

# Keratin as a Filler for Carboxylated Acrylonitrile-Butadiene Rubber XNBR

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**ABSTRACT:** The presented investigations concern the distribution and characterization of keratin waste, derived from the processes of cattle skins, and its use as a filler for carboxylated acrylonitrile-butadiene rubber XNBR. The recovered keratin improves mechanical proprieties such as tensile strength, hardness, resistance to fuel and oil. The addition of keratin in a quantity of five parts per hundred rubber (XNBR) increases the crosslinking density of composites, as testified by the formed ion nodes and the existing

mono and disulphide bonds. Electric conductance and water absorption increased with time for elastomers filled with protein. Therefore these composites are biodegradable after their period of use. As a result hydrophilic-hydrophobic protein-elastomeric composites will have usable properties. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 3674–3687, 2007

**Key words:** biopolymer; composites; rubber; waste; keratin

## INTRODUCTION

The present article concerns the studies on the use of keratin waste, derived from unhairing cow hides as a filler for carboxylated acrylonitrile-butadiene rubber (XNBR). The aim of this article is obtainment biodegradable composites with good usable proprieties; mechanical, with good hardness, elasticity and good resistance to fuels and oils, designed on bottoms to footwear.

This publication is one of the series concerning the beneficial use of keratin and keratin hydrolyzate as fillers for the elastomer (synthetic *cis*-1-4-polyisoprene rubber, IR).<sup>1–3</sup>

The review of literature reports on the use of keratin protein has been given in our previous papers.<sup>1–3</sup>

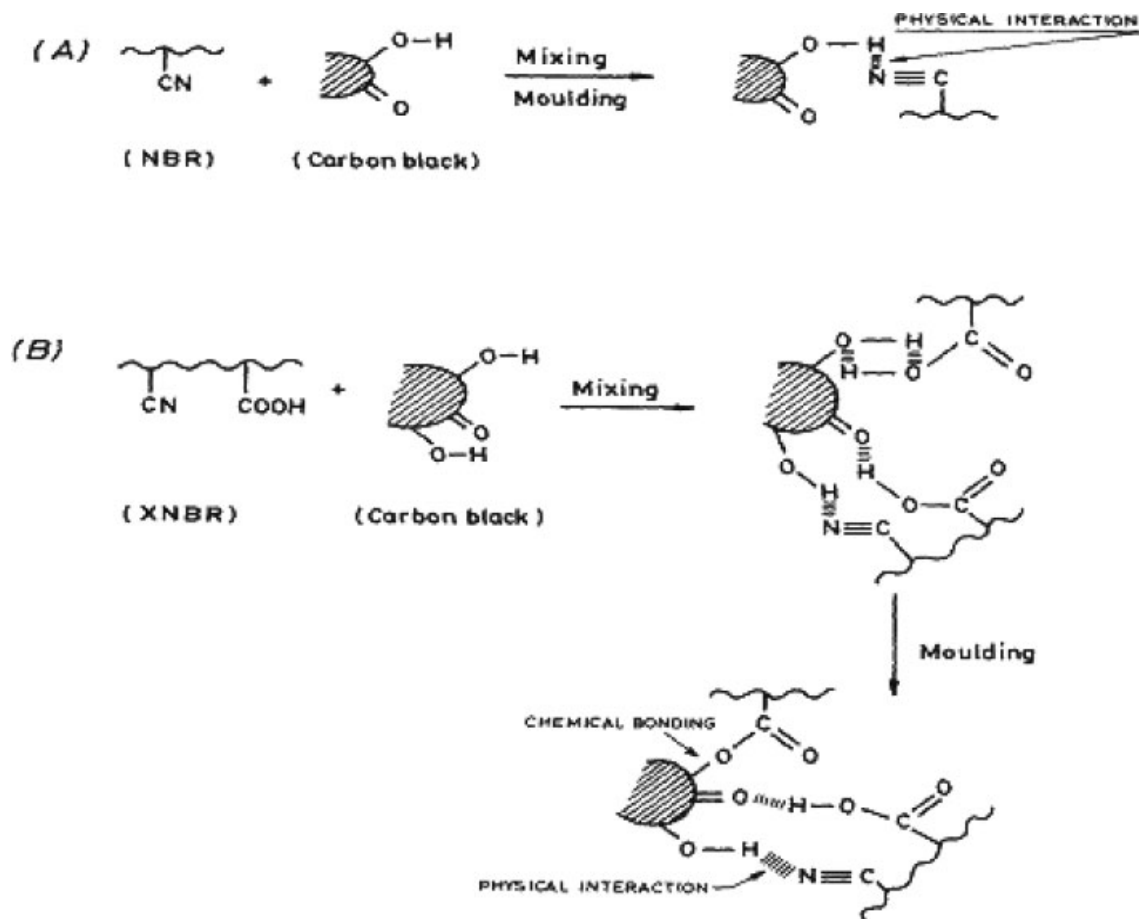
Considering the structure of keratin, this protein has become a subject of numerous studies.<sup>4</sup> The fibrous keratin protein consists of chains coiled into helixes and combined with peptide and hydrogen bonds and sulfide bridges that show a strong hydrophilic character. It is also worth noticing that keratin contains sulfur amino acids such as cysteine (sulfur content: 3.7%) in its composition. The chains of keratin are linked together with short, lateral, disulphide covalent bonds that are very stable, hence

keratin is often called a “vulcanized” protein. This protein easily undergoes reduction, oxidation, and hydrolysis, resulting in various products. Its chemical activity is just due to the presence of cystine in its structure.<sup>5</sup> Murayama-Arai and Takahashi<sup>6</sup> have found that keratin derived from bird feathers possesses 40% of hydrophilic and as much as 60% of hydrophobic groups, which can have important meaning in its use as a filler of hydrophobic rubbers. To improve the properties of elastomer such as synthetic *cis*-1-4-polyisoprene rubber, Goncharova et al.<sup>7</sup> have modified the latter with maleic anhydride to incorporate active epoxide groups that, in turn, facilitate the addition of keratin polar groups to the structure of hydrophobic polyisoprene rubber. Despite many applications of keratin wastes, there are still considerable amounts of unutilized keratin. From the literature it follows that keratin cannot be used as a filler of nitrile rubber yet.

Acrylonitrile-butadiene rubbers, called also nitrile rubbers, are characterized by a high elasticity, tensile strength, a low compressive strain, and resistance to oils. Hence seals made from this rubber are used in applied hydraulics and pneumatics. The vulcanizates of acrylonitrile rubbers with various acrylonitrile unit contents in macromolecules constitute a group of special elastomers commonly used for the manufacture of sealing materials, medical nitrile gloves that are more durable and adherent than latex gloves,<sup>8</sup> high-performance hoses resistant to elevated or low temperatures. Owing to their properties, nitrile rubbers have been widely studied. Bandyopadhyay et al.<sup>9</sup> have investigated the interactions

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**Figure 1** Probable mechanism of bonding in (A) NBR, carbon black and (B) XNBR, carbon black systems.<sup>9</sup>

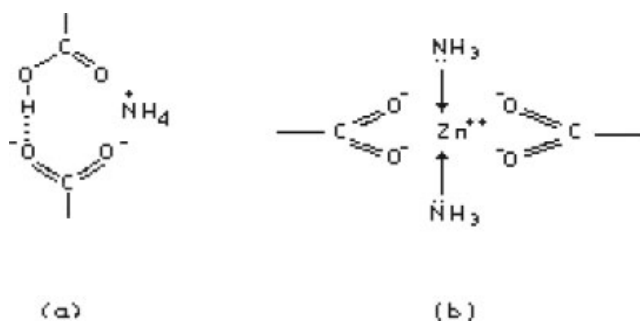
between rubbers such as NBR and XNBR and carbon black as a filler (Fig. 1). The authors have observed a high activity of carbon black and its oxidized surface that increases with increasing the crosslinking degree of the elastomer-filler structure. They expected it was due to the formed chemical and physical (weak hydrogen bonds and Van der Waals' forces) bonds between the carboxylic groups of rubber and the reactive groups on the filler surface. Figure 1 shows a possible linkage between filler and elastomers.

Wu et al.<sup>10</sup> have prepared aluminum nanocomposites containing montmorillonite (MMT) using latices such as styrene butadiene rubber (SBR), natural rubber (NR), nitrile butadiene rubber (NBR), and carboxylated acrylonitrile butadiene rubber (XNBR). The crosslinking with MMT, unlike the conventional crosslinking, has resulted in the improvement in strength properties of the obtained nanocomposites even about 15 MPa increased of tensile strength; moreover, shore hardness number has also increased from 8 to 22.

It is an important task to explain the crosslinking of XNBR rubbers with sulfur and zinc oxide. Sulfur

is a commonly known component of rubber mixes used for conventional crosslinking, while ZnO was used as an activator of such processes. Mandal et al.<sup>11,12</sup> in support about previous publications<sup>13,14</sup> confirmed existence ionic polymer called "ionomer." They have observed that the incorporation of ZnO into XNBR rubber leads to such type of the polymer. It is a polymer, in which carboxylic groups are neutralized by ZnO, which results in ionic crosslinking (Fig. 2). The hardness tests of rubbers have confirmed that the filler-elastomer interactions increase considerably when instead of conventional crosslinking with sulfur the ionic crosslinking with ZnO is used, which results in the formation of clusters.

Interesting literature reports including the mentioned above issues have inspired us to undertake studies that would allow us to characterize the waste protein obtained and to incorporate it as a filler into acrylonitrile-butadiene rubbers as well as to test the properties of the obtained protein-elastomer composites. However, in view of the extensiveness of this problem, the studies have been divided into two parts: the first one, discussed herein, concerns the carboxylated acrylonitrile-butadiene rubber and the



**Figure 2** Schematic diagram of (a) solvation of carboxylic amid by ammonia, (b) co-ordination of ammonia with zinc ion of carboxylic amid salt.<sup>12</sup>

vulcanizates obtained from it, while the other one concerns acrylonitrile–butadiene rubber.

The aim of the present study was to explain the influence of protein on the structure of spatial network of macromolecules and their density of crosslinking, defined as a number of node moles per the elastomer volume. It was also of importance to answer whether ZnO participates in the crosslinking processes as a crosslinker.

The study relates also to the effects of protein structure on the chemical and physical structures of protein–elastomer composites, unincluded as in the literature yet. Such interactions can result in products with interesting and useful properties such as resistance to thermal ageing or the action of oil and fuels. The elastomeric composites containing a hydrophilic protein should show an increased water absorption, which would make them biodegradable. Additionally, the increased resistance to the action of solvents would improve the quality of such composites.

## MATERIALS AND METHODS

Keratin was separated from tannery wastes originating from the liming and unhairing of cow hides, retaining the hair structure. It was dried, disintegrated, powdered followed by sieving through a sieve with 0.1 mm mesh diameter. The obtained keratin was then tested by FTIR spectroscopy (BIO-RAD 175C) with a microscope attachment to measure the particle size by the technique of dynamic light scattering (DLS) using a Zetasizer nano S90. Wider discussion about methods was in publication.<sup>1</sup>

The next step included the incorporation of keratin as a filler into carboxylated acrylonitrile–butadiene rubber, Krynac X7.50 XNBR from Bayer AG (6.7% of carboxylic groups). The scope of investigations at this stage comprised the following:

- The determination of rubber mix composition.

- Rubber mixes were prepared by means of a mixing mill at roll temperature of 30°C and friction of 1.15.

Vulcanization of the mixes was carried out in a vulcanization mold at 150°C for time  $\tau_{90}$  according to the standard PN-ISO 3417 : 1994 (vulcanometer with an oscillating rotor WG-02).

The rubber mixes were vulcanized in steel molds placed between electrically heated press shelves at a temperature of 150°C for  $\tau_{90}$ , the activity of filler was counted on basis of article<sup>15</sup>

- Tensile strength of the vulcanizates was determined by means of a Zwick 1442 tensile testing machine according to the standard PN ISO 37 : 1998.
- Thermal ageing resistance was investigated according to the standard PN-88/C-04,207. The principle of determination consists in exposing an unstressed sample to the action of circulating air at a temperature of 70°C for 100 h. Tensile strength and elongation at break of vulcanizates were measured before and after the ageing procedure. The ageing coefficient was determined from the following relationship in eq. (1):

$$S = \frac{[T_{s_1} E_{b_1}]}{[T_{s_2} E_{b_2}]} \quad (1)$$

where:  $S$  is aging coefficient;  $T_{s_1}$  is tensile strength and  $E_{b_1}$  is elongation at break after thermal ageing,  $T_{s_2}$  is tensile strength, and  $E_{b_2}$  is elongation at break before thermal ageing

- Crosslinking density was determined from equilibrium swelling in the following solvents: toluene, toluene–ammonia vapor and in water (in accordance with the standard PN-ISO 817 : 2001/ap1 : 2002). Four samples with different shapes were cut out from each vulcanizate, weighed, immersed in solvent and stored in a thermostatic chamber at 25°C for 48 h. The samples were then reweighed and dried to a constant weight at 50°C.

The density of rubber was measured according to standard PN-ISO 2781+AC1 : 1996 and the density of lattice was found in accordance with Flory and Rehner's equation.<sup>16</sup>

Symbols in Table III:

$V_T$ —crosslinking density of vulcanizate calculated from the measurement of equilibrium swelling in toluene [mol/dm<sup>3</sup>]

$V_{T/NH_3}$ —crosslinking density of vulcanizate calculated from the measurement of equilibrium swelling in toluene and ammonia vapor [mol/dm<sup>3</sup>]

$\Delta V_{NH_3}$ —concentration of the ion lattice points decomposing under the influence of ammonia [mol/dm<sup>3</sup>]:

$$\Delta V_{NH_3} = V_T - V_{T/NH_3}$$

A—percentage content of the ion lattice points decomposing under the influence of ammonia [%]

$$A = \frac{\Delta V_{\text{NH}_3}}{V_T 100\%} \quad (2)$$

The parameters of rubber–elastomer interactions:  
 $\mu$ —Huggins' parameter (parameter of polymer–solvent interaction);  $\mu = \mu_0 + \beta V_r$

where:

$V_r$ —volumetric content of elastomer in the sample under test

$\mu$ —the parameter of polymer–solvent interaction when  $V_r = 0$

$\mu_0$ —slope of the straight line describing the relationship:  $\mu = f(V_r)$

For XNBR (Krynac X7.50) rubber: in toluene  $\mu_0 = 0.357$ ;  $\beta = 0.201$ ; in toluene and ammonia vapor

$\mu_0 = 0.357$ ;  $\beta = 0.201$

$Q_w$ —equilibrium swelling in water [mg/mg]

C—C, C—S—C carbon and monosulphide bonds

C—S<sub>2</sub>—C disulphide bonds

C—S<sub>x</sub>—C polysulphide bonds

- The assessment of the percentage content of carbon bonds as well as mono-, di and polysulphide bridges in vulcanizates by the use of thiol–amine chemical analysis.<sup>17</sup>
- Dynamic Mechanical Thermal Analysis (DMTA) with the use of a mechanical spectrometer (viscoanalyser), MetraviB VA 815 cooperating with an appropriate software. The parameters: frequency 0.001–1000 Hz, dynamic force  $\pm 150$  N, displacement  $\pm 6000$  nm. Rectangular  $5 \times 30$  mm<sup>2</sup> samples were cut out from the vulcanizates and placed in a measuring chamber cooled with liquid nitrogen. The measurement was taken within the temperature range from  $-150^\circ\text{C}$  to  $+150^\circ\text{C}$ . The following relationships were determined: modulus of elasticity ( $E'$ ) versus temperature, dielectric loss modulus ( $E''$ ) versus temperature and mechanical loss tangent ( $\tan \delta$ ) versus temperature.
- The measurement of electric conduction according to Polish standard PN-85C-04,259/01.
- The determination of hardness by Shore's method according to PN-80C-04,238. The hardness of rubber was measured with the use of hardness tester, type A.
- The determination of elasticity was carried out according to Schob's method (PN-C-04,255 : 1997).
- The determination of absorbing capacity was performed by the percussive method in Schobe's elastometer according to the standard PN-54/C-04,268,
- IR spectroscopy (BIO RAD FTS 175C) of vulcanizates was performed by the transmission method.

Samples in the form of thin films were prepared by pressing under a pressure of 150 MPa.

- The surface of the composites obtained was tested with the use of AFM (Atomic Forces Microscopy).

## RESULTS AND DISCUSSION

The characteristics of keratin recovered from tannery effluents included: particle size, electro-kinetic measurements and IR spectral analysis. Based on previous studies, it was observed that the blending of keratin with zinc oxide makes its incorporation easier with other components into the elastomeric matrix. Therefore, in addition to keratin itself the keratin blended with zinc oxide was also analyzed.

### Measurements of particle size of keratin recovered from effluents and keratin blended with ZnO

It is of both theoretical and practical importance to determine the size of particles and the dispersive character of the substances under investigation. Also the filler particle size and the mode of filler incorporation into mixes exert a considerable influence on the filler dispersion in the elastomeric matrix. The dispersion, in turn, decides about the quality of the mixes obtained and the properties of the resultant vulcanizates. When the dimensions of particles range from 0.01 to 1  $\mu\text{m}$ , one deals with an active filler and consequently with such that performs a reinforcing function. Water was used as a standard disperser. The numerical distributions of keratin particles and ZnO are shown in Figures 3–5.

The numerical distribution curve of keratin particles (Fig. 3) shows the band of fraction with the highest content of particles (about 32%) with dimensions of 469 nm, which indicates that keratin can be an active filler to fulfill its reinforcing function. Once this protein is blended with ZnO (Fig. 4), the particle size decreases to 331 nm (about 46%), giving considerably less fractions of the remaining bands than in the case of pure keratin. Moreover, the polydispersity (the function of particle diameter scatter) considerably decreases in the case of keratin–zinc oxide blend: the scatter of particle diameters is considerably decreased. The ZnO particle size (Fig. 5) is about 664 nm, while the polydispersity considerably increases as compared to that of the substances under investigation. From these results it follows that blending the examined protein with zinc oxide has a positive bearing on the particle size. This is most likely due to the amphoteric character of zinc oxide that counteracts the aggregation of keratin particles.



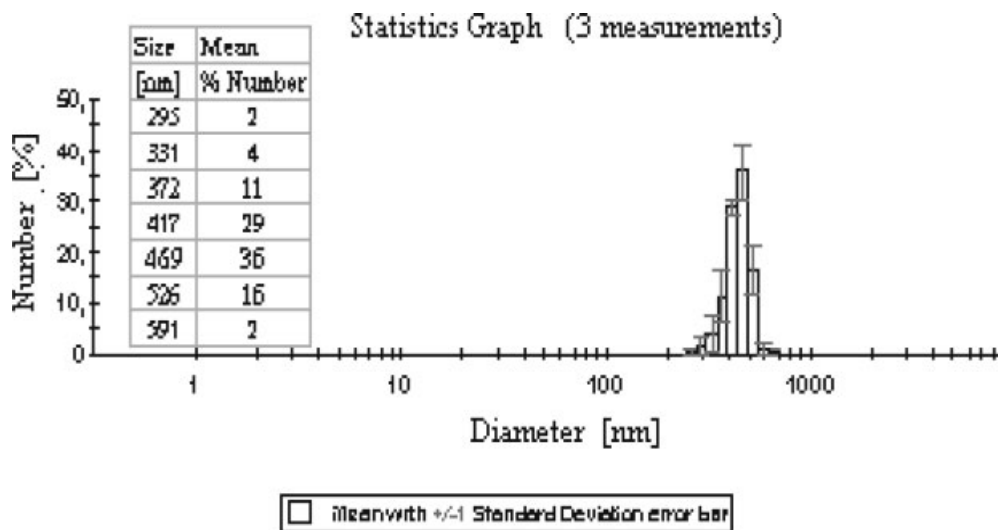


Figure 3 Diameter of keratin particles in water dispersion.

### Spectral FTIR analysis of powdered keratin

The range of characteristic bands of keratin and keratin-ZnO blend originating from particular functional groups is given in Table I.<sup>18</sup> Figure 6 shows the IR spectra of the examined protein powders. At wavelength ranging from 2850 to 2900  $\text{cm}^{-1}$  one can observe  $\text{CH}_2$  and  $\text{CH}_3$  (v) bands with similar intensities, originating from the terminal groups of side chains of keratin and keratin-ZnO blend. In both spectra of proteins are visible bands originating from primary, secondary, and tertiary amides. The spectra of keratin blended with zinc oxide show asymmetric valence vibration at 1580  $\text{cm}^{-1}$  and symmetric valence vibration at 1399  $\text{cm}^{-1}$  originating from ( $\text{COO}_2^-$ ) groups combined with  $\text{Zn}^{2+}$  ions. There is also observed deformation vibration of methylene group combined with peptide bond with similar

intensities for both spectra (1405  $\text{cm}^{-1}$ ). A clear band of valence vibration of  $\text{COO}^-$  and  $\text{SO}^-$  is seen in the spectrum of keratin. Keratin consists of polypeptide chains coiled into helices combined via mono- and disulphide bridges of cysteine.<sup>19</sup> There is also observed a shift of the band originating from the S—S bonds of cysteine due to the mixing of ZnO and keratin.

### Contents and properties mixes rubber

To assess the effect of powdered keratin recovered from cow hair on the properties of keratin-elastomer composites, acrylonitrile-butadiene rubber mixes were prepared, to which either pure keratin or keratin blended with zinc oxide was added. The compositions of the mixes under investigation are given in Table II.

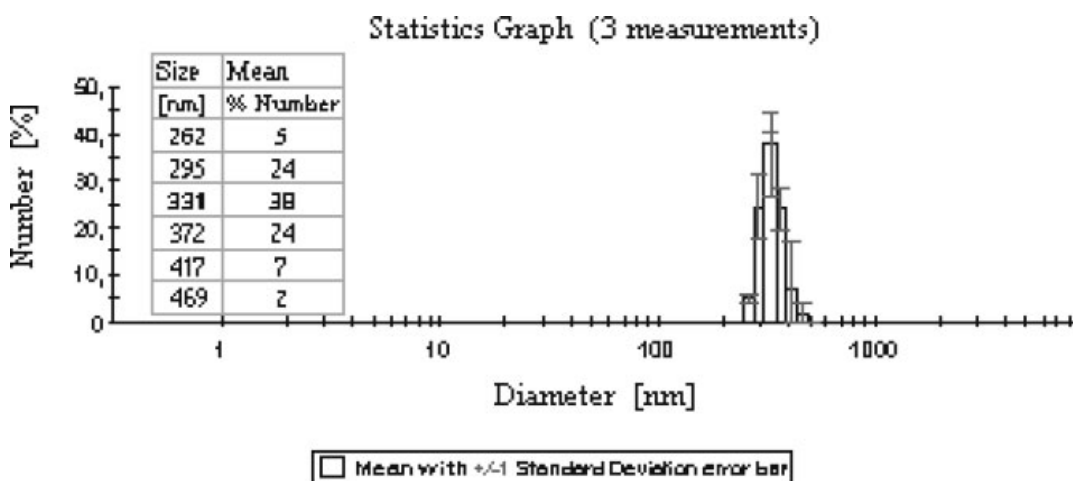


Figure 4 Diameter of keratin particles mixed with ZnO in water dispersion.

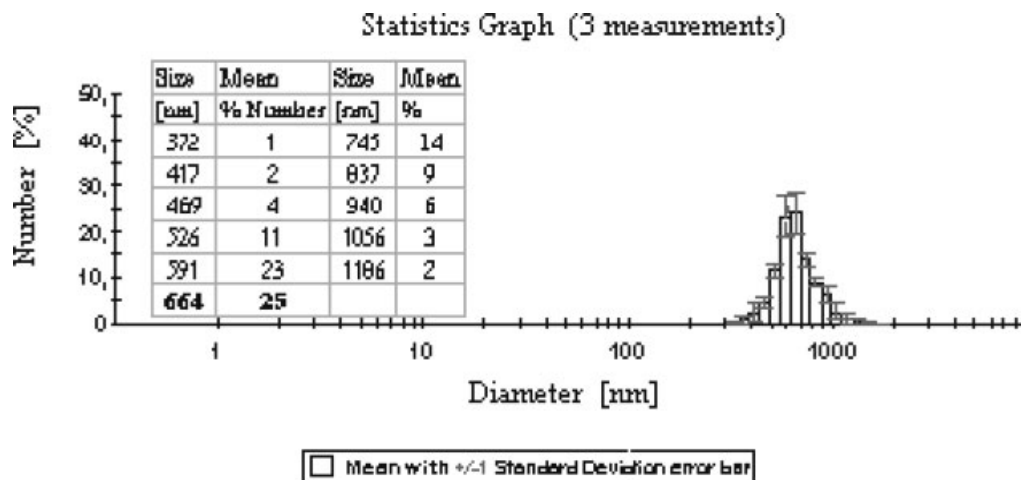


Figure 5 Diameter of zinc oxide in water dispersion.

### Crosslinking density and equilibrium swelling

The difference in the crosslinking densities in toluene and in toluene and ammonia vapor is a measure of the rubber-filler interaction, as the rubber-filler bonds are disrupted under the influence of ammonia vapors. Based on the measurements of equilibrium swelling in toluene and in toluene and ammonia vapors, the following parameters were determined: the crosslinking densities of three vulcanizates, the concentration of network nodes per the given rubber volume, disintegrating under the influence of ammonia vapors. Their percentage content, equilibrium swelling in water, and the percentage content of mono-, di-, and polysulphide bonds. The results are listed in Table III.

From the data given in Table III it follows that the incorporation of protein into the rubber mixes results in increased crosslinking density of vulcanizates in toluene. On the other hand, the crosslinking density in toluene and ammonia vapors is decreased as compared with that of standard vulcanizate. The concen-

tration of disintegrating nodes under the influence of ammonia vapors in keratin-containing vulcanizates is increased. The highest percentage of disintegrating ionic nodes is observed in the vulcanizate containing a powdered keratin blended with zinc oxide. From this it follows that the addition of protein brings about an increase in the ionic nodes in the elastomer matrix even by 10%–11% in relation to the vulcanizate without keratin. This seems to be due to the presence of COOH groups in XNBR rubber, which facilitate the formation of additional polymer-keratin bonds as well as new ionic bonds between zinc oxide and the amine and carboxylic groups of protein and also carboxylic groups of XNBR. Moreover, the keratin filler may limit the vulcanizate swelling as a result of the adhesion of macromolecules to its surface and may change the affinity of polymer to solvent and simultaneously increase the yield of rubber crosslinking, which, in turn, may influence the increase in the number of polymer-polymer bonds according to some, closely unknown mechanism.

**TABLE I**  
The Range of Characteristic Bands of Keratin and Keratin-ZnO Blend Originating from Particular Functional Groups

Characteristic functional groups	The kind of vibration	Wavelength [ $\text{cm}^{-1}$ ]	
		Keratin	Keratin/ZnO
—CH <sub>2</sub> , —CH <sub>3</sub> the side and final chains	$\nu$ - valence	2900–2850	2900–2850
Amide I rz.	$\delta$ - deformation	1658	1658
COO <sup>-</sup> , C=O [(COO) <sub>2</sub> <sup>-</sup> Zn <sup>2+</sup> ] - asymmetric	$\nu$	—	1580
Amide II rz.	$\delta$	1534	1534
—C(=O)—NH—CH <sub>2</sub> —	$\delta$	1405	1405
COO <sup>-</sup> , C=O [(COO) <sub>2</sub> <sup>-</sup> Zn <sup>2+</sup> ] - symmetric	$\nu$	—	1399
COO <sup>-</sup>	$\nu$	1395	—
Amide III rz.	$\delta$	1232	1232
SO sulphone group	$\nu$	1038	—
C—S (sulphides)	$\nu$	650	650
—S—S— (disulphides)	$\nu$	542 (small intensity)	542

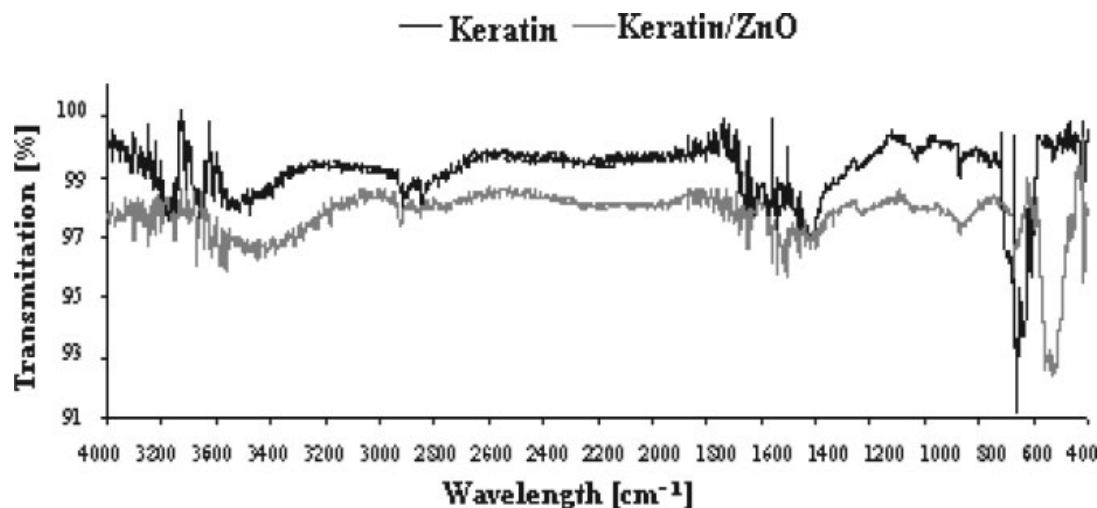


Figure 6 IR spectra of the examined protein powders.

It has been additionally confirmed that the addition of protein increases the affinity of the obtained elastomer-protein composites to water. This is due to the structure of keratin of cow hair. This keratin consists mainly of  $\alpha$ -keratin structures which under the influence of heat extend and transform into  $\beta$ -structure that in turn is capable of forming numerous hydrogen bonds imparting a hydrophilic character to keratin. Therefore, the addition of protein to elastomers facilitates water molecule penetration into the composite structure through the hydration and swelling of keratin. Hence, the transport of enzymes into such composites and their faster decomposition are possible after their service life.

The addition of powdered keratin or keratin blended with ZnO to rubber mixes increases the percentage content of mono- and disulphide bonds in relation to the standard vulcanizate. The mono- and disulphide bonds show the highest stability and will have a higher energy of activation, therefore it will be more difficult to disrupt them as distinguished from polysulphide bonds. The number of more stable bonds (C—S—C and C—S<sub>2</sub>—C) in the vulcanizate containing keratin blended with ZnO increases by 3.3% and 1.6%, respectively, as compared to the standard vulcanizate, while the number of poly-

sulphide bonds decreases by 4.9% in relation to the vulcanizate without protein.

From the performed series of tests it follows that in this protein-elastomer structure, there most likely predominates a trend towards the formation of ionic bonds as indicated by the increase in the percentage content of ionic nodes disintegrating under the influence of ammonia vapors. However, this does not exclude the presence of covalent bonds with sulfur; there also predominates a trend towards increased more stable bonds such as mono- and disulphide bonds.

The mode of incorporating keratin directly blended with zinc oxide influences also the process of vulcanizate crosslinking. It seems that zinc oxide forms complexes with keratin (similarly as with —COOH groups of XNBR), and precisely with —NH<sub>2</sub>, —OH and —COOH groups (crosslinking activators) to accelerate vulcanization processes and to increase the crosslinking density of composites.

### The rheometric and mechanical properties

The data given in Table IV show the effects of keratin incorporated directly into rubber mixes and keratin previously blended with ZnO on the rheo-

TABLE II  
The Compositions of the Mixes under Investigation

Composition	Keratin [parts by wt.]	Keratin/ZnO [parts by wt.]	ZnO [parts by wt.]
I (Standard – without keratin)	–	–	5
II (Keratin)	5	–	5
III (Keratin/ZnO)	–	5/5	–

Other ingredients: Benzothiazyl disulfide – 1,5 parts by wt., Polnox N – 1 part by wt., Stearine – 2 parts by wt., Ethylene glycol – 2 parts by wt., Sulfur – 2,5 parts by wt. per 100 parts by wt. carboxylated acrylonitrile-bitadiene rubber XNBR.

**TABLE III**  
**Crosslinking Density Determined by Measurement of Equilibrium Modul and Equilibrium Swelling**

Content	Parameters						
	$v_T/v_{T/NH_3}$ [mol/dm <sup>3</sup> ]	$\Delta v_1$ [mol/dm <sup>3</sup> ]	$A_1$ [%]	$Q_w$ for water [mg/mg]	Content of bonds [%]		
					C—C, C—S—C	C—S <sub>2</sub> —C	C—S <sub>x</sub> —C
Standard	3.89/2.84	1.05	26.99	0.059	18.22	23.90	57.88
Keratin	3.99/2.49	1.50	37.59	0.068	21.48	24.70	53.82
Keratin/ZnO	4.19/2.58	1.61	38.42	0.082	21.50	25.51	52.99

metric and mechanical properties of rubber mixes and the vulcanisates of carboxylated acrylonitrile-butadiene rubber, XNBR.

The direct blending of keratin and zinc oxide causes a higher increase in the torque that characterizes the degree of vulcanizate crosslinking, a decrease in the vulcanization duration and additionally a decrease in the mix viscosity as compared with the mix, into which keratin was added separately. This indicates an interaction with the accelerant, which prevents the cleavage of lateral bonds. The activator of the crosslinking complex is the presence of -OH and -COOH groups of the protein.

The increase in the torque of keratin-containing mixes is connected with the reinforcing effect of filler. As reported in the literature, Wolff et al.<sup>20</sup> has proved that by means of parameter  $\alpha_f$  one can describe the filler activity and its influence on the mechanical properties of vulcanizates such as stress at an elongation of 300% and tensile strength.

Based on the torque increments  $\Delta L$ , the activity of keratin was determined. The data given in Table IV

show that protein is an active filler and that its activity intensively increases when keratin directly blended with zinc oxide is incorporated into the rubber mixes.

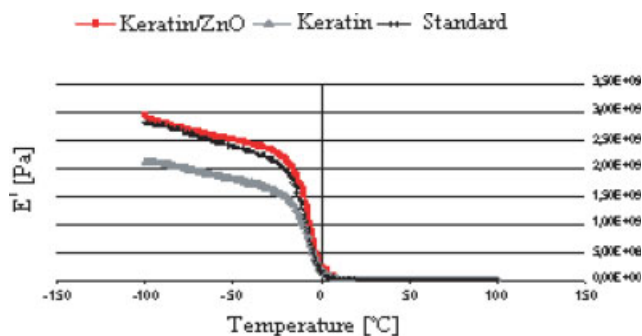
Zinc oxide will probably make the incorporation of keratin into rubber mixes even more beneficial since in addition to its activator function facilitating crosslinking with rubber due to the formation of zinc stearates, it may also act as crosslinker responsible for ionic crosslinking<sup>21</sup> forming structures shown in Figure 2. Additionally, the compounds of divalent zinc will probably form complexes in the keratin structure itself. Keratin can react with metal salts (e.g., zinc stearate) first of all via the active side groups of protein to form lateral bonds, which in turn will participate in the crosslinking of keratin-elastomer composites.

Active fillers usually increase strength, hardness, resistance to abrasion and durability of vulcanizates. The incorporation of five parts by wt. of keratin into XNBR has increased its stress at 300% elongation and tensile strength by about 2 MPa as compared to those of the vulcanizate containing no protein. One may assume

**TABLE IV**  
**Effect of Keratin on the Rheometric and Mechanical Properties Mixes and Vulcanizates XNBR**

Properties \ Content	Standard	Keratin	Keratin/ZnO
$L_L$ – minimal of torque moment [dNm]	7.7	17.7	10.4
$\Delta L$ – the decrease of torque moment ( $\Delta L = L_{HR} - L_L$ ) [dNm]	12.7	26	39.7
$\tau_{0.9}$ – time of vulcanization [min.]	8	8	7
$\alpha_f$ – filler activity	–	20.94	42.52
tensile stress [MPa] at elongation:			
100%	1.99	2.31	2.37
200%	2.99	3.53	3.70
300%	4.90	6.48	6.96
$T_S$ – tensile strength [MPa]	11.44	13.60	14.40
$\epsilon_b$ – elongation at break [%]	406.20	371.67	377.25
$S$ – the ageing coefficient	0.65	0.82	0.90
$\zeta_V$ – volume resistance [ $m \times \Omega$ ]	$2.93 \times 10^{10}$	$1.12 \times 10^9$	$5.98 \times 10^8$
$\zeta_S$ – surface resistance [ $m \times \Omega$ ]	$2.45 \times 10^{12}$	$4.49 \times 10^{11}$	$3.84 \times 10^{10}$
Shore'a hardness A [N]	54	57	58
Shob's elasticity [%] (Scale H = 0.5)	24	11	8
Shock absorption – the number of pendulum lean out	7	5	6
$Q_w$ – equilibrium swelling for patrol [mg/mg] in:			
Ethyline 98	0.39	0.25	0.17
Driving oil ON	0.88	0.58	0.56
Castrol	3.33	2.14	2.27





**Figure 7** The effect of keratin and keratin/ZnO on the conservative modulus  $E'$  versus temperature. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

that in the presence of filler particles the rubber macromolecules become well oriented due to the applied stress, which increases their capability to crystallization. Mechanical properties can be also improved due to small distances between the aggregates of filler in the elastomer matrix and better dispersion of these aggregates. Moreover, keratin itself show good mechanical properties<sup>22</sup> – it is often called “vulcanized protein” – owing to the presence of disulphide bonds, which ensure stable interconnection of chains and consequently strength; besides keratin becomes very reactive.

The incorporation of protein blended directly with zinc oxide into XNBR increases the resistance of this elastomer to thermal ageing. The differences between the tensile strength and elongation at break of keratin-containing vulcanizates measured before and after thermal ageing are considerably lower than those of the standard vulcanizate. One may assume that it is keratin that is responsible for this to a large extent. Keratin has numerous disulphide bridges (cystine), which during ageing can transform into groups of antioxidant character:  $\text{SO}_2$ ,  $-\text{SOH}$  (sulfonic acid radical,  $\text{RSOH}$ ),  $-\text{SH}$  (thiol group derived from  $\text{RSH}$ ), radical:  $\text{RS}\cdot\text{O}$ .

From the measurements of electric conduction (defined as the ratio of intensity of current passing through a conductor to the voltage between it ends) it follows that the standard rubber mix shows a higher resistance, while the mix containing keratin blended with zinc oxide has the highest conduction. This is also confirmed by the tests of equilibrium swelling in water, showing that standard vulcanizates have a lower resistance that of the vulcanizate containing protein.

Considering the possible use of the rubber under investigation for making footwear soles, the analysis of hardness and elasticity of the vulcanizates seemed to be useful. Tests of Shore's hardness have shown that the incorporation of keratin to vulcanizates improves this parameter and decreases Shore's elasticity. This results from the increased crosslinking

density of these vulcanizates as confirmed by previous tests.

The measurements of the capability of shock absorption, e.g., capability to absorb mechanical energy have shown that the vulcanizates containing protein are rather hard materials than elastic. They absorb mechanical energy or show capability to shock absorption, but to a lesser extent than that observed in the standard vulcanizate.

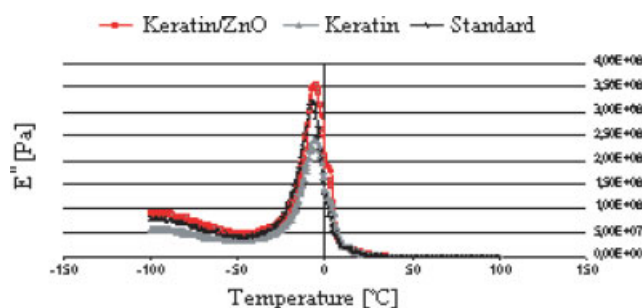
XNBR rubber is a polar rubber, insoluble in non-polar solvents, hence it is resistant to fuels, various paraffin oils and greases. Its resistance to oils such as Castrol motor oil increases when keratin blended directly with zinc oxide is incorporated into it.

Based on the tests discussed above, one can see that the best properties are shown by the vulcanizates of carboxylated acrylonitrile-butadiene rubber containing keratin blended directly with zinc oxide. An additional benefit is the fact that rubber vulcanizates filled with five parts by wt. of keratin recovered from tannery wastes will show equally good properties as those of the rubbers filled with standard fillers, their production costs will be lower and the vulcanizates themselves will be biodegradable.

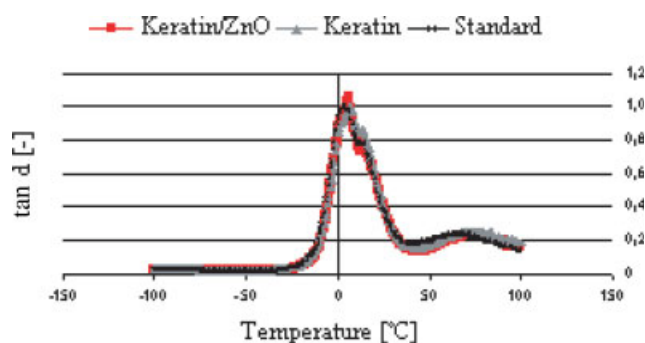
#### DMTA analysis

To find the glass transition temperature,  $T_g$  (e.g., transition from glassy state into high-plastic state; below this temperature follows the set-back of turns of substituent filler-elastomer's macromolecules), of the examined vulcanizates, the dependences of conservative modulus ( $E'$ ), loss modulus ( $E''$ ) and loss tangent ( $\tan \delta$ ) on temperature were determined. The following samples were used for these tests: a rubber conventionally crosslinked with sulfur, a rubber with no filler, rubbers filled with keratin and keratin blended with zinc oxide.

Figure 7 shows the effect of protein on the conservative modulus  $E'$  versus temperature. This modulus is a measure of elastic properties of the exam-



**Figure 8** The effect of keratin and keratin/ZnO on the loss modulus  $E''$  versus temperature. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



**Figure 9** The effect of unfilled and filled samples keratin and keratin/ZnO on the loss tangent  $\tan \delta$  versus temperature. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

ined vulcanizates and it correlates with the stress modulus at 100% elongation, and so also with Young's modulus describing the strength properties of vulcanizates.

The incorporation of keratin blended with zinc oxide into XNBR (Fig. 7) insignificant increases the value of conservative modulus  $E'$  (the curve shifts towards higher values of glass transition temperature). The vulcanizate containing five parts by wt. of keratin/ZnO also shows the highest value of stress modulus at 100% elongation ( $\delta_{100} = 2.37$ ), while the vulcanizate without protein has the lowest value of this parameter ( $\delta_{100} = 1.99$ ) and appropriately lower  $E'$ . As is seen from the course of curves, especially for the vulcanizate filled with keratin/ZnO, the dynamic modulus is violently decreased near the glass transition, which is connected with the increased number of freely moving chain fragments under the influence of absorbed thermal energy. The successive diagram (Fig. 8) concerns the loss modulus,  $E''$  that is a measure of viscous properties and consequently a measure of rubber capability to give up energy.

Greater plasticization of the product due to the addition of protein would be indicated by the shift towards negative values of temperature in relation to the standard, however in this case no shift is observed, but only insignificant increased loss modulus,  $E''$  that is the highest for the vulcanizate containing keratin/ZnO.

The glass transition temperature was determined from the course of  $\tan \delta$  curve, which reaches its maximum at  $T_g$  (Fig. 8).  $T_g$  of protein–elastomer composites is affected by molecular weight, chain rigidity, size and polarity of side groups, molecular interactions, capability to crystallization, type of elastomer, disintegration, and dispersion of filler.<sup>22</sup>

The coefficient of mechanical loss ( $\tan \delta$ ) is a measure of energy lost during work and a measure of the segmental motion of macromolecules describing the filler–rubber interactions; the smaller the mobil-

ity, the smaller are these interactions and the energy loss, so the system is stiffer.

Figure 9 shows that the addition of the examined filler has not some real effect on the glass transition temperature of vulcanisates. The latter was determined by means of the Rheometric Scientific program. The results obtained are given in Table V.

The insignificant change in the values of mechanical loss coefficient of unfilled and filled samples is within the error limits. On the other hand, the value of glass transition temperature of the keratin-containing vulcanisates insignificant increases, which may indicate an insignificant decreased segmental mobility of filler macro-particles and elastomer, therefore there will be a slight loss of energy during work. The rigidity of the systems filled with protein will increase in small range. The test results of mechanical parameters confirms that the elastomer–keratin system is less elastic than that without protein.

#### FTIR analysis of the carboxylated acrylonitrile-butadiene rubber vulcanizates

The IR spectral analysis of the vulcanizates of carboxylated acrylonitrile-butadiene rubber should show the changes in bands resulting from the incorporation of keratin into the structure of the elastomers under investigation and probable crosslinking mechanisms. In Table VI are listed some characteristic bands appearing in the spectra of vulcanizates of XNBR (Krynac 7.50) and XNBR containing five parts by wt. of keratin–ZnO and their IR spectra are shown in Figure 10.

Several overlapping bands are observed within the range  $3033\text{--}2500\text{ cm}^{-1}$ , originating probably from mutual relations between  $\text{NH}_2$  and  $\text{COOH}$  groups of protein incorporated into the elastomer matrix. Broad bands with high intensities appear at  $3615\text{--}3108\text{ cm}^{-1}$  for both elastomers. They originate from free  $\text{—OH}$  groups (moisture and condensed water vapor). There are also observed bands originating from  $\text{—OH}$  ( $\text{COOH}$ ) groups. They seem to be due to the dimers of carboxylic radical formed in the crosslinking process. Additionally, in the spectrum of the protein-containing vulcanizate is observed a band at

**TABLE V**  
The Effect of Keratin and Keratin/ZnO on the Glass Temperature of Vulcanizates

Content	$T_g$ the main of glass transition temperature [°C]	$\tan \delta$ the coefficient of mechanical loss [–]
Standard	3.02	1.01
Keratin	6.64	1.00
Keratin/ZnO	5.99	1.06

TABLE VI  
Characteristic Bands Appearing in the Spectra of Vulcanizates of XNBR

The characteristic functional group	The kind of vibration	Wavelength [ $\text{cm}^{-1}$ ]	
		Standard	Keratin/ZnO
—OH (free groups)	v	3426	3426
$\text{NH}_3^+$	v	—	3079
—OH (COOH), dimers of carboksyatede acides kw. karboksylowego, —CH <sub>2</sub> —	v	3042–2847	
The salts of amines Irz.semi ions, dimers of carboksyatede acides, Sole amin Irz. jonów obojnaczych, dinyry kw. karboksylowego, —CH <sub>2</sub> —	v		3036, 2847
$\text{C}\equiv\text{N}$	$\text{V}_{\text{coupling}}$	2237	2237
—CH=CH <sub>2</sub>	v	1813	—
C=O (COOH)	$\text{V}_{\text{uncoupling}}$	1734	1734
C=C	$\text{V}_{\text{uncoupling}}$	1670 (hight intensity)	1670 (small intensity)
$\text{COO}^-$ , C=O [ $(\text{COO})_2^- \text{Zn}^{2+}$ ] — asymmetric	v	1618–1553	
$\text{COO}^-$ , C=O [ $(\text{COO})_2^- \text{Zn}^{2+}$ ] — asymmetric, /amide Irz.	v/ $\delta$		1597
Amide Irz.	$\delta$	—	1537
—O—C(—O)—CH <sub>2</sub> —	$\delta$	1468	—
—O—C(—O)—CH <sub>2</sub> —	$\delta$	—	1458 (shift, movement)
C=O [ $(\text{COO})_2^- \text{Zn}^{2+}$ ] — symmetric, —O—C(=O)—CH <sub>3</sub>	v	—	1364
—S—CH <sub>3</sub>	$\delta$	—	1317
—CH <sub>3</sub>	v	1192 (small intensity)	1192
MBTS, accelerator	v	1090 (small intensity)	1090
C=C <sub>trans</sub>	$\delta_{\text{trans}}$	—	966 (hight intensity)
C=C	v	920	920
MBTS, accelerator	v	833	833
C=C <sub>cis</sub>	$\delta_{\text{cis}}$	760	760
C—S (sulphides)	v	611	611
—S—S— (disulphides)	v	542–434	542–434

a wavelength of  $2847 \text{ cm}^{-1}$  most likely originating from the salts of primary amines. The probable mechanism of the formation of carboxylic radical dimmers and the formation of additional peptide bonds are shown in Figure 11.

Usually, at a wavelength of  $2237 \text{ cm}^{-1}$  one can observe a band originating from nitrile groups of carboxylated acrylonitrile-butadiene rubber. These groups are likely to react to a very small extent, since once protein is added to the XNBR vulcanizate,

this peak is not shifted at all and its intensity is not changed. At a wavelength of  $1813 \text{ cm}^{-1}$ , is observed a peak of XNBR vulcanizate originating from the terminal ethylene groups. A band of valence vibration of C=O (COOH) and C=C with similar intensities for both spectra is observed at wavelengths of 1734, 1670, and  $920 \text{ cm}^{-1}$ .

The spectrum of XNBR vulcanizate show asymmetric vibration of carbonyl groups ( $\text{v}_C = 0$ ) within the range from 1618 to  $1553 \text{ cm}^{-1}$  that is also visible

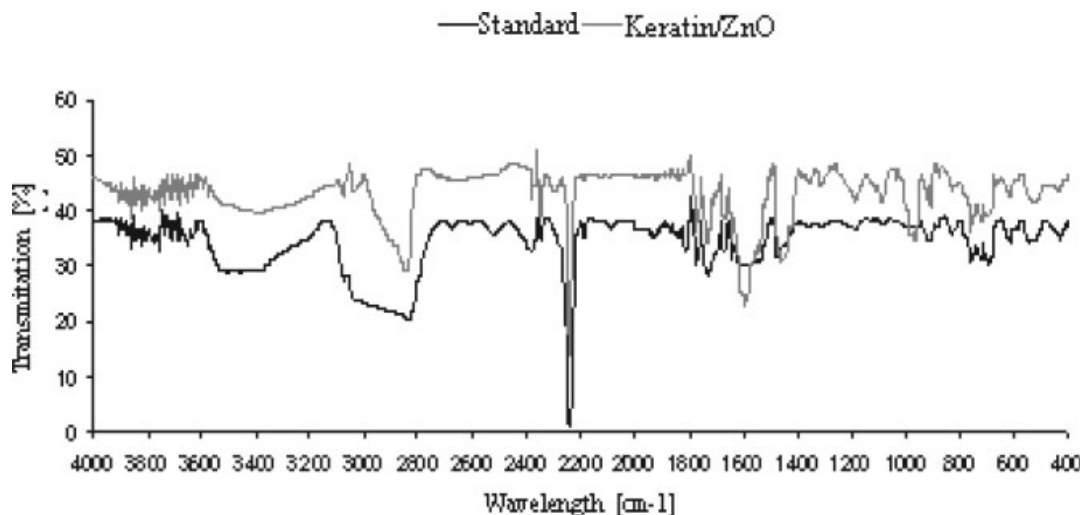


Figure 10 IR spectra of the rubber XNBR vulcanizates without and with keratin.

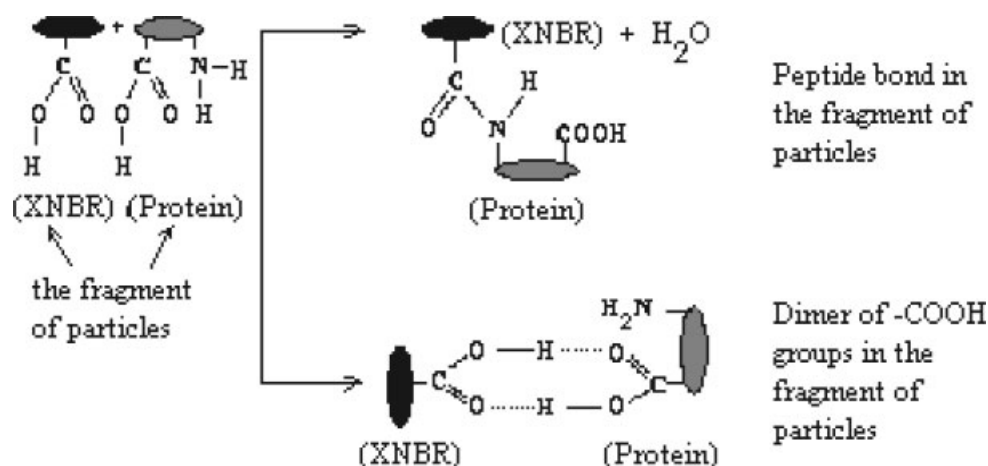


Figure 11 Probable mechanism of bonding—XNBR with protein.

in the spectrum of keratin-containing XNBR vulcanizate at  $1597\text{ cm}^{-1}$ , with symmetric vibration appearing at  $1364\text{ cm}^{-1}$  (in this case, there is an overlapping band originating from primary amides of protein at  $1537\text{ cm}^{-1}$ ). The created structures seem to originate from the formed clusters with zinc cation,  $\text{Zn}^{2+}$  and carboxylic groups of the rubber and protein. In this case, zinc oxide beside mineral sulfur can fulfill the function of ionic crosslinker, as it is commonly known that it can be also combined with other component of the rubber mix such as stearic acid to form its salts, which facilitate and accelerate the crosslinking process. The probable formation mechanism of these structures is shown in Figure 12.

The vibrations of methylene groups present at the carboxylic group are observed in both spectra, with the only difference between them being a small shift, which may results from interactions between fragments:  $-\text{O}-\text{C}(=\text{O})-\text{CH}_2-$  of rubber itself ( $1468\text{ cm}^{-1}$ ) and between these fragments of protein particles and rubber ( $1458\text{ cm}^{-1}$ ).

In the case of keratin-containing vulcanizate is also observed a band originating from methylene groups combined with sulfur, which seems to be due to the protein itself as in the other spectrum such a band is not observed. Methylene groups are observed at a

wavelength of  $1192\text{ cm}^{-1}$ . The signal from sulfur in both spectra with the same intensities is visible for sulfides at  $611\text{ cm}^{-1}$  and for disulphides at  $542\text{--}434\text{ cm}^{-1}$ .

At wavelengths of  $1092$  and  $833\text{ cm}^{-1}$  are observed new bands with slightly higher intensity in the case of keratin-containing vulcanizate. This may result from the structure of MBTS accelerator and its influence on the structure of elastomeric network. Moreover, in the spectrum of XNBR with protein, the intensity of bands  $\text{C}=\text{C}_{\text{trans}}$  ( $966\text{ cm}^{-1}$ ) is increased, while the  $\text{C}=\text{C}_{\text{cis}}$  band shows the same intensity ( $760\text{ cm}^{-1}$ ) in both spectra.

The IR spectra confirm that zinc oxide, beside mineral sulfur, fulfils the function of ionic crosslinker, which is indicated mainly by the bands within the range  $16,618\text{--}1553\text{ cm}^{-1}$  (asymmetric vibration) ( $\nu_{\text{C}=\text{O}}$ ) and symmetric vibration at  $1364\text{ cm}^{-1}$  originating from the complexes formed with  $\text{Zn}^{2+}$  and carboxylic and amine groups.

### Analysis AFM

The surface of the filler under investigation was observed by means of AFM (Atomic Forces Microscopy) and the results are illustrated in Figures 13 and 14.

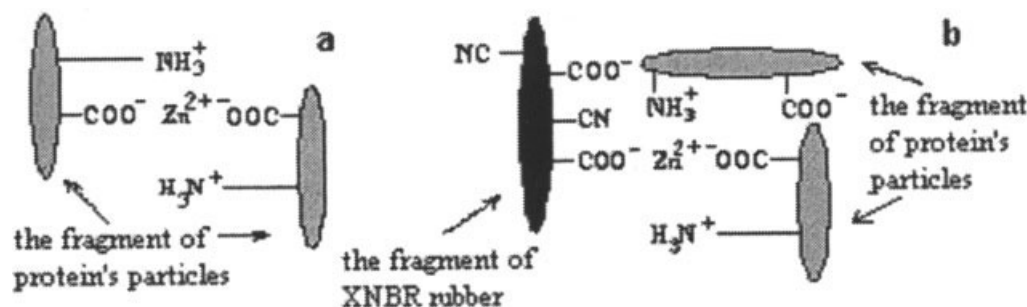
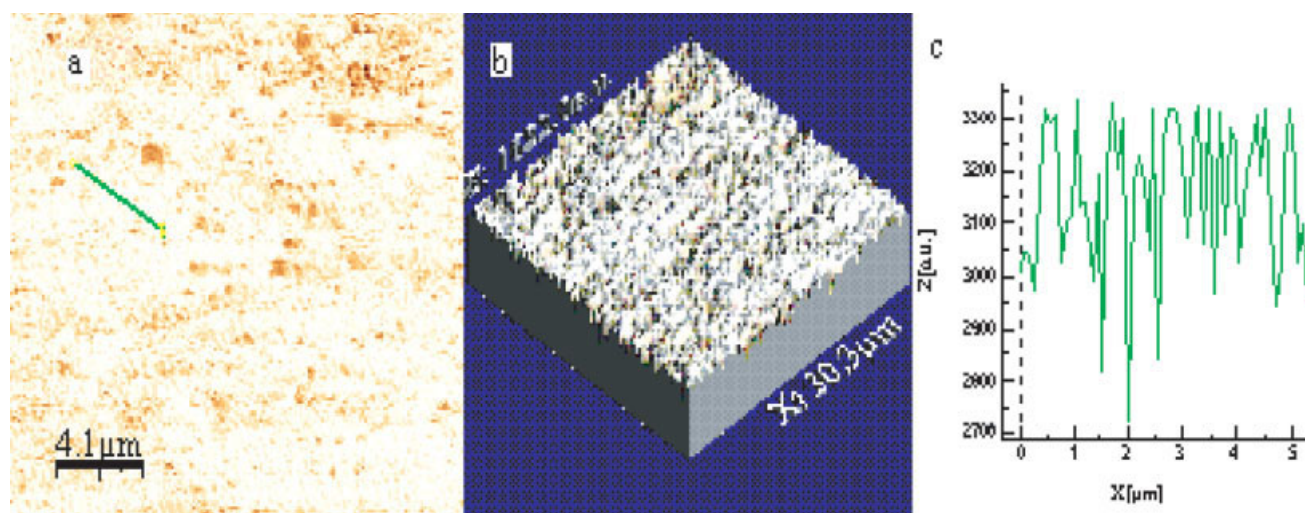


Figure 12 Schematic diagram of: (a) co-ordination of zinc ion with amine and carboxylic groups of proteins and (b) co-ordination of zinc ion with carboxylic group of rubber and amine and carboxylic groups two particles of proteins.





**Figure 13** Topograph of standard vulcanizate XNBR (a, topograph; b, 3D; c, depth of pores on areas 5  $\mu\text{m}$ ). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

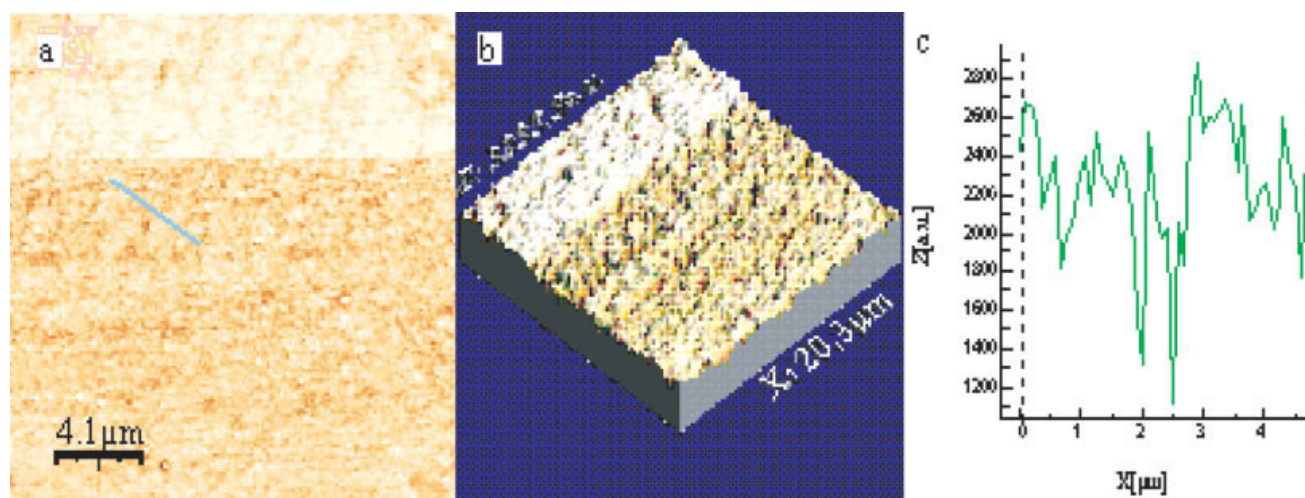
The distribution of dispersion obtained for the vulcanizate containing five parts by wt. of keratin blended with zinc oxide is similar to that of vulcanizate containing no protein. The agglomerates of the filler under investigation show smaller dimensions and more regular shape. One may assume that the vulcanizate containing five parts by wt. of keratin blended with ZnO is characterized by pores with a smaller depth on its surface as compare to the standard. Therefore, less substances from outside will probably be adsorbed on this surface.

### CONCLUSIONS

Based on the performed tests and measurements, it has been found that keratin can be recovered from tannery wastes and identified by means of character-

istic IR bands (peptide bonds—primary, secondary, and tertiary amides, functional groups with sulfur). The small size of particles of the examined protein (469 nm, after blending with ZnO: 331 nm) is a beneficial feature, which certainly improves its dispersion in the elastomeric system.

Keratin as a filler improves the mechanical properties of composites containing this protein and can be used as an exclusive filler for XNBR vulcanizates with the compositions given in this article. From the crosslinking density, based on the concentration of lattice nodes disintegrating under the influence of ammonia vapors and from their percentage content it follows that more ionic nodes formed in this medium were decomposed in the vulcanizates containing protein that in those without protein. This indicates a predominating trend towards the forma-



**Figure 14** Topograph of keratin/ZnO vulcanizate XNBR (a, topograph; b, 3D; c, depth of pores on areas 5  $\mu\text{m}$ ). [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]



tion of ionic nodes in the protein–elastomer structure, and this, in turn, can exert a considerable influence on the properties of protein–elastomer composites. In addition to ionic bonds, there exist also covalent bonds. In the keratin-containing vulcanizates predominate more stable covalent bonds such as mono- and disulphide bridges as distinct from the standard vulcanizates, which possess more polysulphide bonds with a lower stability.

The FTIR analysis of the vulcanizates under investigation has shown that zinc oxide can fulfill the function of an additional crosslinking agent to form ionic lattice nodes. The vulcanizates containing protein show also a good resistance to fuels and oils and therefore they can be used for the production of materials to be applied in contact with these solvents. Keratin-containing vulcanizates considerably better absorb water and show a higher electric conduction due to the hydrophilic properties of the protein.

The vulcanizate containing five parts by wt. of keratin blended with zinc oxide shows the best mechanical properties and a better hardness and slightly worse elasticity as compared to those of the standard mix. Its resistance to thermal ageing is also increases to some extent.

Based on the forced vibration analysis, with the use of the DMTA method, of the protein-containing vulcanizates, one can observe insignificant decreased energy losses in the form of heat resulting from the smaller segmental mobility of the macro-particles of filler and elastomer. Thus, the filler particles seem to be capable of stiffening the elastomer chain to form a more stable structure with the rubber. First of all it confirms the elasticity and hardness investigation.

The AFM photographs of the protein-containing vulcanizate topography show a good dispersion of keratin in the elastomeric medium. It is assumed that the vulcanizates may show a lower adsorption of substances from outside.

The elastomeric composite containing hydrophilic protein (5/5 parts by wt. of keratin/ZnO) shows an increased water absorption, hence it will be easier

biodegraded on waste disposal sites after its service life. Moreover, it increased crosslinking density and resistance to oils and fuels improve the quality of final products. The keratin–elastomer composite obtained from carboxylated acrylonitrile–butadiene rubber seems to be of significant importance in practical applications such as the production of seals and footwear soles.

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