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Estimation of Polymer Compositions Containing Chitosan for Veterinary Applications

Abstract

The aim of the research presented herewith was to obtain a composition of a selected chitosan usability form with poly-N-vinylopyrolidone which could be used as the basis for a modern dressing material or a dressing confection of optimal biological activity. , Microcrystalline chitosan or gels of chitosan salts of diferentiated average molecular weight and similar deacetylation degree were used to obtain the composition with poly-N-vinylopyrolidone. Films were formed from the obtained compositions, which were next tested to assess their mechanical properties. Antibacterial tests were carried out for selected forms of chitosan, proving their activity against the Escherichia coli ATCC 11220 bacteria.

Key words: *chitosan, polymer compositions, bioactivity, dressings, poly-N-vinylopyrolidone.*

Introduction

A great increase in demand for dressing materials and confections, especially those based on natural products, has been observed for many years. This also applies to veterinary products, which apart from their fundamental protective functions could be conductive in wound regeneration, and would be able to shorten the healing process of the epidermis and skin of animals. Dressing materials recently applied in veterinary work are not a universal solution, and do not fulfil all physicians' requirements [1]. This is why new dressing material solutions are looked for, with the aim of fulfilling the demands arising from the need to heal animal wounds quickly and effectively, especially those which are difficult to heal such as scalds, injury wounds, and wounds generated as a result of cancer growth. Application of dressing materials based on natural products, which biostimulate the healing processes and regenerate the damaged skin, will allow doctors to limit the use of antibiotics, the side-effects of which often result in wrong action or even damage to the patient's kidney and liver, and also decrease the immunity and the bacterial microflora necessary for living organisms to function appropriately.

Elaboration of dressing material containing a bioactive layer which consists of a film, ointment or hydrogel requires the selection of a polymer of appropriate molecular and macromolecular structure, and with optimal properties which stimulate the skin and epidermis regeneration processes and wound healing.

Polyaminosaccharides, especially chitosan, the poly[β-(1,4)-2-amino-2-deoxy-D-glucopiranose] and its derivatives, are characterised by excellent biostimulating properties which facilitate reconstruction and vascularisation of damaged tissues, and also compensate the shortcomings of cell components, which are conductive for small scar forming [2].

A special property of chitosan is its bioactivity, which determines the medical and veterinary application of this polymer. This useful feature is a consequence of several processes and phenomena such as biodegradation, influence on living cell membranes, natural conformability with living cells, and lack of toxicity. Chitosan is biologically active thanks to its susceptibility to enzymatic degradation in the presence of lizozyme, an enzyme which exists in organism fluids; this in turn results in the creation of bioactive oligoaminosaccharides, which activate wound granulation and stimulate the healing process [2-3].

A medical dressing material which is of interest for future uses is a gel-like form of microcrystalline chitosan (MKCh), a physicochemical modification of preliminary chitosan. Microcrystalline chitosan has all the positive properties of preliminary chitosan, and at the same time possesses a unique ability to form polymer films directly from a suspension, good miscibility with other polymers and substances, and a high secondary water retention value.

One of the more important application aspects of chitosan and its derivatives in veterinary and medicine is its use for healing different kinds of wounds and dermatosis. Investigation carried out on animals [4-6] proved that applying these biopolymers led to a much faster healing of wounds than in control tests. What is more, the results achieved were better than for the application of pharmaceutic substances of different kinds, e.g. those containing animal collagen. Most often these substances have been tested by being spread directly on the wound surface as ointment, balsam, film, gel, and aerosol. The effect of wound healing and tissue regeneration was better when chitosan compounds were used together with other medicines of anti-inflammatory, anti-bacterial, and anti-pain action, e.g. neomycin, tetracycline, lidokaine, cevitamic acid, and zinc acid.

Several Japanese companies (e.g. JEX KK Co, Katakura Chikkavin Koken Co) manufacture dressings and artificial skin basing on chitosan and its derivatives. These are mainly compositions based on synthetic polymers and chitosan, or materials manufactured from collagen and acetylochitosan [7-8].

The biological properties of chitosan and its derivatives as presented above formed the basis for commencing

investigations related to the application of chitosan in veterinary use in the Institute of Chemical Fibres.

The aim of the research presented in this article was to obtain a composition of a selected chitosan form or its derivative, such as microcrystalline chitosan or a gel from chitosan salt with (PVP). On the basis of these materials, modern dressing preparations with optimal biological activity should be obtained . The development of such new materials, consisting in joining selected chitosan forms with natural or synthetic polymers, should create a possibility for manufacturing new dressings with ointment, hydrogel and aerosol for veterinary use, to protect injured animal skin successfully and ease matters for both the physician and the animal's master.

Development of a composition of the selected chitosan form with poly-N-vinylopyrolidone consisted in appropriate selection and composition modification of these materials, which would secure the obtention of a dressing material that would form a protective layer in the shape of a film of required mechanical properties on the surface of animal skin.

Chitosan of selected physicochemical parameters such as average molecular weight and deacetylation degree was used in order to secure optimal bioactivity, which in this case means biostimulation of the processes of wound healing and dermatosis cure.

Poly-N-vinylopyrolidone is a material widely applied in biomaterial engineering for the construction of gel dressing materials (as thickener) and in manufacturing artificial blood plasma [9]. We expected that poly-N-vinylopyrolidone, together with the selected chitosan usability form of good biological activity, would form compositions which could be used for protecting irritated skin and for healing a variety of wounds.

Experimental Part

Material and methods

For the use of the tests carried out, a preliminary chitosan of shrimp origin manufactured by the Norwegian company Primex Ingredients ASA was used. Its basic physicochemical properties are presented in Table 1.

The following analytic reagents were used for the tests:

- lactic acid 90% pure for analysis (p.f.a.), manufactured by Fluka;
- muriatic acid 35% - 38%, p.f.a., manufactured by POCh S.A., Gliwice;
- sodium hydroxide p.f.a., manufactured by POCH S.A. Gliwice; and
- poly-N-vinylopyrolidone $(CH_2NO)_4$ of molecular weight $\overline{M}_v$=1300000, manufactured by Aldrich.

Table 1. Basic physico-chemical parameters of preliminary chitosans used.

Chitosan denotation	$\overline{M}v$	SD	WRV	Moisture content	Ash content
	kD	%	%	%	%
Chitosan I	346.0	82.2	290.0	5.21	0.28
Chitosan II	144.0	85.3	330.0	6.86	0.30
Chitosan III	89.0	82.2	230.0	7.00	0.20

Table 2. Basic physico-chemical parameters of microcrystalline chitosan.

Test denotation	Polymer content	$\overline{M}v$	SD	WRV
	%	kD	%	%
MKCh I	2.76	292.0	82.2	1350.0
MKCh II	3.50	109.0	85.3	1225.0
MKCh III	3.30	70.0	82.2	1270.0

Table 3. Basic physico-chemical parameters of the gel from chitosan salt.

Denotation of preparation	$\overline{M}v$	SD, %	Polymer content, %
M I	261.0	82.2	1.88
M II	109.0	85.3	1.84
M III	76.0	82.2	1.91
CH I	297.0	82.2	1.95
CH II	97.0	85.3	1.95
CH III	44.0	82.2	1.96

The bacteria *Escherichia coli ATCC 11220* from the American Collection of Pure Cultures (ATCC) were used for testing the anti-bacterial activity of the selected chitosan forms used in compositions with poly-N-vinylopyrolidone.

Obtaining compositions of the selected usability chitosan forms with poly-N-vinylopyrolidone

The batches of preliminary chitosan were transformed onto the microcrystalline form [10] and onto gels of chitosan salts, according to special methods elaborated in the Institute of Chemical Fibres.

For obtaining a composition, an appropriate amount of microcrystalline chitosan or of a gel from chitosan salt was mixed with poly-N-vinylopyrolidone in the weight ratios 2:1, 1:1 and 1:2, and then precisely homogenised by a MPW-120 homogeniser at 3300 r.p.m. over 4 minutes. The compositions prepared in this way were poured out on Teflon plates, and dried at room temperature to obtain samples in the shape of films. The component ratios in the particular chitosan composites with poly-N-vinylopyrolidone are related to dry mass.

Analytic methods

- ***The average molecular weight*** of chitosan was determined on the basis of assessing the intrinsic viscosity (h). The Mark-Houwink equation was used to calculate the molecular weights; the constants $K=8.93x10^{-4}$ and a=0.71 have been accepted [11].
- ***The secondary water retention value (WRV)*** was determined according to the standard method [11].
- ***The deacetylation degree*** of chitosan was assessed with the use of potentiometric titration [12].
- The film samples were tested for determining their ***mechanical properties*** according to Polish standards [13-15] with the use of a 55-44 Instron tester.
- ***Anti-bacterial activity*** against the *Escherichia coli* bacteria of selected chitosan forms was estimated according to a method elaborated at the Microbiological Laboratory of the Institute of Chemical Fibres on the basis of a Japanese standard [16]. A gel-like dispersion of microcrystalline chitosan and of a gel from chitosan salt

was selected for preparing the composition with poly-N-vinylopyrolidone.

Research Results and Discussion

For all tests carried out in the range of this research, microcrystalline chitosan and gels of chitosan salts (i.e. of lactate and hydrochloride) were used. Microcrystalline chitosan (MKCh) was obtained by aggregation of macromolecules from its salt solution, according to the method developed in the Institute of Chemical Fibres.

This method allows for modificating of the obtained microcrystalline chitosan and achieving a broad range of properties and structures. For the purpose of this work, three kinds of MKCh of similar deacetylation degree and different values of average molecular weights were prepared. The basic physicochemical parameters of the microcrystalline chitosan used are presented in Table 2.

For the next research stage, six samples of gels of chitosan salts were prepared according to the method developed at the Institute of Chemical Fibres. The basic physicochemical parameters are presented in Table 3.

The next stage of this research consisted in an attempt to obtain a chitosan composition with poly-N-vinylopyrolidone, which should be the basic component of the dressing material destined for the purpose of veterinary application. The tests performed allowed selection of the quantitative ratios of the following compositions:
- microcrystalline chitosan /PVP, and
- gel from chitosan salt /PVP.

It was assumed that the dressing material used which contained the composition mentioned above would form films on the animal skin surface, which should have been characterised by appropriate mechanical and biological properties.

From the chitosan compositions with poly-N-vinylopyrolidone, films were formed for determination of their strength and elasticity; this is critical for estimating film arrangement on the skin surface. The results of this stage of research are shown in Tables 4-6.

On the basis of mechanical tests carried out, we were able to state that in general, in the case of films from the microcrystalline chitosan / PVP composition (Table 4) for all modified films, an improvement in the mechanical properties (breaking strength by stretching) could be observed for high poly-N-vinylopyrolidone content, independent of the value of average molecular weight. This is especially true for the MKCh samples, where the highest breaking tension could be noted.

The mechanical properties of films from the M chitosan gel / PVP composition are presented in Table 5. In the case of films from the gel M I / PVP and gel M III / PVP compositions, the addition of PVP causes a small improvement in the mechanical property of the films in relation to the properties which are characteristic for the films as unmodified.

On the basis of the results presented in Table 6, it was stated that the addition of PVP to the composition gel CH / PVP does not cause any mechanical property improvement in relation to films without PVP.

According to the programme set, tests of anti-bacterial activity of the selected chitosan forms used in compositions with poly-N-vinylopyrolidone against the *Escherichia coli ATCC 11229* bacteria were also carried out. The *Escherichia coli ATCC 11229* bacteria belong to the most numerous bacteria from the gram-negative intestinal rod-bacteria group. They are a natural physiological content of the alimentary canal of humans and higher animals. The clinical symptoms and the progress of infection caused by these bacteria depend on the place where the infec-

Table 4. *Mechanical properties of films obtained from the composition microcrystalline chitosan/poly-N-vinylopyrolidone (* - fragile films).*

Denotation of preparation	$\bar{M}v$	Composition ratio MKCh/PVP	Film thickness	Breaking tension	Elongation at maximum stretching tension
	kD		mm	MPa	%
MKCh I	292.0	1 : 0	0.035	13.3	3.27
		2 : 1	0.072	16.1	1.01
		1 : 1	0.085	20.6	2.03
		1 : 2	0.095	20.0	3.15
MKCh II	109.0	1 : 0	- *	- *	- *
		2 : 1	0.154	5.19	1.79
		1 : 1	0.125	4.11	1.28
		1 : 2	0.238	5.21	1.19
MKCh III	70.0	1 : 0	- *	- *	- *
		2 : 1	0.106	6.39	2.92
		1 : 1	0.094	13.2	2.81
		1 : 2	0.105	22.3	2.72

Table 5. *Mechanical properties of films obtained from the composition gel from chitosan lactate / poly-N-vinylopyrolidone.*

Denotation of preparation	$\bar{M}v$	Composition ratio M/PVP	Film thickness	Breaking tension	Elongation at maximum stretching tension
	kD		mm	MPa	%
M I	261.0	1 : 0	0.052	8.31	29.7
		2 : 1	0.113	9.99	31.6
		1 : 1	0.078	9.57	38.9
		1 : 2	0.087	9.80	20.9
M II	109.0	1 : 0	0.080	9.37	56.7
		2 : 1	0.058	9.39	40.0
		1 : 1	0.063	7.64	33.6
		1 : 2	0.063	7.53	44.7
M III	76.0	1 : 0	0.040	8.10	29.0
		2 : 1	0.046	9.82	20.2
		1 : 1	0.054	10.7	18.7
		1 : 2	0.067	6.95	30.0

Table 6. Mechanical properties of films obtained from the composition gel from chitosan hydrochloride/poly-N-vinylopyrolidone.

Denotation of preparation	$\bar{M}v$	Composition ratio CH/PVP	Film thickness	Breaking tension	Elongation at maximum stretching tension
	kD		mm	MPa	%
CH I	297.0	1 : 0	0.072	35.3	13.0
		2 : 1	0.128	17.6	5.33
		1 : 1	0.119	16.1	8.90
		1 : 2	0.105	12.5	3.54
CH II	97.0	1 : 0	0.072	34.8	12.1
		2 : 1	0.067	24.8	2.26
		1 : 1	0.054	18.7	2.59
		1 : 2	0.056	17.4	2.37
CH III	44.0	1 : 0	0.083	28.4	10.7
		2 : 1	0.048	24.7	3.85
		1 : 1	0.042	26.0	4.12
		1 : 2	0.075	12.8	3.11

Table 7. Anti-bacterial action of microcrystalline chitosan against Escherichia coli bacteria.

Test denotation	$\bar{M}v$, kD	Concentration of MKCh, %	Bacteriostatic activity	Bactericidal activity
MKCh I	292.0	0.1	0.4	-4.0
MKCh II	109.0	0.1	0.6	-3.8
MKCh III	70.0	0.1	3.1	-1.3

Table 8. Anti-bacterial action of gels from chitosan salts in relation to lactic and muriatic acid (the value with minus sign marks the lack of bacteriostatic and bactericidal activity).

Test denotation	$\bar{M}v$, kD	Concentration, %	Bacteriostatic activity	Bactericidal activity
Lactic acid	-	0.05	1.5	-4.3
M I	261.0	0.1	8.8	3.0
M II	109.0	0.1	8.9	4.5
Muriatic acid	-	0.02	< 2.6	< - 2.5
CH I	297.0	0.1	8.9	4.5
CH II	97.0	0.1	8.7	3.5

tion occurs. These may be inside abscesses, as well as in skin infections in the neighbourhood of wounds [17]. Thus these bacteria were used for the tests as a pathogenic factor which occurs by wound infection. The test results are presented in Tables 7-8.

The concentration of MKCh in the grafted breeding-ground was 0.1% (Table 7). It was stated that microcrystalline chitosan used in microbiological tests in the form of a gel-like dispersion of average molecular weight in the range of 70-292 kD was characterised by bacteriostatic activity against the *Escherichia coli* bacteria. The greatest activity was observed in microcrystalline chitosan of the lowest average molecular weight of 70 kD.

The research results of the anti-bacterial action of the gels from chitosan salts, as well as those from the pure lactic and muriatic acids, are presented in Table 8. The concentration of the gels from chitosan salts in the grafted breeding layer was also 0.1%.

It could be stated that (at the concentration used over the tests) the gel from chitosan lactate as well as of hydrochloride (both of average molecular weight in the range of 97-297 kD) are characterised by high bacteriostatic activity (8.7-8.9) and bactericidal activity (3.0-4.5) against the *Escherichia coli* bacteria. Instead of this, only small bactericidal activity could be stated for the pure lactic and muriatic acids.

An estimation of the anti-bacterial activity of selected compositions of MKCh or gels from chitosan salts will be provided in the foreseeable future.

Conclusions

- An addition of poly-N-vinylopyrolidone to the dispersion of microcrystalline chitosane causes an improvement in the mechanical properties of the films obtained. The mechanical properties of films from gels of chitosan salts with poly-N-vinylopyrolidone depend on the kind of gel applied.
- Microcrystalline chitosan used as a gel-like dispersion at concentration of 0.1% has bacteriostatic activity against the *Escherichia coli* bacteria. The highest (3.1) bacteriostatic activity was stated for the MKCh III of lowest average molecular weight (M=70.0 kD).
- A distinctly higher activity against *Escherichia coli* bacteria was characteristic for chitosan salt gels; at the concentration of 0.1% used over tests, strong bactericidal activity could be stated.

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