

Preparation of Keratin-Modified Chitosan Fibres

Abstract

In this study, a method of preparing fibres of chitosan modified with keratin derived from chicken feathers is presented. Fibre-grade chitosan and an aqueous solution of purified keratin were used in the investigations. The aqueous solution of keratin was added to a 3% solution of chitosan in acetic acid. Properties of the spinning solutions obtained were tested, followed by spinning trials. The fibres of keratin-modified chitosan obtained showed the following properties: tenacity – 13.5 cN/tex, elongation – 33%, wetting angle – 33° and, in comparison to pure chitosan fibres, were less prone to biodegradation under the action of lysozyme enzyme.

Key words: chitosan, keratin, chitosan fibres, fibre properties.

■ Introduction

Thanks to its specific properties, chitosan is increasingly used in various branches of industry, one of the frequent uses being in medical dressings capable of accelerating the wound healing process [1]. There are two ways in which chitosan can be used in dressing: by surface coating onto fibres or ready-made dressing, or by preparing chitosan fibres which are then used in the manufacture of dressings. Chitosan/chitin fibres are usually prepared from solutions of chitosan, chitin or its derivatives. Wet spinning is commonly applied, yet a quasi-dry method is also known to be used, consisting in the extrusion of a highly viscose 3% solution of chitosan in acetic acid into a gaseous ammonia in which the spinning solution solidifies into fibres [2].

One of the wet forming processes yields strong chitosan fibres with a tenacity of up to 35 cN/tex by the extrusion of liquid crystal (LC) chitosan solutions into a coagulation bath [3]. Chitosan fibres made according to the standard wet spinning processes show a rather poor tenacity which can be improved by use of cross-linking agents like glutaric aldehyde or glyoxal [4, 5].

A method of preparing chitosan staple fibres modified with vanillin is also known [6].

In the 90's, a technology was prepared for the manufacture of chitosan fibres from chitosan solutions with a concentration of up to 5% in aqueous acetic acid. From such solutions, fibres were spun in an aqueous coagulation bath containing sodium hydroxide with the possible addition of ethanol [7 - 9].

Wet spinning technology was later improved to provide the manufacture of micro- and nano fibres (microfibrils), multifilament yarn and composite fibres like cellulose-chitosan, chitosan-alginate and fibroin-modified chitosan fibres [10 - 14].

In order to obtain new advantages properties of chitosan fibres our investigations have been directed to search for new modifiers. It seems that keratin taking into account its special properties would be suitable for such an application.

Chicken feathers, composed of ca to 90% keratin, are the cheapest source of the compound. Thanks to specific properties of keratin, it may be employed in the preparation of modified fibres.

Purified keratin derived from chicken feathers was earlier used in the modification of sanitary alginate and cellulose fibres [15 - 17], which was also harnessed in the preparation of microfibrils and sponges [15 - 20]. In 1996 the possibility of using keratin in the production of unique fibrous forms and fibres capable of competing with cellulose fibres in applications such as napkins, body care products and towels was first reported [21 - 23]. A method of preparing keratin-containing fibrous forms from chicken feathers was proposed by J. McCurry et al [24 - 26].

Therefore we have made plans for an attempt to modified chitosan fibres with keratin.

The hydrophilic character and ability to rapidly biodegrade is certainly an advantage of chitosan fibres. However, in some applications, like in hygiene and medical products, a decrease in the biodegradation rate would be desirable to preserve the fibrous structure over a longer time. Adequately purified keratin emerges as the right candidate for this role; it has already found application in a number of cosmetic, hygiene and sanitary products. It was hoped that a proper combination of keratin with chitosan may result in the preparation of fibres with assumed properties.

The objective of the research was to prepare chitosan fibres more hydrophilic but less biodegradable than standard chitosan fibres. For this purpose keratin was admixed to the solution of chitosan. Its impact upon the fibre-forming process and resulting fibres properties was investigated.

■ Materials

Two kinds of chitosan grades (marked A and B) supplied by Primex Ingredients ASA, Norway were used in the trials (**Table 1** see page 40).

Keratin, derived from chicken feathers in the form of an aqueous suspension after dialysis, was used in the investigation. Dialysis was conducted for 48 hours at ambient temperature using a cellulose dialysis sleeve. The concentration of keratin after the dialysis was 1%, which was increased to 3% by evaporation using a UNIPAN 350 P evaporator at 30 °C for 1 hour (**Table 2** see page 40).

Table 1. Selected properties of the chitosan.

Characteristic	Chitosan A	Chitosan B
Deacetylation degree (DD), %	83.2	82.4
Average molecular weight (Mv), kD	338.0	426.0
Water Retention Value (WRV), %	106.0	104.0

Table 2. Properties of the keratin.

Parameter	Property
Nitrogen content, %	15,2
Sulphur content, %	2,07
Humidity, %	5,9
WRV, %	138
Sorption coefficient, %	160
Mw, kDa	144,4
Mw/Mn	6,5
Colour/form	White powder

The acetic acid, sodium hydroxide and ethanol used in this work were supplied by POCH S.A., Gliwice, Poland.

Methodology

Preparation of the spinning solution of chitosan with keratin suspension

Chitosan solutions with concentrations in the range of 4.5 – 5.3% were prepared in a 3% aqueous solution of acetic acid. 10% of glycerol in relation to chitosan was added to the solution, which, after filtration and deaeration, was blended with the keratin suspension at 25 °C. The spinning solution was again aerated during the blending, hence a repeated deaeration was needed before the spinning. Keratin in the form of microparticles does not undergo dissolution and remains a solid, dispersed phase in the spinning solution.

Spinning keratin-modified chitosan fibres

Keratin-modified fibres were spun on a laboratory wet spinning line equipped with a spinning head holding a platinum–rhodium spinneret with 300 openings 80 µm in diameter.

The coagulation bath was composed of 3 wt. parts of ethanol and 10 wt. parts of 3% aqueous sodium hydroxide, which was kept at 30 °C. The spinning speed was 13.0 and 17.5 m/min. The fibres spun were drawn by 21 – 34% in water at 80 °C, next washed in water at 40 °C and then in a water – ethanol bath with 60%

concentration and finally dried without tension in a loose bundle.

Analytical methods

The *dynamic viscosity* of the chitosan and chitosan/keratin solutions was estimated by means of a Brookfield LVT rotation viscometer.

Photos of the chitosan solutions, keratin suspension and component blends were taken with a PZO Biolar polarising microscope, equipped with a digital camera and an image analysing system.

The electron microscope SEM/ESEM, Quanta 200 (W), FEI Co., USA was used to prepare *pictures of the cross-section and surface* of the keratin-modified chitosan fibres.

The *polymer content* in the aqueous solutions was found by gravimetric analysis. In this method a film is formed from a weighed amount of the solution by evaporation of the water and then drying at 60 °C. The film is next coagulated in 2% aqueous sodium hydroxide, then washed and dried to constant weight at 105 °C.

The *nitrogen content* in the chitosan and keratin was estimated by the Kjeldahl method according to Standard PN-EN ISO 5378, while the *content of sulphur* was found by micro- coulometric analysis.

The *hygroscopicity* of the fibres was measured according to Polish Standard PN-80 P-04635 as the capacity to absorb water vapour from the air with a relative humidity of 100%.

The *water retention value* was estimated according to ISO FDIS 23714.

Mechanical properties of the fibre were tested according to PN-ISO 1973:1997 and PN-EN ISO 5079:1999. The tests were carried out in an air conditioned room at 65 ± 4% RH and 20 ± 2 °C.

The *biodegradability* of the fibres was carried out under static conditions at 37 °C. The samples were sterilised at 121 °C for 15 minutes. The biodegradation lasted 21 days with the use of Lysozyme Merck at a concentration of 80 µg/cm³ in the medium of a phosphate-citric buffer at a pH of 7.23. After the biodegradation, the sample tested was, first washed with distilled water at 50 °C and then with 70% ethanol, and finally dried

to a constant weight. The mass loss was estimated.

The *wetting angle* was estimated with respect to glycerol, whose polar properties and surface tension are close to those of water. The wetting angle was measured after 60 seconds from the deposition of the glycerol droplet.

Results and discussion

Preparation of chitosan solutions

Aqueous solutions of chitosan in 3% acetic acid were prepared for investigations into the spinning of keratin-modified fibres. Properties of the solutions are shown in **Table 3**. Due to technical problems and the high value of the apparent dynamic viscosity, the solutions could only be prepared with a chitosan concentration in the range of 4.58 – 5.28%.

The dissolving of chitosan proceeded smoothly for the solution with the highest chitosan concentration (5.28%) and dynamic viscosity of 65 Pa·s.

Few mechanical impurities and insoluble polymer particles can be seen in the microscopic images of the non-filtered chitosan solution. By comparing the images of the Chit-26 and Chit-29 chitosan solutions, it was possible to conclude that a prolonged dissolving time and decreased chitosan concentration positively influence the reduction in the amount of insoluble particles in the solution. On the other hand, neither the dissolving time nor the kind of chitosan displayed any influence on the pressure during filtration of the spinning solution (**Figure 1**).

Chitosan solution with keratin suspension

In **Figure 2** microscopic images of the keratin aqueous suspension after dialysis are presented.

The particle size of keratin is in the range of 5 to 10 µm in a 1% suspension; the particles are susceptible to agglomeration during the concentration of the suspension. Mixing by means of an ultrasonic 35 W homogeniser for 10 minutes was applied to prevent agglomeration. The composition and properties of the solutions can be found in **Table 4**.

Chitosan solutions with a keratin content of 0.08 to 0.93% and dynamic viscosity in the range of 3.3 to 17.5 Pa·s were pre-

pared for the spinning process. Admixing the keratin suspension with the chitosan solution resulted in a slight decrease in the total content of the fibre-forming polymer in the solution and a considerable reduction in the apparent dynamic viscosity in comparison to the initial chitosan solution. The spinning solutions made of chitosan B reveal a higher apparent viscosity, which can be explained by the higher average polymer weight of the polymer. The small decrease in the total polymer content results from the addition of the aqueous part of the keratin suspension. A quite different problem was the unforeseeable great decrease in the dynamic viscosity, which was in no relation to the addition of the aqueous suspension. May be the impact of keratin particles on the solutions measurements could be a partly but not satisfying explanation. This interesting phenomenon could be the subject of future investigation.

The spinning of keratin-modified chitosan fibres

Spinning conditions dictated by the methodology were adopted to examine the impact of the keratin presence on the spinning process. Initially a spinning speed of 17.5 m/min and draw ratio of 34% were applied.

The Keratin contained in the chitosan solution badly disturbed the stability of the spinning process. Despite the use of ethanol (30% v/v) in the alkaline coagulation bath and prolonged washing in 60% aqueous ethanol, the tenacity of the fibres obtained in wet conditions dropped considerably, which could have been caused by the lower solidifying rate of the polymer stream.

The forming of chitosan fibres modified with the keratin marked F/ChK 1 was unstable at the conditions assumed, ac-

Table 3. Conditions for the preparations of chitosan solutions and some of their properties.

Symbol of the solution	Symbol of chitosan	Dissolving time min.	Chitosan concentr. %	Dynamic viscosity/ temp. Pa·s/ °C
Chit-26	A	60	5.18	38 / 25
Chit-29	A	130	4.58	32 / 25
Chit-35	B	90	5.28	65 / 22

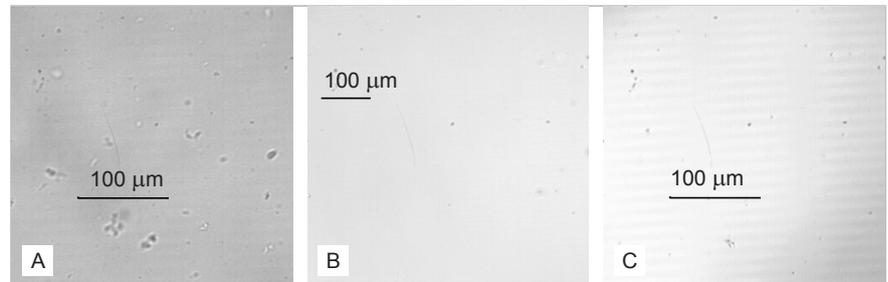


Figure 1. Images of chitosan solutions: A – Chit 26, B – Chit 29, C – Chit 35.

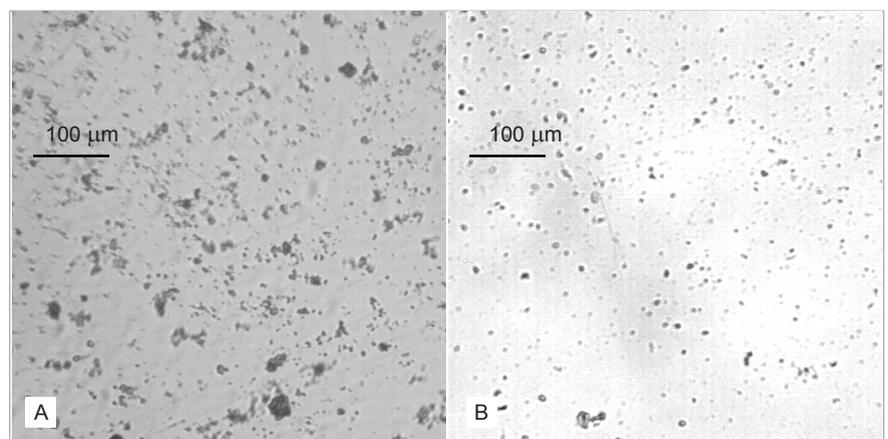


Figure 2. Images of the keratin suspension after dialysis; A - after concentration to 3%. B- with 1 % concentration.

companied by disturbances in the spinneret area and breakages of fibres, which necessitated the application of a lower draw ratio of 25%. This change enabled a non disturbed preparation of fibres under the conditions applied. The spinning of fibres marked F/ChK 2 and F/ChK 3 had a positive run enabling a draw ratio of 34%, unlike F/ChK 4 and F/ChK 5,

which were unstable, necessitating the lowering of the draw ratio to 30%. F/ChK 6 and F/ChK 7 were unstable alike with the blocking of the spinneret and fibre breakages bringing about a lower spinning speed of 13 m/min and draw ratio 21% accordingly, which improved the spinning stability and resulted in the preparation of keratin-modified fibres.

Table 4. Properties of the blended chitosan/keratin spinning solutions; * 0.3 % Triton X-100.

Symbol of the spinning solution	Symbol of the chitosan solution	Keratin concentration in the aqueous suspension %	Properties of the spinning solution		
			Total content of polymer, %	Keratin concentration, %	Dynamic viscosity at 25°C, Pas
ChK 1	Chit 26	1.00	4.14	0.08	2.30
ChK2	Chit 29	1.00	3.79	0.10	7.50
ChK 3		1.00	3.97	0.28	6.00
ChK 4		3.26	4.36	0.64	8.00
ChK 5		3.26	3.58	0.93	5.25
ChK 6*	Chit 35	3.00	4.55	0.59	16.75
ChK 7		3.00	4.58	0.92	11.75
ChK 8*		3.00	4.85	0.57	17.50

Table 5. Selected properties of the keratin-modified chitosan fibres.

Symbol of the fibres	Symbol of the chitosan solution	Hygroscopicity, %	WRV, %	Sulphur content, %
F/Chit 29 (100% of chitosan)	Chit 29	35.2	160	0
F/Chit 35 (100% of chitosan)	Chit 35	36.9	207	0
F/ChK 2	Chit 29	35.6	159	Not tested
F/ChK 3		37.1	149	Not tested
F/ChK 4		41.2	163	0.030
F/ChK 5		40.0	169	0.027
F/ChK 6	Chit 35	42.0	210	0.032
F/ChK 7		42.9	221	0.036
F/ChK 8		41.5	225	0.028

The disturbance in the spinning can be explained by the presence of keratin particles as a discontinuous phase, causing an agglomeration clogging tendency in the spinneret opening. The supplementary mixing of the keratin suspension using the ultrasonic homogeniser and the addition of the agglomeration-preventing substance - Trinitron improved the quality of the solution; however, it did not rectify the solidification of fibres. It was only the lower spinning speed and draw ratio that contributed to the improvement in spinning stability and coagulation of the keratin-containing chitosan fibres.

Determination of the hygroscopicity, WRV and sulphur content of the keratin-modified chitosan fibres

The sorption properties of selected chitosan and keratin-modified chitosan fi-

bres were determined by testing their hygroscopicity and WRV (see **Table 5**).

Chitosan and keratin-modified chitosan fibres formed from the Chit 29 (chitosan A) solution had an WRV of 149 - 169%, while for fibres made of Chit 35 (chitosan B) the WRV was in the range of 210 - 225%. A strict relation between the keratin content in the fibres and the WRV fibre could not be found.

Chitosan fibres with a higher keratin content (F/ChK 4, F/ChK 5) are characterised by a higher hygroscopicity - at about 41%. Despite the insignificant differences in keratin content in the fibres, the hygroscopicity was at the level of 42%, irrespective of the kind of chitosan used.

The nitrogen content in the chitosan and keratin-modified chitosan fibres was in

the range of 7.5 - 7.7%. Slight differences in nitrogen content result from the quality of the chitosan and low accuracy of the analytical method. Assessment of the amount of keratin contained in the fibres is more accurate when based on the measurement of sulphur content.

The highest sulphur content, 0.036%, was found in the fibres marked F/ChK 7. Considering the sulphur content of 2.07% in the starting keratin (**Table 1**) and assuming that no chemical transformation proceeds during the processing that causes a decrease in the sulphur content of the keratin, the theoretical calculation of the sulphur content in the keratin-modified chitosan fibres marked F/ChK 7 leads to a figure of 1.74%.

Mechanical properties of the keratin-modified chitosan fibres

Mechanical properties of the chitosan and keratin-modified fibres are presented in **Table 6**.

The tenacity of the keratin-modified fibres (F/ChK 1) is two-fold lower than that of the chitosan fibres (F/Chit 26), while the linear density and variation coefficients of the elongation are higher for the modified fibres (see **Table 6**). Such properties are evidence of the disturbances which occur during the spinning. Contemplating the results presented in **Table 6**, it was concluded that the keratin-modified fibres F/ChK 2 and F/ChK 3

Table 6. Mechanical properties of the keratin-modified chitosan fibres; *100% of chitosan.

Parameter	Symbol of fibre								
	F/Chit 26*	F/ChK 1	F/Chit 29*	F/ChK 2	F/ChK 3	F/ChK 4	F/ChK 5	F/Chit 35*	F/ChK 6
Linear mass, dtex	3.91 ± 0.05	4.43 ± 0.82	3.96 ± 0.07	3.69 ± 0.08	5.81 ± 0.10	6.56 ± 0.21	5.43 ± 0.25	3.72 ± 0.04	4.48 ± 0.10
Variation coefficient of linear mass, %	1.01	5.08	1.14	1.71	1.38	2.62	3.74	0.815	0.874
Confidence region of linear mass, %	± 0.013	± 6.30	± 1.42	± 2.13	± 1.72	± 3.25	± 4.64	± 1.01	± 1.08
Breaking force conditioned, cN	2.46 ± 0.20	1.44 ± 0.16	3.98 ± 0.22	2.45 ± 0.15	4.37 ± 0.24	2.14 ± 0.19	1.86 ± 0.10	5.09 ± 0.23	8.77 ± 0.56
Variation coefficient of breaking force conditioned, %	19.9	26.9	13.2	15.1	13.4	21.5	12.8	10.8	15.5
Confidence region of breaking force, %	± 8.20	± 11.1	± 5.44	± 6.22	± 5.52	± 8.86	± 5.27	± 4.45	± 6.39
Tenacity conditioned, cN/tex	6.29	3.25	10.0	6.75	7.52	3.26	3.43	13.7	13.5
Elongation at break conditioned, %	33 ± 3	30 ± 7	41 ± 4	20 ± 4	21 ± 2	22 ± 5	28 ± 5	27 ± 2	33 ± 4
Variation coefficient of elongation conditioned, %	24.0	54.7	21.0	14.0	17.6	36.0	32.9	26.8	27.9
Confidence region of elongation, %	± 9.89	± 22.5	± 8.65	± 18.1	± 11.4	± 24.7	± 17.7	± 11.0	± 11.5

Table 7. Wetting angle of the keratin-modified chitosan fibres; *100% of chitosan.

Parameter	Symbol of fibre						
	F/Chit 29*	F/ChK 2	F/ChK 3	F/ChK 4	F/ChK 5	F/Chit 35*	F/ChK 6
Wetting angle, °	28.6	33.0	-	29.5	30.1	28.9	32.9
Variation coefficient of wetting angle, %	11.8	11.8	-	11.0	7.54	10.6	7.47

Table 8. Percentage of mass loss in chitosan and keratin-modified chitosan fibres during the 21 days of biodegradation: * (100% of chitosan).

Symbol of fibre	Biodegradation time -days							
	3		7		14		21	
	buffer	enzyme	buffer	enzyme	buffer	enzyme	buffer	enzyme
F/Chit 35*	0.32	0.86	Not estimated	Not estimated	Not estimated	Not estimated	2.52	8.55
F/ChK 6	0.11	1.88	0.44	4.46	0.39	5.74	1.05	6.90

spun at a 34% draw ratio are characterised by higher tenacity and an elongation of 20%. In the case of fibre F/ChK 3, the linear density increased to 5.81 dtex with the variation coefficient of the parameter at a very low level. Such values may be evidence of blockages in the spinneret openings and/or the tendency of fibres to shrink while drying in a loose bundle.

The keratin-modified fibres F/ChK 4 and F/ChK 5 revealed a tenacity in the range of 3.26 - 3.43 cN/tex (Table 6). Spinning these fibres was greatly disturbed by the higher amount of keratin (0.93%) introduced to the chitosan solution, and probably by its insufficient dispersion. The excessive amount of keratin is also the reason for the lower tenacity of the fibres. In further steps the content of keratin in the fibre was reduced, and chitosan with a higher molecular weight was applied.

As regards the spinning trial of the chitosan fibres marked F/Chit 35, a tenacity of 13.7 cN/tex was attained.

Spinning the keratin-modified fibres F/ChK 6 led to a tenacity and elongation close to those of the chitosan fibres (F/Chit 35), but with a distinctly higher linear density of 4.48 dtex. The latter may be evidence of the blocking of spinneret openings during the forming of fibres. The F/ChK 6 fibre revealed a comparatively high elongation of 33%. The kind of chitosan used in the trial probably had a considerable influence on stability during the spinning of the keratin-modified chitosan fibres and their mechanical properties.

The results of the wetting angles measurements are presented in Table 7.

Although the wetting angles of the keratin-modified fibres was insignificant higher in all trials, considering the high variation coefficient of wetting angle results we can state, that hydrophilic feature of the fibres did not change. This can be confirmed by the comparison of the WRV and hydrophilicity values obtain

for of both chitosan and keratin-modified chitosan fibres.

Biodegradation of the keratin-modified chitosan fibres

The Chitosan fibres F/Chit 35 and keratin-modified chitosan fibres F/ChK 6 were tested for biodegradation. The latter is characterised by a 0.032% sulphur content and a 32.9° wetting angle. The biodegradation was carried out in a warmer-shaker at 37 °C for 21 days, during which the mass loss was estimated. In Table 8 results of the biodegradation testing are presented

After the first three days of biodegradation, the keratin-modified chitosan fibres revealed a two-fold mass loss compared with the pure chitosan fibres, while after 21 days the loss was 23% higher for the chitosan fibres. As can be see in Table 8 the differences of the biodegradability of chitosan fibres and keratin-modified chitosan fibres are small insignificant from the view of practical application. This lead to the conclusion that modifying chitosan fibres with keratin with the aim to decrease the biodegradability mass loss is not reasonable.

Morphology of the keratin-modified chitosan fibres

Figures 3 and 4 present SEM images of the side views and cross-sections of selected chitosan and keratin-modified chitosan fibres

A regular oval cross-section of the chitosan fibres can be seen; the side views reveal some regular cavities and grooves.

The keratin-modified fibres are characterised by an oval albeit less regular cross-section compared with 100% chitosan fibres. Tiny light dots can also be seen in the cross-sections, probably reflecting keratin particles or they agglomerates. The side views show a less characteristic surface with shallower cavities and grooves. The surface of the cross-section of the keratin-modified fibres is smoother, which may result from the forming of a thin sheath on the fibre surface from keratin which dissolved in the spinning.

Conclusion

- The possibility of manufacturing keratin modified chitosan fibres was confirmed (the keratin content was con-

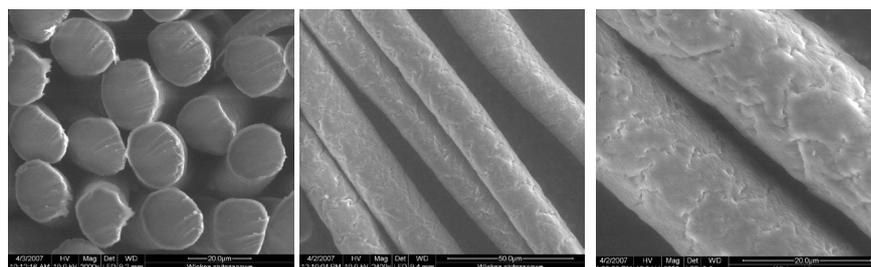


Figure 3. SEM images of chitosan fibres marked F/Chit 35.

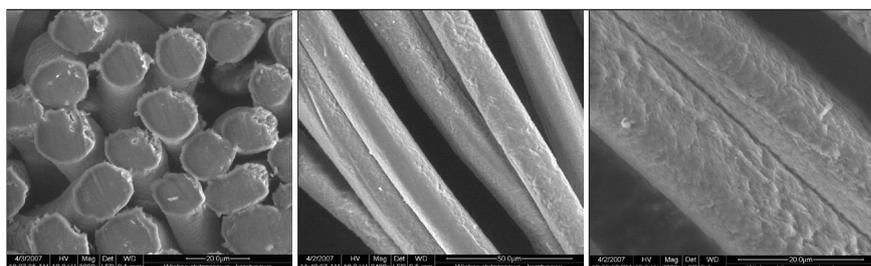


Figure 4. SEM images of keratin-modified chitosan fibres marked F/ChK 6.

firmed by sulphur content of 0.032%). The fibres obtained were characterized by a tenacity of up to 13.5 cN/tex and elongation at break of up to 33%.

- The keratin content added as a suspension to the chitosan spinning solution, could not be dissolved in it and the fibres were spun with keratin particles within the range of micrometers.
- Manufacturing keratin modified chitosan fibres with a sulphur content of more than 0.32% is accompanied with great difficulties, especially disturbances of the spinning process.
- Keratin modified chitosan fibres can be manufactured with mechanical hydrophylic and biodegradability properties compatible with those of pure chitosan fibres.
- Manufacturing keratin-modified chitosan fibres with the aim to decrease the susceptibility to biodegradation is not justified from a technological point of view, as the property differences of the fibres obtained are insignificant.



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