeptide has an intrinsic propensity to form an α-helix. Alanine shows the greatest tendency to form α-helices in most experimental model systems. A constraint on the formation of the α-helix is the presence of proline residues, which have the least proclivity to form α-helices. In proline, the nitrogen atom is part of a rigid ring, and rotation about the N—C bond is not possible. Thus, a proline residue introduces a destabilizing bend in an α-helix. Besides, the nitrogen atom of a proline residue in a peptide linkage has no substituent hydrogen to participate in hydrogen bonds with other residues. For these reasons, proline is only rarely found in an α-helix [44].

Consequently, the decrease in alanine content and an increase in proline content of alkali-treated wool (*c.f.* Table **2**) assure the transformation of the α-helical structure of wool into the β-conformations.

Conclusion

There are palpable discrepancies among alkali metals and alkaline metals hydroxides regarding their effect on the wool structure. The brightest decrease in the amino acid composition of alkali-treated wool keratin was taken place in cystine, glycine, and the basic amino acids histidine, arginine, and lysine. Only the proline content of alkali-treated wool increased significantly relative to the untreated wool. We assumed that the decrease in alanine content coupled with the increase in proline content of alkali-treated wool assures the transformation of the α-helical structure of wool into the β-conformations. Treatment of wool with alkalis led to the rupture of most of the chemical and physical bonds between the polypeptide chains of wool keratin; consequently, its alkali as well as urea-bisulphite solubility was augmented. Elemental analysis proved that some elimination reactions are involved during the alkaline

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treatment of wool. These reactions would include decarboxylation, desulphydration, and deamination. Alkaline hydrolysis of the peptide bonds of some parts of the biopolymer resulted in the formation of new carboxylic and amino groups and led to an increase in the acid and base combining capacity. TGA and DSC investigations proved that the residual part of wool after being treated with alkali exhibited thermal characteristics that are somehow similar to that of untreated wool.

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