ADVANCED CELLULOSIC MATERIALS

Kinga Brzoza-Malczewska[1*], Magdalena Kucharska[1], Maria Wiśniewska-Wrona[1], Krystyna Guzińska[1], Jolanta Jóźwicka[1], Anna Bacciarelli-Ulacha[2]

[1] Institute of Biopolymers and Chemical Fibers
ul. Skłodowskiej-Curie 19/27, 90-570 Łódź, POLAND
e-mail: biomater@ibwch.lodz.pl

[2] University of Technology, Faculty of Material Technologies and Textile Design, ul. Żeromskiego 116, 90-924 Łódź,

Abstract

Aim of the work was to prepare a method of producing chitosan and chitosan-alginate nanoparticles designed for the modification of textile cellulosic products in hygiene and medical application. Spectrophotometry was used in the estimation of the prepared nanoparticles; analyzed, too, was the particle size and antibacterial and antifungal activity.

Key words: *nanoparticles, chitosan, complex chitosan/alginate, cellulosic products*

Received: 03.02.2015
Accepted: 05.05.2015

1. Introduction

In the last years, there has been a remarkable increase of diseases caused by microbiological and hospital infections which has in turn triggered extensive investigation in new materials and procedures which would warrant durable bioactive effects along with safety use for patients. Fungi and Gram positive and Gram negative bacteria are commonly found in cellulosic materials, textiles and paper in particular. Many of these microorganisms are pathogens that are often responsible for hospital infections. When applied in medical and hygiene devices, textiles usually come into contact with much bacterial-contaminated media which may cause degradation of the materials and give an unpleasant smell of. This is the reason why a lot of textile medical and hygiene products appeared on the market bearing antimicrobial properties. Such properties are normally conferred upon the materials by the processing with salts of silver or nano-silver [1-17]. The substances are presently a target of criticism because of their negative impact upon the environment and human health [18-20].

By including alternative natural materials, the development in nano-technologies has recently aroused more interest. Polysaccharides like cellulose, chitosan and alginates make a large group of biopolymers. Except of being renewable materials, they are readily biodegradable and show good adhesive properties as well as lack of toxicity.

Chitosan is a renewable, biodegradable biopolymer with good film-forming and adhesion properties. It reveals a strong antibacterial action mainly in the form of its salt derivative, and , moreover. it is well miscible with many substances inclusive polymers. Chitosan antimicrobial activity encompasses fungi, yeast and bacteria while Gram (+) are more affected than Gram(-) bacteria [21-23].Chitosan properties depend upon molecular mass, deacetylation degree, concentration, pH and composition of the medium in which the substance is applied [24]. Thanks to good adhesion and film-forming properties is chitosan fit to provide antibacterial coating or act as carrier for other antimicrobial compounds [25].

Presented in this article is a method to produce and properties of nanoparticles of chitosan and chitosan-alginate for uses in medical and hygiene materials. It was an aim of the research to prepare a method of producing chitosan and chitosan –alginate complex with particles in nano dimensions designed as antimicrobial substances. The elaborated nano-polymers may find their use in hygiene and dressing materials for everyday use as well as in hospitals (hygiene tissue, wadding, active layer of inserts and sanitary napkins, wound dressings and compresses for contaminated wounds).

2. Materials and methods

2.1Materials

DOI: 10.15259/PCACD.20.01

Chitosan Chito Clear HQG was delivered by Primex Co. Characterized by: average molecular mass ($\bar{M}_v$) =372 kD, deacetylation degree (DD)= 81 %, ash content= 0,22%, content of heavy metals: Ash <0,1%; Cd <0,1%; Pb = 0,27%; Zn = 0,80%; Hg <0,05- 0,0%.

Sodium alginate Protanal 10/60FT delivered by FMC Biopolymer Enginering, Inc.

2.2Analytical methods

Estimation of average molecular mass of chitosan (M_v)-Viscometric method

The viscometric average molecular mass was calculated based on the limiting viscosity number [η]. Viscosity was measured by means of a dilution viscometer with capillary nr1, K≈ 0,01 according to internal test method IBWCH.

Estimation of deacetylation degree of chitosan (DD) – method of first derivative of UV spectrum

Deacetylation degree was analyzed by spectrophotometric method consisting in the estimation of the maximum of the curve of first derivative of UV spectrum followed by a mathematical calculation of DD according to internal IBWCh procedure.

Estimation of ash content

Ash content was estimated at 800^0 according to internal IBWCh testing procedure.

Estimation of heavy metals

The content of heavy metals was estimated with the use of Absorption Atomic Spectrometry according to procedure NL-13/2008 ed. IV.

Estimation of chitosan and chitosan/alginate nanoparticle size.

The estimation of nanoparticles size in suspension was made with the use of DSL (Dynamic Light Scattering) with the apparatus Nicomp 380.

2.3Methods

Assessment of antibacterial activity of microcrystalline (MCC) and chitosan/algNa nanoparticles

The antibacterial activity against *E. coli and S. aureus was assessed according to standard* JIS L 1902:2002, "Examination of Antibacterial action of textiles. Quantitative test"

Assessment of antifungal activity of microcrystalline (MCC) and chitosan/algNa nanoparticles

The antifungal action against *C. albicans* was assessed according to standard ASTM: E2149-01 „Standard Test Method for Determining

the Antimicrobial Activity of Immobilized Antimicrobial Agent under Dynamic Contact Condition" – Shaking Flask Method.

Spectrophotometric examination of microcrystalline (MCC) and chitosan/algNa nanoparticles

The spectrophotometric examination of nano-polymers was made by transmission spectrometry in IR with Fourier transform (FTIR). The spectrophotometric ultra- red spectra were prepared by means of the apparatus Genesis Series FTIR^{TM} of UNICAM Co equipped with specialized software WinFIRST of Mattson Co USA. The spectra were prepared in the wave number range of 4000 - 500 cm^{-1}, with resolution of 4 cm^{-1} and scan number of 16. The samples were analyzed in the form of tablets with KBr at constant proportion of 1 mg sample and 300mg KBr.

Preparation of nan-microcrystalline (MCC) and nano-chitosan/algNa complex in powder form

Nano-polymers in powder form were obtained by freeze-drying by means of the laboratory lyophilizing cabinet type ALFA 2-4 by Christ Co. Freeze drying was made in the temperature range of from (-20) to 10 ^{0}C at vacuum from 0,1 to 0,7 mbar. The total drying time under such conditions was from 20 to 24 hours depending upon quantity of charge.

Sterilization of the powdered nano-polymers.

Sterilization of the powdered nano-polymers was made at the Institute of Radiation Technique by irradiation of the samples with fast electrons at dose of 25 kGy.

3. Results and discussion

3.1. Method to prepare nanoparticles of chitosan and chitosan/alginate

Preparation of microcrystalline chitosan (MCCh) with particle size below 1 μm was the main goal of the investigation. The ultrasonic reactor Hielscher UP 200S equipped with sonotrode S14L2D was used to that end. Aqueous chitosan lactate with concentration of 0.2 wt.% and 0.1wt.% aqueous sodium hydroxide were used in the preparation of MCCh. The method of preparing the polymer runs as follows: aqueous solution of sodium hydroxide is by means of a peristaltic pump continuously admixed to the solution of chitosan lactate which is ultrasonically treated with acoustic density in the range of 40÷60W/cm^2. The coagulation of the chitosan lactate was conducted directly in the ultrasound stream produced by the sonotrode until the pH of the suspension reached the 6.7-6.8 level at process temperature in the 20÷30 ^{0}C range. Coagulation process finished, the obtained polymer suspension was still treated for 20 minutes with ultrasounds at acoustic density in the range of 30÷50 W/cm^2.

Within the research, attempts were also made to prepare a chitosan/sodium alginate complex (chitosan/algNa). Aqueous chitosan lactate with concentration of 0.2 wt.% and an alkaline solution of sodium alginate with concentration

of 0,044 wt.% were used in the preparation of the chit/algNa nanoparticles. The coagulation process of the chitosan lactate with alkaline solution of sodium alginate was conducted the same way as the preparation of chitosan nanoparticles. Outcome of the reaction was a chit/algNa complex with 95% content of chitosan

Assessment of particle size of microcrystalline chitosan and complex chitosan/algNa prepared by ultrasound treatment

Particle size was assessed in the prepared suspensions of microcrystalline chitosan (MCCh) and the complex chitosan/algNa. The examination was accomplished by Dynamic Light Scattering (DLS) technique with the use of the apparatus Nicomp 380. The NICOMP distribution of solid particles of the examined polymers is presented in Table 1. It has been documented in the examination that the particles size of both MCCh and complex chitosan-algNa falls below 1 micrometer. MCCh varied in size and number content, three fractions for MCCh are shown and two fractions for chit/algNa (Table 1). In the tested sample, particles about 25 nm in diameter make the vast majority in MCCh (about 97%) and those with 60nm diameter amount to 67% in the complex chitosan/algNa. The results testify in favour of the prepared method employing an ultrasound reactor. The method enables the preparation of nano-sized particles of MCCh and complex chi/algNa.

Table 1. Dimensions of nanoparticles analyzed by DLS method, NICOMP distribution

Sample	No. of fraction	Volume Weighting		Number Weighting		Intensity Weighting	
		Diameter, nm	Share %	Diameter, nm	Share %	Diameter, nm	Share %
MCCh	1	25,0	81,0	24,5	97,3	26,9	2,2
	2	137,3	10,7	132,1	2,3	158,2	38,9
	3	548,7	0,3	528,7	0,4	528,7	58,9
Chit/Alg	1	57,4	91,1	60,4	66,7	67,3	5,9
	2	297,1	8,9	308,5	33,3	307,5	94,1

Spectrophotometric analysis of nano-MCCh and nano-complex chitosan/algNa

Spectrophotometr**i**c examination according to described method. was made of the prepared nano-polymers. Comparative spectra were drawn for the virgin chitosan, sodium alginate and complex chitosan/algNa (Fig. 1), and for the virgin chitosan in comparison to nano-chitosan (Fig. 2)

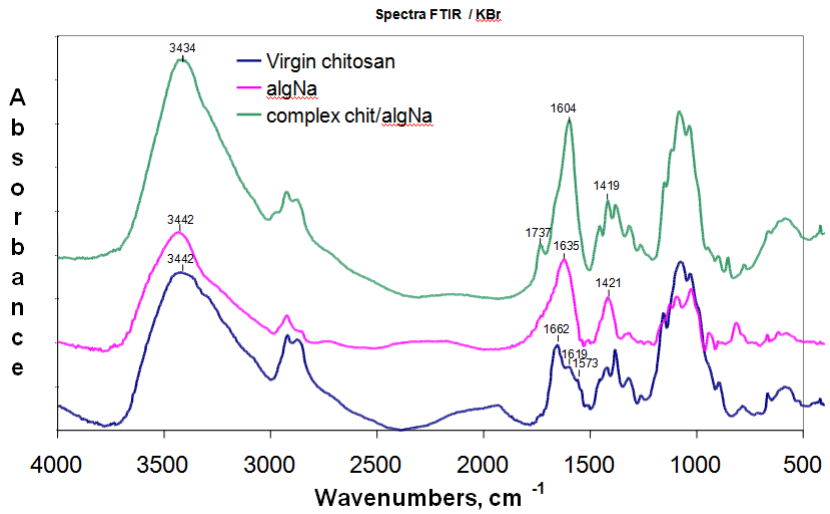

Figure 1. FTIR spectra of algNa, virgin chitosan and complex chit/algNa

In the chitosan FTIR spectrum following bands are seen: 1662cm^{-1} - originating from the stretching vibrations C=O in the amide group (amide band I), 1619cm^{-1}-originating from covalent vibrations N-H in the $-NH_2$ group, and 1573cm^{-1} –originating from the bending vibrations N-H in the amide group [25]
In the spectrum of sodium alginate two characteristic bands can be distinguished: 1635cm^{-1} and 1421cm^{-1} responding to the covalent vibrations C-O in the carboxylate ion [25].
Discernible in the spectrum of the complex chit/algNa is a new absorption band at 1737cm^{-1} that responds to the deformation deformacyjne symmetric vibrations N-H in the NH_3^+ ion. That band points the presence of ion bonds in the complex.
Bands of the chitosan spectrum at wave numbers 1619 i 1573cm^{-1} disappear. The disappearance of the 1573cm^{-1} band may be explained with the contribution of protonized amine groups to the creation of the chitosan-alginate complex. Except of the ion bonds, appearing are also inter-and intra-chain hydrogen bonds that are formed between the elements of the chitosan chain structure and between chitosan and alginate [26-27].The presence of the bonds is also confirmed with a broad and intensive absorption band with a maximum at 3434cm^{-1}, appearing in the 3000-3500cm^{-1} range which indicates the occurrence of associated –OH, and -NH_2 groups in chitosan, and –OH groups in alginate [26,27].

DOI: 10.15259/PCACD.20.01

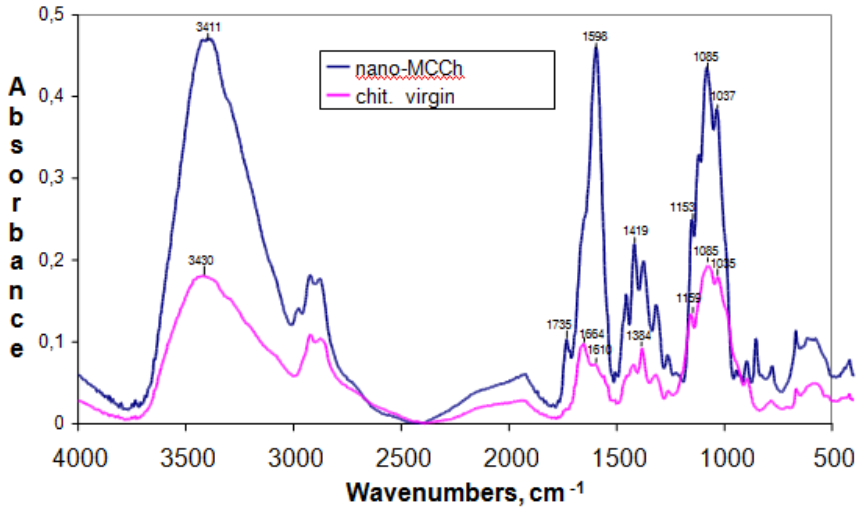

Figure 2. FTIR spectra of virgin chitosan and MCCh nano-particles

In the FTIR spectrum of virgin chitosan there are two characteristic to the substance two absorption bands: 1662cm^{-1} (Amid I) and 1610cm^{-1} (Amid II) [25]. The absorption band originated from stretching vibrations of the –CH bond at 1384cm^{-1} for nano-MCCh is displaced to1419cm^{-1}. In the nano-MCCh spectrum there appeared a new band at 1733cm^{-1} originating from the ester group.

Assessment of biological activity of nano-MCCh and nano-complex chit/algNa

It was assumed by the authors the elaborated nano-polymers be used in the modification of cellulosic hygiene and dressing materials for everyday uses and in hospitals (hygiene tissue, wadding, active layer of inserts and sanitary napkins, wound dressings and compresses) to confer antimicrobial properties. Consequently, the prepared nano-polymers were submitted to the testing of their antimicrobial activity against bacteria *Escherichia coli and Staphylococcus aureus* as well as the fungus *Candida albicans* according to standards JIS L 1902:2002 and ASTM: E2149-01. Prior to the testing, the nano-polymers were powdered by freeze-drying and sterilized by irradiation with fast electrons at dose of 25 kGy. The obtained results (Tables 3-5) show that both nano-polymers exert a very good antibacterial and antifungal activity. The bacteriocidal activity against bacteria *Escherichia coli and Staphylococcus aureus* is very good falling into the range of 2,7-3,2 (Table 3,4). What concern the antifungal assessment, the nano-polymers revealed a close to 100% reduction against the fungus *Candida albicans* (Table 5.)

Table 3. Estimation of antibacterial activity of nano-biopolymers against bacteria *E. coli*

Sample symbol	Time [h]	No. of bacteria [jtk/Pr]	Bacteriostatic activity against E.*coli*	Bacteriocidal activity against E. *coli*	Value of growth
Reference	0	$1.1x10^4$	-	-	-
Reference	24	$1.5x10^8$	-	-	4.2
MCCh	24	<20	6.9	2.7	-
Reference	0	$2.2x10^4$	-	-	-
Reference	24	$8.6x10^7$	-	-	3.6
chit/algNa	24	<20	6.6	3.0	-

Table 4. Estimation of antibacterial activity of noano-biopolymers against bacteria *S. aureus*

Sample symbol	Time [h]	No. of bacteria [jtk/Pr]	Bacteriostatic activity against S. *aureus*	Bacteriocidal activity against *S. aureus*	Value of growth
Reference	0	$3.4x10^4$	-	-	-
Reference	24	$2.8x10^6$	-	-	1.9
MCCh	24	<20	5.1	3.2	-
Reference	0	$6.3x10^4$	-	-	-
Reference	24	$8.2x10^6$	-	-	2.1
chit/algNa	24	<20	5.6	3.5	-

Table 5. Estimation of antifungal activity of nano-biopolymers against fungus *C. albicans*

Sample symbol	Time [h]	No. of bacteria [jtk/Pr]	Bacteriostatic activity against S. *aureus*	Bacteriocidal activity against *S. aureus*	Value of growth
Reference	0	$3.4x10^4$	-	-	-
Reference	24	$2.8x10^6$	-	-	1.9
MCCh	24	<20	5.1	3.2	-
Reference	0	$6.3x10^4$	-	-	-
Reference	24	$8.2x10^6$	-	-	2.1
chit/algNa	24	<20	5.6	3.5	-

DOI: 10.15259/PCACD.20.01

4. Conclusions

1. The method prepared for the production of microcrystalline chitosan and complex chitozan/algNa in the stream of ultrasounds with acoustic density of 52.5 (W/cm^2) yields biopolymers with dimensions in the nano-metric range.
2. Dimensions of the obtained chitosan particles fall in the 25-550nm range. while more than 80% of the nanoparticles reveal a 25nm diameter. Nanoparticles of the complex chitosan-alg are characterized by diameter in the range of 57-300nm. with 90% of the nanoparticles having a diameter of 57nm
3. FTIR analysis of the nano-complex chit/algNa has documented the presence of a new absorption band at $1743cm^{-1.}$ responding to the symmetric deforming vibration N-H in the $-NH_3^+$ ion. It witnesses the appearance of hydrogen bonds in the complex. Derived from the ester group, the $1735cm^{-1}$ band emerged in the nano-chitosan spectrum.
4. The prepared nano-biopolymers have shown a very high bacteriostatic and bacteriocidal activity against bacteria gram (-) *Escherichia coli* and gram (+) *Staphylococcus aureus.* as well as antifungal action against fungus *Candida albicans*.

5. Acknowledgement

The presented part of investigations was carried out within the research project no NCBiR/ERA-NET-MATERA/01/2011 supported by the Ministry of Science and Higher Education

6. References

1. Arora S. Jain J. Rajwade J. Paknikar K.; (2008) Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol Lett 179.93–100. **DOI**: 10.1016/j.toxlet.2008.04.009
2. Chi Z. Liu R. Zhao L. Qin P. Pan X. Sun F. Hao X; (2009) A new strategy to probe the genotoxicity of silver nanoparticles combined with cetylpyridine bromide. Spectrochim Acta 72. 577–581. **DOI**: 10.1016/j.saa.2008.10.044
3. Choi O. Hu Z; (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol 42. 4583–4588. **DOI:** 10.1021/es703238h
4. Hwang E. Lee J. Chae Y. Kim Y. Kim B. Sang B. Gu M; (2008) Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. Small 4. 746–750.
5. Kim J (2007) Antibacterial activity of Ag+ ion-containing silver nanoparticles prepared using the alcohol reduction method. J Ind Eng Chem 13. 718–722.

6. Kim J. Kuk E. Yu K. Kim J. Park S. Lee H. Kim S. Park Y. Park Y. Hwang C. Kim Y. Lee Y. Jeong D. Cho M (2007) Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol 3. 95–101. **DOI**: http://dx.doi.org/10.1016/j.nano.2006.12.001
7. Kim K. Sung W. Moon S. Choi J. Kim J. Lee D; (2008) Antifungal effect of silver nanoparticles on dermatophytes. J Microbiol Biotechnol 18. 1482–1484.
8. Kim Y. Kim J. Cho H. Rha D. Kim J. Park J. Choi B. Lim R. Chang H. Chung Y. Kwon I. Jeong J. Han B. Yu I; (2008) Twenty-eight-day oral toxicity. genotoxicity. and genderrelated tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhal Toxicol 20. 575–583. **DOI:**10.1080/08958370701874663
9. Kvitek L. Panacek A. Soukupova J. Kolar M. Vecerova R. Prucek R. Holecova M. Zboril R; (2008) Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). J Phys Chem C 112. 5825–5834. **DOI:** 10.1021/jp711616v
10. Lok C. Ho C. Chen R. He Q. Yu W. Sun H. Tam P. Chiu J. Che C; (2006) Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res 5. 916–924. **DOI:** 10.1021/pr0504079
11. Raffi M. Hussain F. Bhatti T. Akhter J. Hameed A. Hasan M; (2008) Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224. J Mater Sci Technol 24. 192–196.
12. Schrand A. Braydich-Stolle L. Schlager J. Dai L. Hussain S; (2008) Can silver nanoparticles be useful as potential biological labels?. Nanotechnology 19. No23. 235104. **DOI**:10.1088/0957-4484/19/23/235104
13. Sondi I. Salopek-Sondi B; (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275. 177–182. **DOI**: 10.1016/j.jcis.2004.02.012
14. Vertelov G. Krutyakov Y. Efremenkova O. Olenin A. Lisichkin G; (2008) A versatile synthesis of highly bactericidal Myramistin stabilized silver nanoparticles. Nanotechnology 19. No23.
15. Choi O. Deng K. Kim N. Ross L. Surampalli R. Hu Z (2008) The inhibitory effects of silver nanoparticles. silver ions. and silver chloride colloids on microbial growth. Water Res 42. 3066–3074. **DOI**: 10.1016/j.watres.2008.02.021
16. Cowan M. Abshire K. Houk S. Evans S; (2003) Antimicrobial efficacy of a silver-zeolite matrix coating on stainless steel. J Ind Microbiol Biotechnol 30. 102–106. **DOI**: 10.1007/s10295-002-0022-0
17. Zhang Y. Peng H. Huang W. Zhou Y. Yan D; (2008) Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles. J Colloid Interface Sci 325. 371–376. **DOI**: 10.1016/j.jcis.2008.05.063
18. Marambio-Jones C. Hoek EMV.; (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J. Nanoparticle Res. 12. 1531 – 1551. **DOI**: 10.1007/s11051-010-9900-y

19. Boholm M. Arvidsson R; (2014) Controversy over antibacterial silver: implications for environmental and sustainability assessments. J Cleaner Prod. 68. 135-143. **DOI**: 10.1016/j.jclepro.2013.12.058
20. Blaser SA. Scheringer M. MacLeod M. Hungerbühler K.; (2008) Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. Science of the Total Environm. 390. 396-409. **DOI**: doi:10.1016/j.scitotenv.2007.10.010
21. Rhoades J.. Roller S; (2000) Antimicrobial Actions of Degraded and Native Chitosan against Spoilage Organisms in Laboratory Media and Foods. Appl. Environ. Microbiol.. 66. 80-86. **DOI**:10.1128/AEM.66.1.80-86.2000
22. No HK.. Park NY.. Lee SH.. Meyers SP; (2002) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol. 74. 65-72. **DOI:**10.1016/S0168-1605(01)00717-6
23. Jeon YJ.. Park PJ.. Kim SK; (2001) Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr. Polym.. 44. 71-76. **DOI**:10.1016/S0144-8617(00)00200-9
24. Gottfriedl K.. Sztuka K.. Statroszczyk H.. Kołodziejska I.; (2010) Biodegradowalne i jadalne opakowania do żywności z polimerów naturalnych. Opakowanie. 8. 26–36.
25. Smitha B.. Sridhar S. Khan AA; (2005) Chitosan-sodium alginate polyion complexes as fuel cell membranes. European Polym. J. 41. 1859-1866. **DOI**:10.1016/j.eurpolymj.2005.02.018
26. Gierszewska-Drużbińska M. Ostrowska-Czubenko J; (2007) Synteza i właściwości membran hydrożelowych na podstawie chitozanu oraz alginianu sodu. Polimery 52. 517.
27. Satori C. Finch DS. Ralph B. (1997) Determination of the cation content of alginate thin films by FTiR spectroscopy. Polymer 38. 43.