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Fibrous Keratin-Containing Composite

Abstract
Increasing demand for dressing and hygiene care products boosts innovation and investigations into novelty materials. One example of a promising modern bio-composite is fibrids in the form of chitosan – keratin and alginate – keratin sponges. Chitosan, alginates and keratin, which are themselves distinguished by valuable properties, may, when combined into a composite material, reveal interesting useful features. As a result of the conducted research, novelty materials were prepared that have not yet been announced in adequate literature: chitosan – keratin and alginate – keratin bio-composites in sponge form. The sponges were made by employing freeze drying, which enables the preparation of a highly porous internal structure. The keratin additive contributed to the enhancement of sorption properties of the preparations obtained. The new materials do not exert any cytotoxicity or irritating and allergisation action, which is a precondition in hygiene and medical uses. The advantage of utilising a burdensome, cheap by-product – chicken feathers – for the preparation of materials with high added value is unquestioned.

Key words: *keratin, chitosan, alginate, sponge.*

Introduction

Natural polymers and their derivatives are a valuable resource for uses in fibres, film sponges and increasingly for the preparation of fibrids [1, 2].

Fibrids and fibres made of natural polymers such as alginates and chitosan have, thanks to their specific features, found a wide application in cosmetics, medicine and environmental protection [3-6]. Both alginates and chitosan are enjoying an increasing interest in the preparation of composites and other fibrous material-with enhanced absorption, designed for medical devices. Attractive, too, is the biopolymer keratin, which is amply available from, amongst others, chicken feathers [7]. Keratin, thanks to its hydrophilic properties, is seen as a candidate for the preparation of highly absorptive materials suitable for medical dressings, and for some technical and textile applications.

Chitosan reveals bio-stimulating properties that ease the reconstruction and vascularisation of tissue and the equalisation of cell components, and, moreover, it accelerates wound healing. Chitosan is a cationic polyeloctrolyte of which high viscosity solutions can be prepared suitable for the forming of fibres, films and coatings. It stimulates the formation of monocytes and inhibits the growth of fungi and bacteria, thus limiting the risk of wound infection. The cationic character of chitosan provides blood clotting capability. The excellent bioactivity predisposes chitosan to the preparation of modern dressings, including haemostatic ones. Chitosan bioactivity depends among others on the hydrolytic degradation induced by enzymes that are present in body fluids (lysozyme and N-acetyloglucosamidase), and on the possibly emergent products of its degradation in the form of oligomers, which accelerate the healing [8, 9]. Also popular are dressings based on alginates, featured by haemostaticity and very good absorption properties [10-12].

Exploiting the advantage of the hydrophilic properties of feather keratin, alginate/keratin fibrids were prepared. The preparation and properties of such fibrids were presented in earlier publications [13].

At the Institute of Biopolymers and Chemical Fibres (IBChF), investigations are being conducted devoted to new alternative methods of preparing microfibrids and fibres from natural polymers [14]. Several useful forms of chitosan were elaborated, like microcrystalline chitosan (MCCh), which modifies gels, fibres, fibrids and sponges [15-18]. Thanks to beneficial biological properties, a possible medical and veterinary use in medicine emerges for these forms of chitosan, mainly as dressings [18-21].

Objective

In research conducted so far in the preparation of keratin-containing bio-composites such as fibres and fibrids, it has been documented that the fibrous forms are a good method of utilisation of keratin obtained from chicken feathers.

Having this in mind, investigations were begun into the preparation of bio-composites in sponge form containing keratin for uses in hygiene materials. By combining the biomedical properties of alginates and chitosan with the high hydrophilicity of keratin, it was hoped to prepare a unique composite material for medical and hygiene uses.

Materials and methods

Materials

Chicken feathers

The starting material was feathers from a chicken slaughterhouse. The material was characterised by:

- Sulfur content 2.9%
- Nitrogen content 15.5%
- Ash content ca. 1%

The keratin obtained from the feathers [22] was characterised by:

- Sulfur content 2.3%,
- Nitrogen content 15.2%,
- Cysteine content 16.9%
- WRV 155.5%
- Absorption coefficient 188.5%
- Mw 144.6 kDa
- Mw/Mn 2.6
- Colour white powder

Chitosan

Chitosan Chito Clear FG90 made of shrimps was used, delivered by "Primex Ingredients ASA" (Avaldsnes, Norway), and was characterised by:

- Deacetylation degree DD 83.2%
- Average molecular weight Mv 338.0 kD
- Water retention value WRV 106.0%
- Viscosity at 20 °C 78.6 cP
- Content of heavy metals 0.02%
- Ash content 0.4%

Sodium alginate

Sodium alginate marked Protanal LF 10/60 LS was used. It is designed for the

preparation of medical alginate fibres. The sodium alginate was characterised by:

- Appearance powder
- Colour white to light yellow
- Content: guluronic acid 40-45%
  mannuronic acid 55-60%
- Moisture 10.0%
- Viscosity (1% at 20 °C) 52 mPas
- Calcium content 1.5%
- pH of 1% solution, 20 °C 6.5
- Insoluble particles 0.01%
- Total aerobic bacteria content 125 cfu/g

Methods

Preparation of keratin fibrids with content of other natural polymers

The preparation of fibrids was investigated with the use of apparatus (proprietary IBChF construction) for the vertical wet forming of fibres, equipped with an agitator situated above the spinneret.

Preparation of alginate/keratin fibrids

For the preparation of alginate/keratin fibres [13], a coagulation bath was employed, containing 3 g/l of $CaCl_2$ with the addition of HCl to provide a pH of the solution equal to 4. The obtained fibrids separated from the coagulation bath were immersed in methanol for about 1 h, then centrifuged, washed several times with water and eventually freeze dried.

Preparation of chitosan/keratin fibrids

Filtered and deaerated 5.56% chitosan (Chit.26) solution in 3% acetic acid solution was mixed with an aqueous suspension of keratin (RK). From such prepared blends (MCCh), fibrids were formed in a coagulation bath containing a 3% aqueous sodium hydroxide solution, at vigorous agitations (15000 rpm). The temperature of the coagulation bath was 35 °C.

The obtained fibrids, separated from the coagulation bath, were immersed in methanol for about 1 h, then centrifuged, washed several times with water and eventually freeze dried.

Preparation of alginate-keratin sponges in the course of freeze drying

Wet fibrids (Fib AK) with a dry mass of 1.28% were blended with glycerol in the proportion of 1:0.6 (on the dry mass of the fibrids). After homogenisation, the blend was left for one day. After that time, the alginate solution LF 10/60 Sm = 0.5% was added and blended in a homogeniser (attachment G 45G 5000 r.p.m.) for 5 min.

After many trials, the content of the polymer AK was assumed to be 90.6%, and that of alginate LF 10/60 9.4.

The freeze drying lasted about 20 h.

Preparation of chitosan-keratin sponges in the course of freeze drying

For the preparation of samples, 150 g of wet fibrids were used, formed into large circles. The sponges were prepared from a mixture containing an aqueous suspension of chitosan-keratin fibrids (content of solid components at about 2.2%) and glycerol. The components were blended in the weight proportion of 1:0.6 (on the dry mass of the solid matter). The blends were carefully homogenised, and after 24 h dried in a lab freeze dryer, type ALFA 1-4, made by Christ Co. Freeze drying was accomplished in the temperature range of -20 to +10 °C and vacuum from 0.1 to 0.7 mbar. The drying time was 20 to 24 hours depending upon the weight of the sample. The drying conducted that way allowed polymeric materials to be obtained in sponge form with an even, faultless surface.

Analytical methods

Water retention value (WRV)

The WRV was estimated according to a method described in [23].

Sponge absorption coefficient

In this analysis, a method described in [24] was applied.

Sponge moisture imbibition

Moisture imbibition was examined with keratin samples that were dried up to a constant weight in an exsiccator at 65% RH (NH_4NO_3) at an ambient temperature (20-21 °C). The imbibition was estimated by measuring the time-dependent weight of the sample. On attaining a constant weight, which indicates moisture saturation at given conditions, the keratin samples were placed in an exsiccator at 93% RH ($KN0_3$), and the time-dependent weight of the sample was measured. On reaching a constant weight, the samples were again put into the exsiccator at 65% RH, thus enabling the desorption to be estimated.

Sulfur and nitrogen content in the sponges

The sulfur content was estimated according to the Sheniger standard method.

The nitrogen content was estimated according to the Kjejdahl method [25, 26].

Microscopic inspection of the spinning solutions

Spinning solutions were inspected by using the polarising microscope Biolar (ZPO, Optical Works Warsaw) with a photographic attachment. The images were recorded by means of the computer analyser IMAL.

Assessment of the appearance of sponges by means of scanning electron microscopy (SEM)

The Quanta 200 SEM microscope made by FEI was used for this purpose at ×2000 magnification. The assessed sample was placed on the table and fixed with carbon adhesive. The structure was assessed in a low vacuum without gold powdering.

Sterilisation of the chitosan/keratin sponges

The samples were irradiated with a dose of 25 kGy.

Biomedical tests

The biomedical testing was concerned with the cytotoxic, irritating and allergenic behaviour of the keratin/chitosan fibrids. The cytotoxicity was tested *in vitro* according to standard PN-EN ISO 10993-5 using L929 cells. The irritating action was tested on albino New Zealand rabbits according to standard ISO 0993-10 after on-skin application. About 7 g of chitosan/keratin fibrids in sponge form were delivered for the testing.

For the allergenic tests, 110 sterilized chitosan/keratin sponge samples sized 2.5 × 2.5 cm (altogether 690 cm^3) were provided. The testing was carried out on 20 healthy, in puberty guinea pigs of both sexes, according to ISO 10993-10:2002 (the Buehler method). The testing was done in the National Laboratory of Medical and Biocidic Products of the National Drug Institute. In this phase of the research, it was decided to refrain from biological testing of the alginate/keratin preparations. Alginate is a polymer that is commonly used in medicine; therefore, the testing of the chitosan/keratin products was thought to be more justified.

Table 1. *Alginate/keratin solutions for the preparation of fibrids.*

Sample mark	Polymer content, %	Alginate content, %	Keratin content, %
Alg 2 [1)]	5.56	5.56	–
RAK 1	1.76	0.93	0.83
RAK 2	1.60	0.93	0.67
RAK 3	1.76	0.93	0.83
RAK 4	1.60	0.93	0.67
RAK 5	1.34	1.01	0.33

[1)] *– spinning solution of sodium alginate*

Table 2. *Properties of alginate/keratin sponges.*

Sample mark	Nitrogen content, %	Absorption coefficient, %	WRV, %
Fib Alg 2	–	175.0	170
Fib AK 1	1.9	194.4	179
Fib AK 2	2.0	198.2	180
Fib AK 4	2.4	200.5	188
Fib AK 5	2.1	195.2	184

Results and discussion

Properties assessment of the keratin--containing fibrids in sponge form

Composites in sponge form were prepared, employing the freeze-drying technique by which spongy preparations can be obtained with a highly porous surface and internal structure. Composites of keratin with chitosan, sodium alginate and a plasticizer were used. The composition of the polymers was selected to match the content of dry mass on the level of 2.4-3.0%.

Properties assessment of the keratin--alginate composites in sponge form

The properties of the spinning solutions used for the forming of fibrids are shown in ***Table 1***, while the characteristics of the alginate/keratin fibrids can be found in ***Table 2*** and ***Figure 1***. For the alginate/keratin sponges, the sulfur content and absorption properties were estimated; SEM photos were taken, too.

Depending upon the amount of keratin in the spinning solution, the content of nitrogen in alginate/keratin fibrids in sponge form differs between the limits of 1.9 to 2.4%. Their absorption coefficient oscillates between 190 and 200% and the WRV value between 180 and 188%. The two parameters in the case of alginate sponge amount to 170% and 150%, accordingly. An increasing amount of keratin enhances the absorption properties of alginate/keratin in the form of sponges.

One other method of assessment of the absorption properties is the measurement of moisture imbibition. The kinetics of the process was investigated for selected samples of the alginate/keratin fibrids, and, to compare, for alginate fibrids in sponge form. Curves illustrating the sorption and desorption processes are presented in ***Figure 1***.

The alginate/keratin fibrids in sponge form reveal a moisture content higher by about 40% when compared with alginate sponges.

Properties assessment of chitosan/keratin composites in sponge form

The properties of the spinning solutions used for the forming of fibrids are shown in ***Table 3***, while the characteristics of the chitosan/keratin fibrids can be found in ***Table 4*** and ***Figure 2***. For the chitosan/keratin sponges, the sulfur content and absorption properties were estimated; SEM photos were taken, too.

Table 3. *Chitosan/keratin spinning solution for the preparation of fibrids.*

Sample mark	Polymer content, %	Chitosan content, %	Keratin content, %
Chit 26	5.56	5.56	–
RK 3	3.26	–	3.26
MKCh3	1.70	1.00	0.70
RK 4	0.98	–	0.98
MKCh4	1.70	1.00	0.70

Table 4. *Properties of chitosan/keratin sponges.*

Sample mark	Sulfur content, %	WRV, %
Fib-Chit 26	–	205
Fib-KCh3	0.48	284
Fib-KCh4	0.40	238

The content of keratin in the chitosan/keratin sponges was assessed based on the sulfur content. The WRV of the keratin-containing products is higher than for pure chitosan sponges.

The chitosan/keratin fibrids in sponge form display moisture absorption higher by up to 90% than chitosan sponges.

SEM assessment of the appearance of keratin-containing fibrids

To assess the appearance of the sponges, SEM inspections were made. The photos are presented in ***Figure 3***. Clearly visible in the structure are fibrids forming the pore walls.

Biomedical testing of chitosan/keratin fibrids in sponge form

Modern hygiene and dressing materials, except for their protective functions,

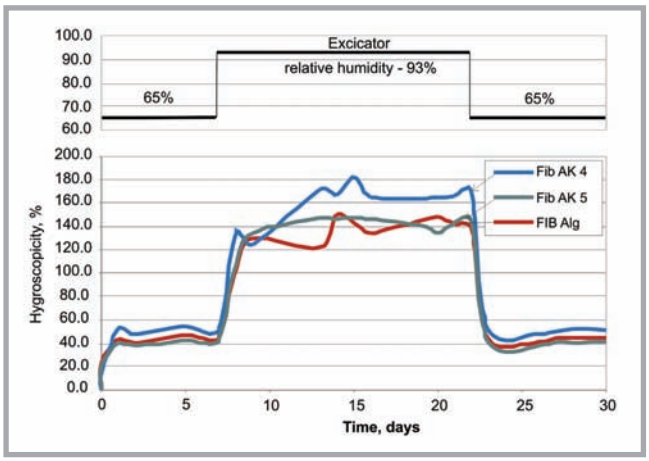

Figure 1. *Sorption and desorption curves of alginate/keratin fibrids in sponge form.*

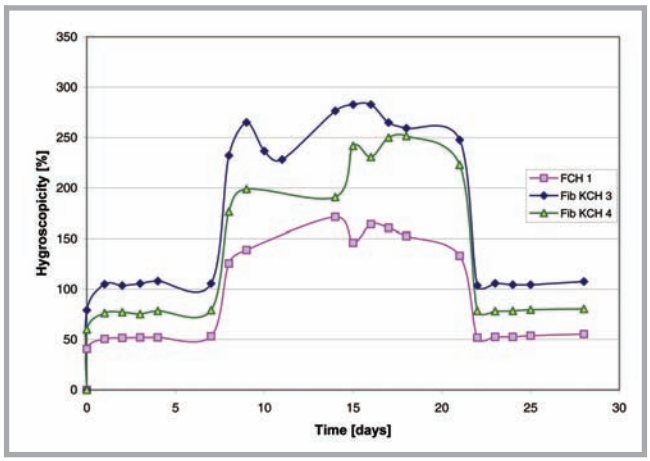

Figure 2. *Sorption and desorption curves of chitosan/keratin sponges.*

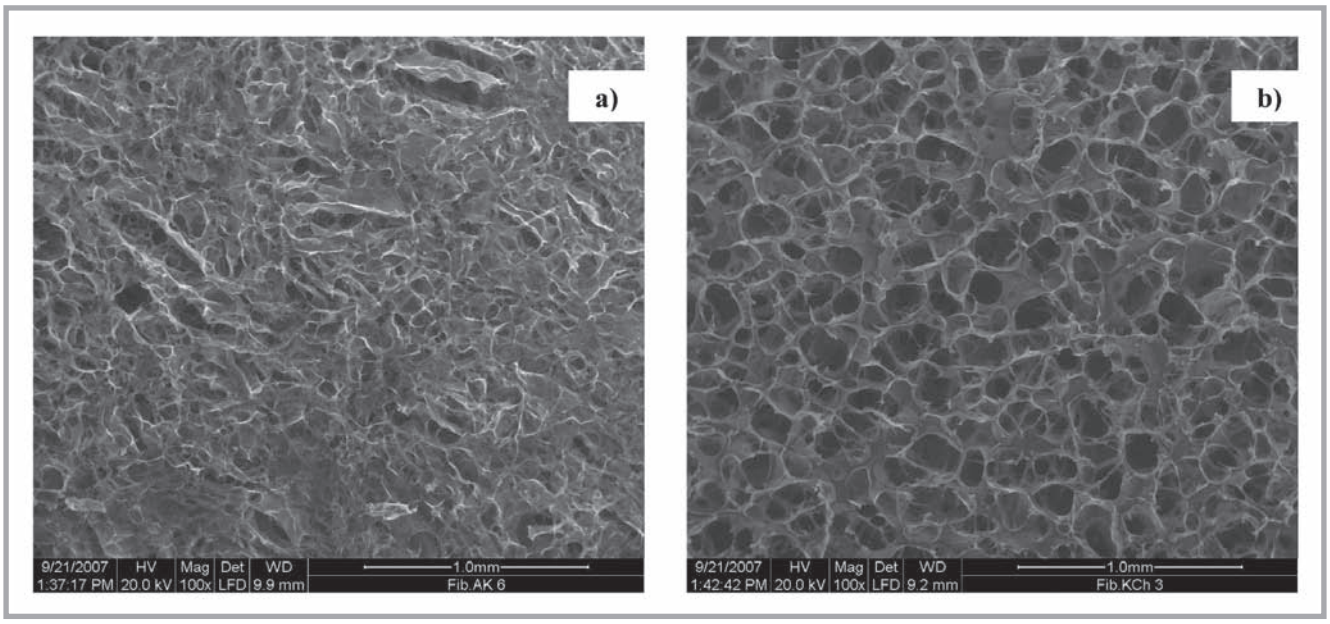

Figure 3. *SEM micro-photos of the sponge surface: a) alginate/keratin, b) chitosan/keratin.*

must be made of biocompatible materials featured by specific biological characteristics. Conditional is the lack of cytotoxicity and irritating and allergenic actions. Taking this into consideration, biomedical testing was carried out of the KCh-3 preparation containing the highest amount of keratin. The tests were carried out in the National Drug Institute in Warsaw, producing the following results:

- ***Cytotoxicity*** tests gave the result of "0" degree.
- ***Irritation tests*** – during the entire observation time (72 hours), no irritation on the rabbits' skin could be seen.
- ***Allergenic testing*** – no allergenic action was displayed in the tests with guinea pigs, hence there is practically no risk of allergenic effects in humans.

An exhaustive report from the National Drug Institute is available from IBChF, comprising the results of the three tests.

Summary

As a result of the investigations, innovative bio-composite materials were prepared in sponge form based on alginate/keratin and chitosan/keratin polymers. Materials of that kind have not yet been reported in professional literature. It was found that alginate/keratin and chitosan/keratin fibrids formed into sponges display enhanced absorption properties when compared with those without keratin content. The prepared composites do not display cytotoxicity or any irritating and allergenic action. Such features combined with outstanding absorption properties make the materials suitable for hygiene and medical applications. One more asset is the chance of utilising the burdensome feathers from poultry farming.

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