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# Preparation and Characterization of Keratin-Polyvinyl Alcohol Composite Film

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AN ECONOMICALLY feasible method for preparation of keratin/ polyvinyl alcohol composite was proposed. Keratinous materials; namely wool waste, feathers, camel hair, and human hair were dissolved in selected reagents. Dissolution of the said keratinous materials was carried out using alkali metal hydroxide or alkaline earth metal hydroxide in presence of swelling agent and reducing agent. A keratin-polyvinyl alcohol composite film was prepared by casting both materials in aqueous solution. Polyvinyl alcohol acts as a carrier for the keratinous material. The range of viscosity of the obtained keratin-polyvinyl alcohol composite, which is suitable to be spread into films, was assessed. The effect of crosslinking of keratin-polyvinyl alcohol film on its mechanical properties as well as hygroscopic properties was monitored. Liquid chromatography mass spectrometric analysis elucidated that the molecular mass of the obtained soluble keratin is not less than 2 Dalton. The elemental analysis of the said composite divulges that only limited change in the carbon, hydrogen, sulphur and nitrogen contents was recorded, compared with the native keratinous substrates.

**Keywords:** Keratin, Feather, Film, Plasticizer, Polyvinyl alcohol and Wool.

Biologically, keratin is a type of insoluble proteins that associate as intermediate filaments and form the bulk of cytoplasmic epithelia and epidermal appendageal structures; Viz. hair, wool, horn, hooves and nails[(1)]. As one of the most abundant renewable biomaterials, keratin has many important commercial applications in the fiber, membrane, polymer, adhesives, and paints industries [(2)]. However, keratin waste is not adequately utilized at the present time [(3)]. Huge amounts of keratin waste are produced annually from slaughterhouses as well as the tanning industry. Poultry feathers constitutes up to 8.5 % of chicken weight and represent a considerable amount of almost pure keratin; a considerable amount of which is classified as environmentally unacceptable waste. About 0.9 to 1.5 billion kg of waste feather are produced in the United States in 2009 [(4)]. A restriction on the use of protein, however, is the limited number of useful solvents in which they are soluble.

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Among other proteins; Viz. collagen, albumin, gelatin and fibroin; keratin-based materials have the advantage of being biocompatible, biodegradable, mechanically durable, and naturally abundant.

Keratin-based composites were utilized in many applications such as regenerated fibres[5–8], ocular surface reconstruction[9], drug permeation[10], biosensing and biolabeling [11], hydrogels [12], scaffold for tissue engineering [13], cell culture [14] and absorbent for toxic substances such as heavy metal ions and formaldehyde [15].

Nevertheless, the practical use of keratin-based products was eventually inadequate owing, mainly, to their poor mechanical properties. Consequently, a lot of research was devoted to optimize the physical strength and flexibility of keratin films. This problem was overcome by blending keratin with other biopolymers such as gelatin [16], silk fibroin [17], and chitosan [14]. For the sake of better physicomechanical properties, keratin has been blended also with synthetic polymers such as poly ethylene oxide [18] and polyamide [19].

In the present work, keratin/PVA composite was prepared from renewable keratinous waste or cheap materials. The said keratin/PVA film was plasticized using glycerol and crosslinked by glutardialdehyde or iron II chloride. Chemical, physical, and mechanical properties of the said composite are assessed to assign its preliminary use whether in clothing and non-clothing fields.

## Materials and Methods

*Materials*

Scoured crossbreed wool fibres were purchased from Misr Company for Spinning and Weaving, El-Mehalla El-Kobra, Egypt. The tensile strength is 390 kg/cm$^2$ and elongation at break 80 %.

Raw Egyptian wool fleece (Barki) was kindly supplied from small enterprise in, Fowwa, Egypt. The tensile strength is 75.8 kg/cm$^2$ and elongation at break 60%.

Chicken feather, camel hair and human hair, were collected from the local market. The tensile strength and elongation at break of human hair are 494 kg/cm$^2$ and 110%; respectively.

Spectra/Por® 1 dialysis membrane (MWCO 68000 Da) was purchased from Spectrum (Rancho Dominguez, USA).

*Reagents*

Sodium hydroxide and thiourea were purchased from El-Nasr Pharmaceutical Chemicals Company, Egypt. Lithium hydroxide monohydrate was provided by Sisco Research Laboratories, Bombay, India. Strontium hydroxide was supplied by Acros Organics, New Jersey, USA. Barium hydroxide monohydrate was

purchased from Fluka, Steinheim, Germany. Urea was supplied by Merck, Germany. Low molecular weight polyvinyl alcohol (PVA) was purchased from TEXCHEM, Egypt. Glutardialdehyde (25 % aqueous solution w/w) was provided by Aldrich, Steinheim, Germany. Iron II chloride tetrahydrate was purchasd from BDH Laboratory Supplies, Poole, England. Egyptol PLM nonionic detergent based on nonyl phenol ethoxylate supplied from Starch and Brewer's Company, Alexandria, Egypt. All other chemicals are of laboratory grade and used without further purification.

*Methods*

*Scouring*

Raw wool fleece, crossbreed wool, chicken feather, human hair, or camel hair, were scoured using 2 g/l sodium carbonate and 1 g/l non-ionic detergent at 60 °C for 15 min followed by thorough rinsing with cold water then squeezed and finally air dried at ambient temperature.

*Dissolution*

Table 1 summarizes the methods of dissolution of crossbreed wool in different concentrations of aqueous solution of NaOH/urea/ thiourea, LiOH/urea/thiourea, $Sr(OH)_2$/urea/thiourea, or $Ba(OH)_2$/urea/thiourea, for different period of times at 30–90 °C; the material-to-liquor ratio (MLR) was 1:20. Other keratinous materials; namely chicken feather, human hair, and camel hair, were dissolved in aqueous solution of NaOH/urea/thiourea. The obtained soluble material was filtered through sintered glass crucible, and finally dialyzed against demineralized water (100 ml extract in 5000 ml water) using an MWCO 6–8000 Da (Spectra/Por®) cellulose membrane for 24 hr at 20 ºC. This procedure was repeated six times. The keratin dialysate was centrifuged at 10,000 g for 30 min to remove coarse aggregates and immediately used to prepare the keratin films.

**TABLE 1. Reagents and conditions used for dissolution of crossbreed wool as well as other keratinous materials [a] (MLR: 1:20).**

| Reagent | Conc. (M) | Temperature (°C) | Time (hr) |
|---|---|---|---|
| NaOH/urea[b]/thiourea[c] | 0.5 | 30/50/70 | 1.0/0.15/0.03 |
| | 0.75 | 30/50/70 | 0.83/0.11/0.03 |
| | 1.0 | 30/50/70 | 0.75/0.1/0.05 |
| LiOH/urea[b]/thiourea[c] | 0.5 | 30/50/70 | 3.5/0.5/0.25 |
| | 0.75 | 30/50/70 | 2.0/0.15/0.10 |
| | 1.0 | 30/50/70 | 1.0/0.5/0.04 |
| $Ba(OH)_2$/urea[b]/thiourea[c] | 0.1 | 30/50/70/90 | 48/3.5/0.75/0.33 |
| | 0.2 | 30/50/70/90 | 20/0.9/0.33/0.25 |
| | 0.3 | 30/50/70/90 | 15/0.33/0.16/0.08 |
| $Sr(OH)_2$/urea[b]/thiourea[c] | 0.1 | 30/50/70/90 | 168/4.0/3.0/1.0 |
| | 0.2 | 30/50/70/90 | 48/3.0/1.75/0.25 |
| | 0.3 | 30/50/70/90 | 21/2.5/1.5/0.1 |

a: Chicken feather, human hair, and camel hair, were dissolved in sodium hydroxide/urea/thiourea mixture.

b: 8 % (w/w) c: 6.5 % (w/w)

*Film formation*

Soluble protein (2 g) was mixed with 1 g polyvinyl alcohol at room temperature with occasional stirring for few minutes until complete mixing. The obtained viscous material was spread into a film on a glass plate (20 x 20 cm) at room temperature and left to dry overnight. PVA acts as a carrier or supporter for the keratinous material to form more assembled film.

*Crosslinking*

The dried keratin/PVA film (about 6 g) was incubated in 100 ml bath containing, 20 ml (25 % w/w) gluraldehyde, 10 ml glycerin, and 70 ml phosphate buffer pH 7 for 60 min at room temperature. The film was washed thoroughly with running distilled water and finally left to dry at ambient temperature. Glycerol acts as a plasticizer for the said keratin/PVA film.

Crosslinking of the film obtained by dissolution of crossbreed wool in aqueous solution of 0.3 molar strontium hydroxide in presence of urea and thiourea, was carried out using iron II chloride tetrahydrate. A one gram of the keratin/PVA film was refluxed with 0.05 g $FeCl_2$ in about 20 ml of 1-butanol till boiling for about 15min. The sample was left to dry in air.

*Analyses*

*Apparent viscosity*

The apparent viscosity of selected viscous material obtained from the dissolution of keratin, after being mixed with PVA, was assessed using Programmable Rheometer Model DV III (Brookfield Engineering Labs. Stoughton, MA, USA). The less viscous materials were determined with spindle 21, and the more viscous materials were determined with the 27 spindle. About eight ml of each sample was used. Slurry temperature was maintained at 25°C with a jacketed water bath.

*Solubility in water*

The degree of solubility of the obtained keratin/PVA films was assessed according to the method of Turhan and Sahbaz with minor modifications[20]. In our study, the film solubility (%) in water was assumed to be the ratio of water-soluble solids, after 15, 30, and 60 min immersion in water at 30 and 40 °C, to the initial solid content. For that, three film samples with an area of 2x3 $cm^2$ were cut, weighed and placed in an Erlenmeyers with 30 ml of distillated water, which were sealed with parafilm. During the assays, the Erlenmeyers were kept at 25°C in a thermostatic bath with mild stirring. These films, taken from Erlenmeyers, and original films were dried in a desiccator over phosphorous pentoxide for 48 hr for determination of solid contents. The film solubility was the average value of three measurements.

*Swelling test*

The swelling of keratin film was measured according to the method described by Tanabe *et al.* [6]. The examined samples are those prepared by dissolution of

crossbreed wool in various concentration of caustic soda in presence of urea and thiourea at 70 °C using MLR 1:20, followed by mixing with PVA and finally crosslinking with glutardialdehyde. Keratin/PVA film samples, prepared by dissolution of crossbreed wool in NaOH were cut into square pieces (10 x 10 $mm^2$) and immersed in acetate buffer (*pH* 4.0), distilled water (*pH* 6.0) or phosphate buffer (*pH* 8.0) at room temperature until the film reached to constant size. Typically, complete equilibration was obtained within 2 hr. The equilibrium-swelling ratio was calculated according to the following equation: $[(L_{wet} - L_{dry}) / L_{dry}]$ x100, where $L_{dry}$ and $L_{wet}$ are the length of a side of the dry and swollen film, respectively.

*Water absorption test*

Water absorption was measured gravimetrically according to the method of Reichl *et al* [9]. Samples were incubated in distilled water at 37°C and their weights were measured after 24 hr of immersion time. Prior to weighing, the water on the surface was wiped off with tissue paper. The amount of water absorbed was compared to the dry weight.

*Elemental analysis*

The amounts of carbon, hydrogen, nitrogen and sulphur in the keratinous materials as well as selected samples of the obtained films were assessed using the device Elementar CHNS Analyser, Model Vario EL III, Germany.

*Liquid chromatography mass spectrometry (LCMS)*

The molecular weight of the obtained soluble keratins was determined by electro spray ionization (ESI) on the negative mode, LC MS MS, LCQ Advantage MAX (Thermo electron Corporation, USA).

*Base combining capacity*

The base combining capacity was estimated by measuring the amount of alkali combined with the keratinous material as follows [21]:

(a) The sample was soaked in 2% hydrochloric acid for 3 to 4 hr with occasional shaking. The sample was filtered and washed several times with ethanol/water mixture (60–40) until chloride ions are free. Then the sample was filtered and dried.

(b) The dry sample (0.5 g) was precisely weighed and introduced in 250 ml Erlenmeyer flask, followed by 50 ml 0.1 N sodium hydroxide solution containing 5% sodium chloride. The flask was stoppered and allowed to stand overnight with occasional shaking. The content of the flask was back-titrated with 0.05 N hydrochloric acid using phenolphthalein as indicator. Blank titration was carried out on an untreated sample, and the carboxyl content of the sample was determined as follows:

$$\text{Carboxyl content} = \frac{(X - Y)\, N_A}{W} \times 100 \text{ meq/100 g fibre}$$

where *X*: volume of HCl solution used in titration of control sample,
*Y* : volume of HCl solution used in back titration,
$N_A$: normality of HCl solution, and
W : weight of sample (in g).

*Tensile properties*

The tensile strength and elongation at break of the keratin/PVA films, as well as PVA film as a control sample, were assessed using "Instron 5500R Universal Testing Machine" at 22 °C and 65 % relative humidity. This type of Instron has a self calibration, zero adjusting and automatic balance, which are done daily before testing or during testing. This testing instrument is accompanied by a highly reliable system for evaluating the mechanical properties.

## Results and Discussion

Scoured crossbreed wool fibres were dissolved in different concentration of alkali metal hydroxides (sodium hydroxide and lithium hydroxide) or alkaline earth metal hydroxides (strontium hydroxide and barium hydroxide), in presence of swelling agent (urea) and reducing agent (thoiurea). The alkali concentrations, reaction time and temperature depend on the used alkali (*c.f.* Table 1). The use of lower concentrations of strontium and barium hydroxides in dissolution of keratin than those of sodium and lithium hydroxides, is due to the relatively higher molecular weights and lower water solubility of the former ones. On the other hand, coarse wool, chicken feather, human hair, and camel hair were dissolved only in aqueous solution of NaOH/urea/thiourea.

The soluble keratin was mixed with polyvinyl alcohol (PVA) as a supporter for keratin. Films prepared from keratin only are stiff and brittle and practically useless. The keratin/PVA films were incubated in glycerol to impart better plasticity to the films. The strength of the obtained films was enhanced by crosslinking with glutardialdehyde.

*Apparent viscosity*

The apparent viscosity of the prepared keratin/PVA mixtures was evaluated to assign the proper viscosity at which the keratin/PVA composite might be suitable for spinning into fibres or spreading into film. Data of this investigation, shown in Fig. 1, illustrate that the viscosity of the keratin/PVA mixture that can be spread into film ranges between 650 cp (centipoises) and 1950 cp. For the same reagent, the viscosity of keratin/PVA mixture decreases in the order: coarse wool > crossbreed wool > feather > camel hair > human hair.

This figure revealed also that, irrespective to the used substrate, the viscosity of keratin/PVA mixture followed the order: NaOH/urea/thiourea> LiOH/urea/thiourea > $Sr(OH)_2$/urea/thiourea > $Ba(OH)_2$/urea/thiourea.

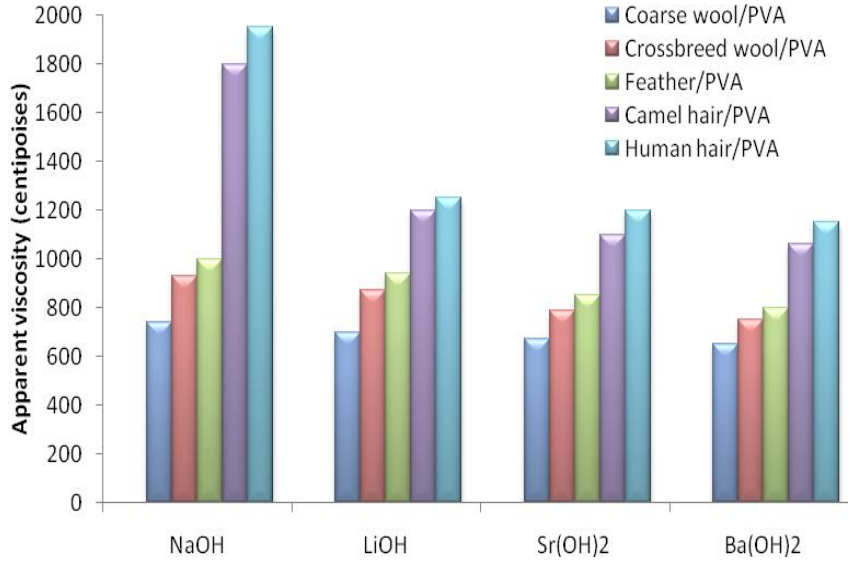


**Fig. 1. Apparent viscosity of soluble keratin/PVA mixtures (MLR: 1:20).**
Soluble keratin was prepared by dissolution of different keratinous substrates using NaOH (1 M solution, 70 °C, 2 min), LiOH (1 M solution, 70 °C, 2.5 min), $Sr(OH)_2$ (0.3 M solution, 70 °C, 9.5 min), or $Ba(OH)_2$ (0.3 M solution, 70 °C, 90 min), in presence of urea and thiourea. The apparent viscosity of 10 % PVA (then diluted with water by ratio of 1:2 PVA:water; respectively, is 450 centipoises.

*Hygroscopic properties*

The hygroscopic properties of the prepared keratin/PVA films are of prime importance to assign their possible applications whether in traditional textile application or in unconventional non-textile uses. These properties include the degree of solubility in water, the swellability in aqueous solutions at different pH values, and the water absorbability.

*Solubility of keratin/PVA films in water*

The degrees of solubility of crosslinked as well as uncrosslinked keratin/PVA films in water at 30 and 40 °C for 15, 30 and 60 min, was investigated. Results of this investigation (not shown here) revealed that keratin/PVA films, obtained by dissolution of keratinous material in NaOH/urea/thriourea, LiOH/urea/thriourea, or $Ba(OH)_2$/urea/thriourea, have variable degrees of solubility depending on the temperature of the bath and the incubation time in a direct relationship. Maximum solubility in water (43.5 %) was encountered at 40 °C for 60 min upon dissolving keratin/PVA film, prepared by dissolution of crossbreed wool in 0.3 Molar $Ba(OH)_2$ in presence of urea and thiourea at 90 °C.

Results of this investigation indicate also that, in all cases, treatment of keratin/PVA films with glutardialdehyde hindered its degree of solubility in

water, presumably due to formation of new crosslinks between the polypeptide chains by virtue of their amino and carboxylic groups. Minimum solubility (1.59 %) in water was recorded at 30 °C for 15 min for keratin/PVA film, prepared by dissolution of crossbreed wool in 0.5 Molar NaOH solution in presence of urea and thiourea at 70 °C followed by crosslinking with glutardialdehyde. Glutardialdehyde has been reported as one of the best crosslinker of proteins [(22)]. Pyridinium crosslink structure was proposed to be derived from glutardialdehyde and proteins[(23)].

On the other hand, Keratin/PVA films obtained by dissolution of coarse wool, crossbreed wool, chicken feather, human hair, and camel hair in $Sr(OH)_2$/urea/thiourea mixture, were found to be dramatically soluble in cold water. Although strontium hydroxide has lower basicity than the other used alkalis, yet it needs prolonged time, up to 168 hr, for dissolution of keratin. The higher concentration and prolonged time used during dissolution of keratin in strontium hydroxide may have destructive action to protein substrates and resulted in its partial hydrolysis, and hence solubility in water.

To lower degree of solubility of keratin/PVA films in water, the film was crosslinked with iron II chloride in nonaqueous medium (1-butanol). Crosslinking of keratin/PVA film with $FeCl_2$ decreases its solubility in water by about 20%, relative to the uncrosslinked films. This finding might be attributed to the presence of vacant orbital in iron II ions of iron II chloride makes them electron acceptors that are able to form coordinate bond with any electron donor atom (*e.g.* oxygen and nitrogen atoms) along the keratin macromolecule. The newly formed coordinate bonds, in turn, decrease the solubility of keratin-based films significantly.

On the other hand, the solubility test was undergone on unplasticized keratin/PVA films. It has been reported that the degree of solubility of keratin-based films increased by two folds when plasticizer was added. The relatively low solubility of keratin/PVA films without plasticizer suggests that some of the –S–S– bridges were formed during drying of films, although other interactions such as hydrogen bonds and electrostatic and hydrophobic interactions could also be improved. The results also indicated that the addition of glycerol avoided the formation of these bridges, and hence, increasing the films' solubility[(24)].

*Film swelling and water absorption of keratin/pva films*

It is noteworthy to mention that the samples examined in this investigation were selected on the basis of their limited solubility in water (not more than 2%). Hence, the reported results for the swellability and absorbability are representative data away from the solubility data.

Swelling of keratin–PVA films, obtained by dissolution of crossbreed wool in NaOH/urea/thiourea, in water was investigated at pH 4.0, 6.0 and 8.0. Results of this investigation, shown in Table 2, clarify that the swelling ratio of keratin–PVA

film depends on the pH value of the medium and/or the concentration of sodium hydroxide. Maximum swelling ratios (120 %) and water absorption (253.5 %) were recorded in case of keratin/PVA film prepared by dissolution of crossbreed wool in 1.0 Molar sodium hydroxide solution in presence of urea and thiourea.

**TABLE 2. Swelling and absorption ratios of keratin/PVA films in aqueous solutions at different pH values at room temperature using MLR (1:100).**

| Film | Swelling ratio (%) | | | Water absorption (%) |
|---|---|---|---|---|
| | pH 4 | pH 6.0 | pH 8.0 | |
| Keratin/PVA film prepared by dissolution of crossbreed wool in 0.5 M NaOH/urea/thiourea | 87.6 | 10.6 | 110.5 | 239.4 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in 0.75 M NaOH/urea/thiourea | 97.6 | 10.1 | 114.3 | 253.3 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in 1.0 M NaOH/urea/thiourea | 99.7 | 10.9 | 120.0 | 253.5 |

While PVA is a neutral compound with no ionized groups, keratin is an acidic protein whose isoelectric point is between 4.9 and 6.1[25]. Therefore, keratin/PVA film is positively charged at pH 4.0 and negatively charged at pH 8.0, resulting in the extensive swelling because of the repulsion between polypeptide chains of similar charges. On the other hand, limited swelling ratio of keratin/PVA film was observed at pH 6.0 due to the presence of most of the amino acid resides along the keratin macromolecule in the *Zwitter ion* form.

Data of Table 2 reveal also that keratin film absorbs about 130 % water of its mass when immersed in water for 24 hr at room temperature. This may be rationalized on the basis of the presence of polar groups along the keratin macromolecules; viz. amino, carboxylic, and hydroxyl groups, which are able to form hydrogen bonds with water molecules. The water absorbability of keratin was even enhanced by blending with PVA, presumably due to the presence of polar hydroxyl groups along the PVA polymer chains.

*Elemental analysis*

The carbon, hydrogen, sulphur and nitrogen contents all the used keratinous substrates as well as the keratin/PVA films obtained by dissolution of the said keratins in different solubilising media, were measured and tabulated in Tables 3 and 4.

**TABLE 3. C, H, S, and N contents of some keratins as well as keratin/PVA (K/PVA) films obtained by their dissolution in NaOH/urea/thiourea at 70 °C and LMR: 1:20.**

| Sample | C% | H% | S% | N% |
|---|---|---|---|---|
| Crossbreed wool | 44.8 | 10.8 | 3.6 | 14.8 |
| Coarse wool | 43.9 | 8.6 | 3.7 | 14.4 |
| Feather | 44.1 | 9.8 | 2.9 | 13.9 |
| Human hair | 43.8 | 10.2 | 4.0 | 13.7 |
| Camel hair | 44.6 | 9.8 | 3.1 | 14.4 |
| K/PVA film prepared by dissolution of *CBW* in 0.5 M NaOH | 37.9 | 13.8 | 2.9 | 12.7 |
| K/PVA film prepared by dissolution of *CBW* in 0.75 M NaOH | 38.2 | 13.4 | 2.5 | 13.4 |
| K/PVA film prepared by dissolution of *CBW* in 1.0 M NaOH | 38.0 | 12.4 | 3.2 | 12.4 |
| K/PVA film prepared by dissolution of *CW* in 0.5 M NaOH | 38.0 | 9.6 | 3.3 | 13.4 |
| K/PVA film prepred by dissolution of *CW* in 0.75 M NaOH | 38.4 | 10.2 | 3.1 | 11.9 |
| K/PVA film prepared by dissolution of *CW* in 1.0 M NaOH | 36.8 | 11.4 | 2.5 | 12.7 |
| K/PVA film prepared by dissolution of *feather* in 0.5 M NaOH | 37.7 | 13.0 | 2.1 | 13.6 |
| K/PVA film prepared by dissolution of *feather* in 0.75M NaOH | 39.5 | 13.2 | 2.6 | 13.4 |
| K/PVA film prepared by dissolution of *feather* in 1.0 M NaOH | 39.3 | 13.8 | 2.2 | 12.5 |
| K/PVA film prepared by dissolution of *HH* in 0.5 M NaOH | 37.6 | 12.0 | 2.8 | 12.0 |
| K/PVA film prepared by dissolution of *HH* in 0.75 M NaOH | 38.3 | 11.2 | 2.5 | 12.8 |
| K/PVA film prepared by dissolution of *HH* in 1.0 M NaOH | 40.5 | 10.8 | 2.9 | 13.2 |
| K/PVA film prepared by dissolution of *CH* in 0.5 M NaOH | 40.0 | 12.2 | 3.4 | 12.8 |
| K/PVA film prepared by dissolution of *CH* in 0.75 M NaOH | 37.9 | 14.6 | 3.0 | 11.9 |
| K/PVA film prepared by dissolution of *CH* in 1.0 M NaOH | 39.1 | 10.4 | 2.8 | 13.2 |

CBW: crossbreed wool, CW: coarse wool, HH: human hair, and CH is camel hair.
All reagent were used in presence of urea and thiourea (c.f. experimental part).

**TABLE 4. C, H, S and N contents of crossbreed wool as well as keratin/PVA films obtained by its dissolution in different reagents at 70 °C and LMR: 1:20.**

| Sample | C% | H% | S% | N% |
|---|---|---|---|---|
| Crossbreed wool | 44.8 | 10.8 | 3.6 | 14.8 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.5 M NaOH*/urea/thiourea | 37.9 | 13.8 | 2.9 | 12.7 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.75 M NaOH*/urea/thiourea | 38.2 | 13.4 | 2.5 | 13.4 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *1.0 M NaOH*/urea/thiourea | 38.0 | 12.4 | 3.2 | 12.4 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.5 M LiOH*/urea/thiourea | 38.1 | 10.8 | 2.4 | 14.8 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.75 M LiOH*/urea/thiourea | 45.8 | 13.6 | 3.2 | 13.7 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *1.0 M LiOH*/urea/thiourea | 37.1 | 12.8 | 2.4 | 14.2 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.1 M* $Ba(OH)_2$/urea/thiourea | 40.3 | 10.0 | 2.9 | 14.1 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.2 M* $Ba(OH)_2$/urea/thiourea | 38.0 | 12.0 | 3.1 | 14.2 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.3 M* $Ba(OH)_2$/urea/thiourea | 38.6 | 12.2 | 3.0 | 12.1 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.1 M* $Sr(OH)_2$/urea/thiourea | 37.5 | 10.6 | 3.6 | 13.6 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.2 M* $Sr(OH)_2$/urea/thiourea | 32.2 | 11.2 | 3.5 | 13.9 |
| Keratin/PVA film prepared by dissolution of crossbreed wool in *0.3 M* $Sr(OH)_2$/urea/thiourea | 34.7 | 12.8 | 3.2 | 13.0 |

Data of Table 3 divulge that preparation of keratin film by dissolution of crossbreed wool, coarse wool, feather, camel hair and human hair 0.5 – 1.0 Molar aqueous sodium hydroxide solution in presence of urea and thiourea, caused, in most cases, slight decrease in the carbon, nitrogen and sulphur contents. This is due, most probably, to a limited desulphydration (in a form of $H_2S$ gas), decarboxylation (in a form of $CO_2$ gas) and deamination (in a form of $NH_3$ gas) of some amino acid residues along the keratin macromolecules takes place during dissolution of keratin under the used reaction conditions.

Similar results were attained in case of keratin/PVA films obtained by dissolution of crossbreed wool in different alkaline solutions in presence of urea and thiourea (Table 4).

*Liquid chromatography mass spectrometry (LCMS)*

The molecular masses of the soluble keratin obtained by dissolution of crossbreed wool in aqueous solution of NaOH/ urea/ thiourea, LiOH/urea/ thiourea, $Ba(OH)_2$/ urea/ thiourea and $Sr(OH)_2$/urea/ thiourea were determined using liquid chromatography mass spectrometric (LCMS) analysis. The mass spectra of these samples clarify that the molecular mass of any sample exceeds 2 kDa. Putting into consideration the fact that the maximum molecular mass of any amino acid in keratins is 240, for cystine, while the lowest one is 70, for glycine, we may assume that the average molecular weight for each amino acid is 100, and hence the number of amino acid residues along any oligopeptide chain of the obtained soluble keratin is at least 20 amino acid residues [26]. Based on previous studies, that use severe alkaline conditions to obtain soluble keratin of molecular mass of 6–9 kDa, the authors would predict that the molecular mass of the obtained soluble keratin using either milder dissolution parameters or weaker bases would exceed this value [27].

*Base-combining capacity*

The base-combining capacity of keratin is a function of the free acidic groups found along its macromolecules. Data of Table 5 illustrate that the carboxylic content decreases remarkably upon dissolution of crossbreed wool in the used alkaline solutions. This is a clue that decarboxylation of amino acid residues took place during dissolution of keratin. The carboxylic content of keratin film prepared by dissolution of crossbreed wool in $Ba(OH)_2$/urea/thiourea is about one fifth that of native crossbreed wool. Barium hydroxide was reported as a decarboxylating agent for α-amino acids [28].

**TABLE 5. Carboxylic content of crossbreed wool as well as some of keratin/PVA films prepared thereof at 70 °C and LMR: 1:20.**

| Sample | Base combining capacity (meq./100 g sample) |
|---|---|
| Crossbreed wool | 132.4 |
| Film prepared by dissolution of wool in NaOH/ urea/thiourea | 73.4 |
| Film prepared by dissolution of wool in LiOH/ urea/thiourea | 83.2 |
| Film prepared by dissolution of wool in $Ba(OH)_2$/ urea/thiourea | 28.8 |

*Mechanical properties*

The mechanical properties of the obtained material play an important role in assigning the possible usage of the proposed keratin-based fibre or composite. Table 6 summarizes the tensile strength and elongation at break of keratin-based films as well as raw keratinous substrates.

**TABLE 6. Tensile strength and elongation at break of various keratinous materials as well as those of keratin-based films (at 70 °C and LMR: 1:20).**

| Sample | Tensile strength ($kg/cm^2$) | Elongation at break (%) |
|---|---|---|
| PVA film | 108.1 | 51 |
| Crossbreed wool/LiOH/urea/thiourea/PVA | 50.5 | 246 |
| Crossbreed wool /LiOH/urea/thiourea/PVA/ glutardialdehyde | 79.1 | 265 |
| Crossbreed wool/$Ba(OH)_2$/urea/thiourea/PVA | 41.9 | 175 |
| Crossbreed wool/$Ba(OH)_2$/urea/thiourea/PVA/ glutardialdehyde | 66.8 | 203 |
| Crossbreed wool/$Sr(OH)_2$/urea/thiourea/PVA | 25.4 | 235 |
| Crossbreed wool /$Sr(OH)_2$/urea/thiourea/PVA/ $FeCl_2$ | 45.8 | 185 |
| Crossbreed wool/NaOH/urea/thiourea/PVA | 27.2 | 208 |
| Crossbreed wool /NaOH/urea/thiourea/PVA/ glutardialdehyde | 59.9 | 300 |
| Coarse wool/NaOH/urea/thiourea/PVA | 46.7 | 225 |
| Feather/NaOH/urea/thiourea/PVA | 56.0 | 200 |
| Camel hair/NaOH/urea/thiourea/PVA | 54.9 | 170 |
| Human hair/NaOH/urea/thiourea/PVA | 55.5 | 155 |

Data of this table elucidate that the tensile strength of all keratin/PVA films is lower than that of PVA film and much lower than that of the native keratinous material. On the other hand, the elongation at break of all keratin-based films is much higher than that of PVA films.

The reduction in the tensile strength of all keratin/PVA films may be attributed to the rupture of disulphide bonds in keratin macromolecules by the influence of alkali [29], as well as cleavage of the hydrogen bonds by urea [30].

Data clarify also that the tensile strength of any crosslinked keratin/PVA film is higher than its non-crosslinked analogue. This finding assures formation of new crosslinks among the keratin macromolecule which were after-treated by glutardialdehyde or iron II chloride; results which are in harmony with the degree of solubility of crosslinked keratin/PVA films in water.

Close investigation of the results in Table 6 reveals that within the same dissolution medium, the tensile strength varies from keratinous substrate to another in the order: feather ≈ human hair > camel hair > coarse wool> crossbreed wool. Crosslinking of crossbreed/ PVA film resulted in increasing its tensile strength to a value higher than that of the non-crosslinked films obtained from the other substrates.

## Conclusion

Keratins can be solubilized in dilute solutions of alkali metals or alkali metal hydroxides in presence of swelling and reducing agents without severe degradation of the keratin macromolecules. The soluble keratin can be spread into film after being blended with PVA and a trihydric alcohol (glycerol) as a plasticizer. The hygroscopic properties as well as the mechanical properties of the produced keratin/PVA films imply some possible unconventional applications of these films in medicine and pharmacology. Further studies are directed towards the feasibility of using such keratin/PVA materials in scaffolds for tissue engineering, bacteria culture, and surgical threads. Further investigation will be directed towards studying the morphological structure of the prepared composite will be extensively investigated to assign its proper use.

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## تحضـــير وتوصيف متـــراكب الكيـــراتين/عديـد الكحـــول الفينيلي

**أميرة أبوالخير، سلوى موافي، مروى أبوطالب و حسام السيد**
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يهدف هذا البحث إلى استخدام وسيلة مجدية إقتصاديا في تحضير متراكب من الكيراتين/عديد الكحول الفينيلي باستعمال بقايا المواد الكيراتينية مثل الصوف المصري الخشن، الريش، الشعر. ولإذابة هذه النفايات تم إستخدام خليط من هيدروكسيدات عناصر الأقلاء أو هيدروكسيدات عناصر الأقلاء الأرضية، مادة مسببة لإنتفاخ الشعيرات، عامل مختزل. ثم يتم خلط الكيراتين الذائب مع عديد الكحول الفينيلي لتكوين متراكب قابل للتشكُّل في صورة فيلم. تم تحضير تركيزات مختلفة من عديد الكحول الفينيلي لتقييم اللزوجة المناسبة لتكوين الفيلم ثم تم دراسة تأثير إضافة مواد قادرة على تكوين رباطات عرضية على كل من الخواص الميكانيكية والإسترطابية للفيلم المتحصل عليه. وقد أمكن باستخدام جهاز تعيين الكتلة إثبات أن الكتلة الجزيئية للمادة الكيراتينية الذائبة لا يقل عن 2 دالتون. كما أثبت التحليل الكيميائي للعناصر لبعض عينات للمتراكبات المتحصل عليها أن هناك تغييرات طفيفة في نسبة كل من الكربون، الكبريت، النيتروجين والهيدروجين بالمقارنة بالمواد الكيراتينية الخام.