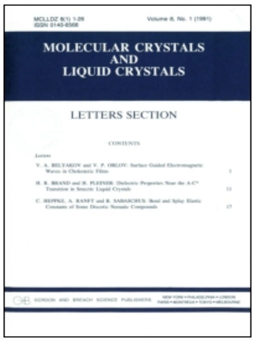

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Physico-chemical properties of three-component mixtures based on chitosan, hyaluronic acid and collagen

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ABSTRACT
The aim of this work was preparation of biopolymer materials based on blends of chitosan, hyaluronic acid and collagen. The properties of blend films have been investigated by contact angle measurements and atomic force microscopy. The contact angle measurements showed that chitosan films are more polar after addition of hyaluronic acid and collagen. The AFM results showed that the addition of chitosan to hyaluronic acid led to the increase of surface roughness. Moreover, miscibility studies of biopolymeric blends were also done using the viscometric method. Viscometric studies indicated that two-component and three-component blends are partially miscible.

KEYWORDS
chitosan; hyaluronic acid; collagen; blends

1. Introduction

Biopolymers play a very important role in tissue engineeringand are widely used as biomaterials [1-4]. According to their degradation properties, biopolymers can be further classified into biodegradable and non-biodegradable biopolymers [5]. In recent years there is a need of development new materials for tissue engineering and regenerative medicine. Natural polymers have the potential of biological recognition that may positively support cell adhesion and function [6]. The blending of biopolymers has received a considerable attention due to the new possibility of materials preparation for several applications. Two or even more biopolymers can be mixed together and such the blends can lead to new materials. The physico–chemical properties of polymer blends are important from both scientific and practical point of view [7,8]. An important aspect of the properties of a blend is the miscibility of its components. Miscibility in polymer blends is assigned to specific interaction between natural polymers [7].

Chitosan (CTS), hyaluronic acid (HA) and collagen (COL) are compounds, which belongs to the group of biopolymers. Their synthesis generally involves enzyme-catalyzed, chain growth polymerization reactions of activated monomers, which are typically formed within cells by complex metabolic processes [8].

Chitosan is a polysaccharide and it is obtained mainly due to the deacetylation of chitin, which is one of the most abundant renewable natural product. It occurs in marine crustaceans armor, armor of insects and the cell walls of fungi. Chitosan can also occur naturally in some

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species of mushrooms, but its content is much lower than that in chitin [9,10]. This polymer is soluble in dilute solutions of organic acids, for example: acetic acid, formic acid, alcohols, and glycerol. It can be dissolved also in some dilute solutions of inorganic, for example: hydrochloric acid. Chitosan is not dissolved in a solution of alkaline and neutral solutions [11,12]. Chitosan, due to its interesting properties (biological, physical, chemical ones) is used in various kinds of fields. This versatile polymer is used in medicine, engineering, water treatment, food industry, agriculture and cosmetic industry [11,12].

Hyaluronic acid (HA) is also known as hyaluronan, because in physiological conditions it existsin the form of a sodium salt, therefore negatively charged [13,14]. Hyaluronic acid is a glycosaminoglycan copolymer of d-glucuronic acid and n-acetyl-d-glucosamine. This acid is a linear polysaccharide naturally occurring in mammalian tissues [15]. Hyaluronic acid acts as a major intracellular component of connective tissues such as the synovial fluid of joints, vitreous fluid of the eye, and the scaffolding within cartilage and the umbilical cord [14].It is a material of increasing importance in biomaterials science and it can be applied in tissue culture scaffolds and in cosmetic materials [16]. HA has been shown to play an important role in lubrication, cell differentiation and cell growth. Its properties, both physical and biochemical, in solution or hydrogel form, are extremely attractive for various technologies concerned with body repair [17-20].

Collagen is the most abundant polymer in the animal life [7]. At present there are known 29 genetically distinct types of collagen characterized by a unique structure and the presence of tissue-specific properties [21]. Collagen exists in several organs acting as the bonding material of the cell. It maintains the structural integrity of tissues provides the hardness, stiffness and elasticity of tissues. Thanks to its supermolecular structure, collagen connects a large amount of water and consequently is characterized by great flexibility. Collagen plays a fundamental role in wound healing, blood clotting and tissue repair and regeneration of bone. One of the functions of collagen is interaction with specific receptors which affect the processes of adhesion, differentiation, survival and cell growth [22-25].

In the present study, we aimed to prepare the miscible CTS/HA blend with different additions of collagen. CTS/HA blends were prepared from mixed polymer solutions. The composition of CTS/HA was 50/50. In order to obtain three-component mixtures, collagen was added as the third component. Thin films were obtained after evaporation of solvent. The obtained binary and ternary blends were characterized by contact angle measurements, atomic force microscopy and viscomertic method.

2. Material and methods

2.1. Preparation of blends

Chitosan and hyaluronic acid were supplied by the company Sigma-Aldrich (Poznan, Poland). The deacetylation degree (DD, %) of chitosan was 78%, and a viscosity average molecular weight was 0.59×10^6. Hyaluronic acid had a viscosity average molecular weight of 1.8×10^6. This parameter was measured with the Ubbelohde viscometer and calculated from the viscosity of solutions according to the Mark-Houwink equation [26]. Collagen was obtained in our laboratory from tail tendons of young rats. Tendons were excised and washed in distilled water, and dissolved in 0.1M acetic acid for three days in 4°C. The undissolved parts were removed by centrifugation for 10 min at 10,000 rpm. Soluble fraction was decanted, frozen and lyophilized. CTS/HA blends were prepared from mixed polymer solutions. Chitosan was solubilised in aqueous 0.1M CH_3COOH/0.2M NaCl, hyaluronic acid in aqueous 0.01M HCl, and collagen in aqueous 0.1M CH_3COOH. The composition of CTS/HA was 80/20, 50/50, 20/80. The collagen solution was added in the different ratios based on the CTS/HA blend in 50/50 weight ratio. Polymers in aqueous solution were mixed together by magnetic stirring for 24 h. These solutions were used to prepare the polymer films.

2.2. Contact angle measurements

The contact angle of two liquids: diiodomethane (D) and glycerol (G) on the surface of chitosan, hyaluronic acid, collagen and their blend films were measured at room temperature, using the DSA10 goniometer equipped with the system drop-shape analysis (Krüss GmbH Germany). The liquid drop of 2-3 μl was placed onto the polymer surface with a microsyringe. The drop image was recorded by video camera and digitalized. The profile of a single drop was numerically solved and fitted by appropriate mathematical functions using instrument software. Each accepted result of contact angle is the average value of a maximum of 10 measurements. The surface free energy and its polar and dispersive components were calculated using the same method as shown in previous paper [27].

2.3. Atomic force microscopy

The topographic imaging was performed using a multimode scanning probe microscope with a NanoscopeIIIa controller (Digital Instruments, Santa Barbara, CA) operating in the tapping mode, in air, at room temperature. Surface images, using scan widths ranging from 1 μm to 5 μm, with a scan rate of 1.97 Hz were acquired at fixed resolution (512 × 512 data points). The roughness parameter was calculated for scanned area (5 μm × 5 μm) using Nanoscope software. The AFMimages and roughness calculations were obtained for different sample places and the most typical areas are presented.

2.4. Viscosity measurements

Viscosity measurements of chitosan, hyaluronic acid, collagen and their blend solution ($c = 0.1\%$) were performed at $25 \pm 0.1°C$ using the Ubbelohde capillary viscometer. The flow times were recorded with an accuracy ±0.01 s. Before measurements the solutions were filtered through G1 sintered glass filters. The intrinsic viscosity and the values of interaction parameter were determined according to Huggins equation [28] using solution of 5 concentrations. The miscibility was estimated by comparison of the experimental and ideal values of b_m and $[\eta]_m$. The values of interaction parameters (b_m) were obtained using the same methods as shown in previous papers [29-30].

3. Results and discussion

3.1. Contact angle measurements

An interesting property of blend films is the wettability, which was evaluated by means of contact angle determination. The contact angles for two different liquids (diiodomethane (D) and glycerol (G)) on the surface of chitosan, hyaluronic acid and chitosan/hyaluronic acid films were measured before and after the addition of collagen. The results are shown in Tables 1 and 2. It can be noticed that glycerol contact angles for used films are almost the same for HA, CTS and their blends (~67-70°), while diiodomethane contact angles

Table 1. Values of contact angles (deg), surface free energy [γ_S] and its polar [γ_S^p] and dispersive [γ_S^d] components for CTS, HA and their blends.

	contact angle [°]					
W_{CTS}	G	D	γ_s[mJ/m^2]	γ_s^D[mJ/m^2]	γ_s^P[mJ/m^2]	γ_s^P/γ_s^D
0.0	69.6 ± 1.46	54.5 ± 0.94	35.28	23.88	11.40	0.48
0.2	69.2 ± 1.00	53.8 ± 0.69	34.57	25.76	8.80	0.34
0.5	68.1 ± 0.89	59.5 ± 1.00	33.75	23.11	10.63	0.46
0.8	65.9 ± 0.87	52.2 ± 0.99	36.90	25.91	10.99	0.42
1.0	66.8 ± 1.87	55.7 ± 0.89	32.94	24.41	8.52	0.35

of blend with $w_{CTS} = 0.8$ surface is somewhat lower (~52°) than those of all other films (~54-56°). This may indicate that the 80/20 CTS/HA blend surface is slightly more polar than the surface of others films. The surface free energy is often recommended for characterization of polymer hydrophilicity. Values of the surface free energy as well as thedispersive and polar components were determined according to Owens – Wendt equation [31]. The obtained values indicate that the chitosan surface has the lowest polarity because it has the lowest polar component of its surface free energy (Table 1). For the CTS/HA blends, the values of the dispersive component are much bigger than those of the polar component, which indicates mainly hydrophobic surface. This behavior indicates that there are fewer interactions between the polar groups of chitosan and hyaluronic acid in the blend. Therefore, the polar groups of the polymers were hidden below the surface of the film. In the case of ternary CTS/HA/COL blends (Table 2), the values of surface free energy and polar component are reduced compared with pure hyaluronic acid and collagen films. The exception is the CTS/HA (50/50) blend with 10% of collagen film. This surface possesses the highest polarity.

3.2. *Atomic force microscopy*

The surface morphology of films obtained for pure components and their binary and ternary blends are shown in Figures 1–2. The corresponding roughness parameters are presented in Tables 3 and 4. As it can be seen, the AFM images show differences in the surface properties of films for pure polymers and their blends. In our previous study we found that for CTS/HA blends (when HA was dissolved in $0.1\text{mol}\cdot\text{dm}^{-3}$HCl solution) the chitosan component was found to be concentrated on the surface of the blends [25]. In both, previous and present

Table 2. Values of contact angles (deg), surface free energy [γ_S] and its polar [γ_S^p] and dispersive [γ_S^d] of CTS/HA (50/50) blend containing different addition of collagen.

	contact angle [°]					
w_{COL}	G	D	γ_s[mJ/m^2]	γ_s^D[mJ/m^2]	γ_s^P[mJ/m^2]	γ_s^P/γ_s^D
0.0	68.1 ± 0.89	59.5 ± 1.00	33.75	23.11	10.63	0.46
0.1	54.4 ± 0.58	67.0 ± 0.74	38.70	15.53	23.17	1.49
0.2	69.3 ± 0.91	56.3 ± 1.07	34.28	24.84	9.44	0.38
0.3	68.1 ± 0.90	58.4 ± 1.01	32.95	22.36	10.59	0.47
0.4	65.9 ± 0.67	67.9 ± 1.45	32.18	18.28	13.90	0.76
0.5	74.7 ± 0.90	69.1 ± 1.15	27.25	16.72	10.52	0.63
0.6	81.5 ± 0.69	64.6 ± 0.84	27.50	22.32	5.18	0.23
0.8	77.6 ± 0.35	52.6 ± 0.29	33.25	28.20	5.06	0.18
1.0	65.0 ± 0.70	49.8 ± 0.78	37.23	26.60	10.63	0.40

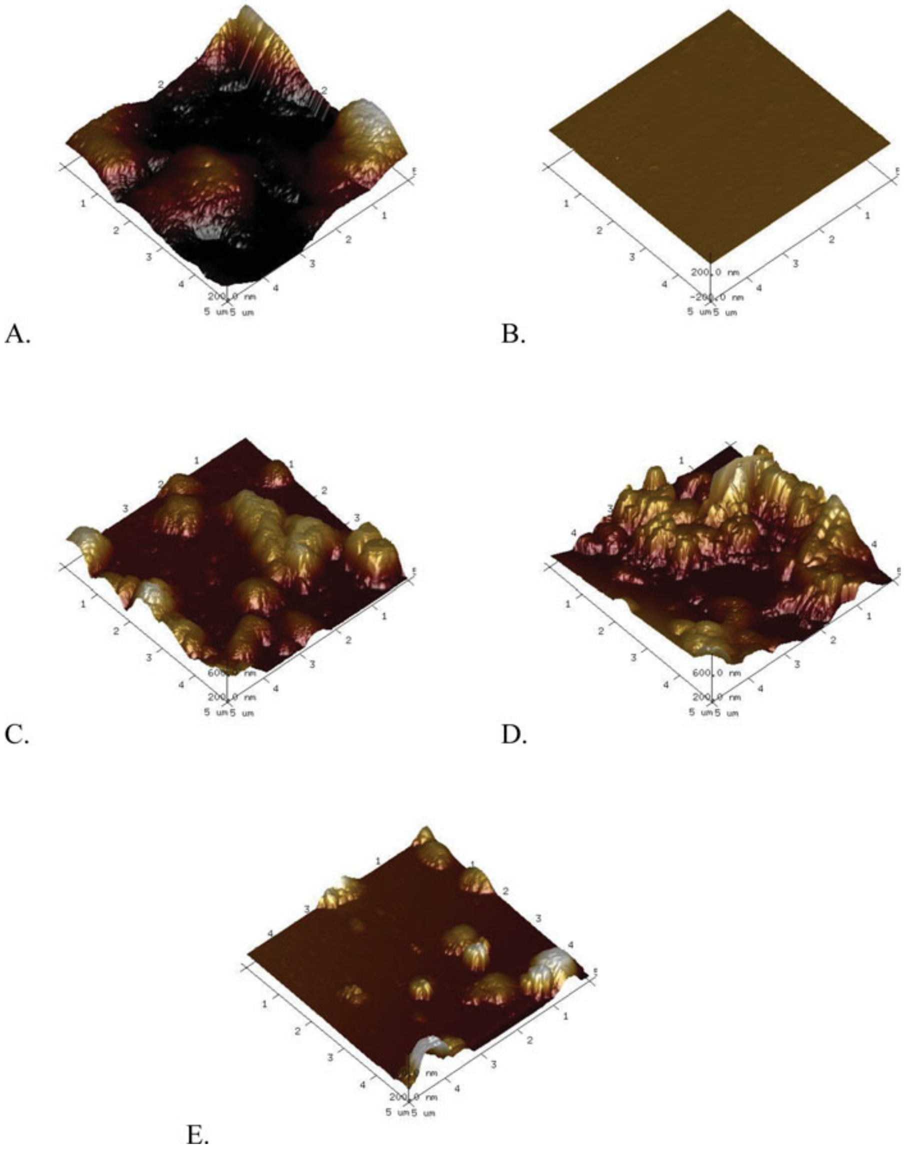

Figure 1. AFM images of the surface of unmodified polymer films: A. CTS, B. HA and CTS/HA polymer blends: C. 80/20, D. 50/50, E. 20/80.

Table 3. The roughness parameters (R_q) of CTS, HA and their blends.

w_{CTS}	R_q (nm)
0.0	2.46
0.2	68
0.5	184
0.8	181
1.0	190

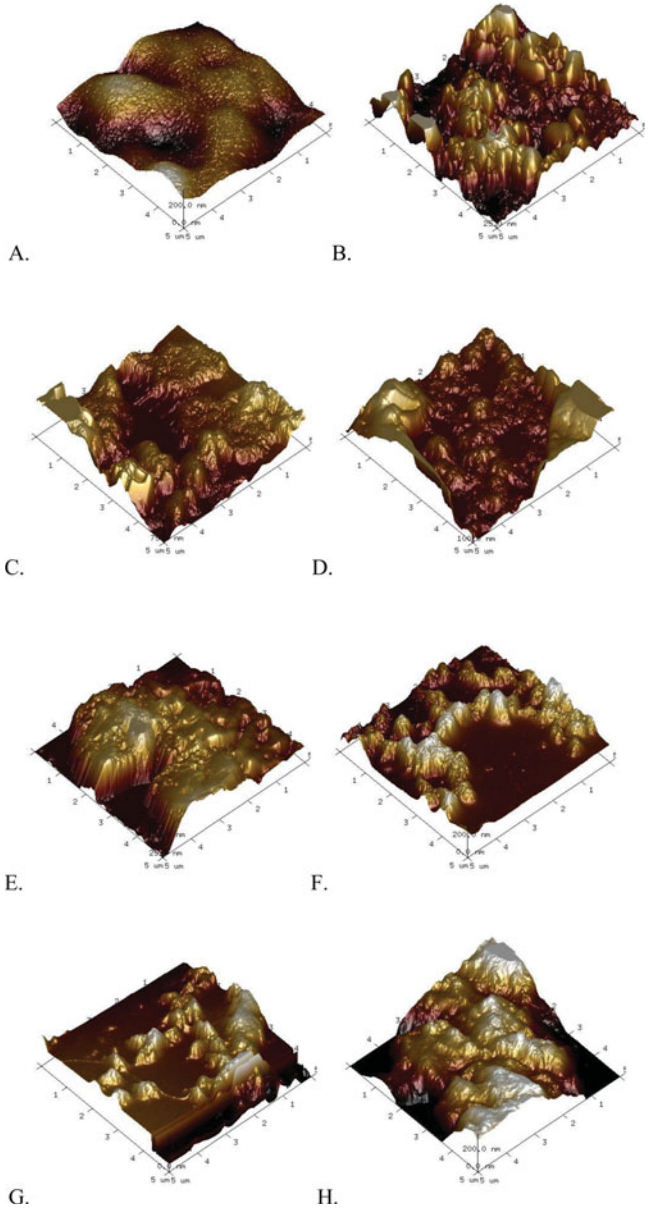

Figure 2. AFM images of the surface of A.COL and films made of CTS/HA (50/50) blend containing different addition of collagen: B.10%, C.20%, D.30%, E.40%, F.50%, G.60%, H.80%.

Table 4. The roughness parameters (R_q) of COL and three component blends.

w_{COL}	R_q (nm)
0.1	143
0.2	156
0.3	179
0.4	123
0.5	60
0.6	72
0.8	160
1.0	67

Table 5. Values of interaction parameters for CTS/HA blends, (w_{CTS} – weight fraction of chitosan).

w_{CTS}	$[\eta]_m^{exp}$ [dl/g]	$[\eta]_m^{id}$ [dl/g]	$\Delta[\eta]$	b_m^{exp} $[dl/g]^2$	b_m^{id} $[dl/g]^2$	Δb_m
0.2	2.29	3.14	– 0.84	4.00	9.13	– 5.13
0.5	2.57	3.33	– 0.76	9.39	5.16	4.23
0.8	2.99	3.52	– 0.52	6.73	4.92	1.82

studies we have shown that the surface of hyaluronic acid film is rather flat and does not contain any symptom of surface roughness when the HA was dissolved in $0.1 mol\cdot dm^{-3}$HCl solution or in $0.01 mol\cdot dm^{-3}$HCl solution (Figure 1B). After the addition of chitosan, the surface of film is altered with visible roughness. The surface roughness increases with increasing contents of chitosan in the blend films but it is smaller than for chitosan film (Figure 1 and Table 3). The roughness of film made of chitosan is rather big and results from the crystallinity of the sample. For the ternary blend films, the surfacesare slightly more folded than the surfaces of binary CTS/HA blends. The values of the roughness of the CTS/HA blend with 50 and 60% of collagen are the lowest among studied specimens (Table 4). This may indicate an increase in the homogeneity of these blends in comparison with other compositions.

3.3. Viscosity measurements

In recent years, viscometric method has been widely used to study the polymer – polymer interaction and miscibility because this technique is a simple, quick and an inexpensive [25, 26, 29, 30]. Many researchers proposed criteria to determine polymer-polymer miscibility by the viscometric method [32-36]. As published by Garcia et al. [33], the Δb_m parameter calculated by the equation: $\Delta b_m = b_m^{exp}$ - b_m^{id}, can be utilized to evaluate the presence of interactions. If $\Delta b_m > 0$, attractive forces are dominant which proves the miscibility of the components. If $\Delta b_m < 0$, repulsive forces prevail and immiscibility is expected. The parameters of the miscibility criterion proposed by Garcia et al. [33] are tabulated in Tables 5–6 and Figure 3. As it can be observed, for CTS/HA blend solutions the parameter Δb_m values are positive with the exception of the composition of low content of chitosan in the blend ($w_{CTS} = 0.2$). Thus, it can be suggested that the CTS/HA blends are miscible at the weight fraction of chitosan in the blend $w_{CTS} \geq 0.5$ as $\Delta b_m > 0$ and $\Delta[\eta] < 0$. The miscibility criteria are satisfied.It is in accordance with previously reported data for the solutions of CTS/HA blends [25]. As it can be observed in Table 6 and Figure 3 for the ternary CTS/HA/COL blends, the Δb_m values are positive for $w_{COL} \leq 0.2$ and are negative for the weight fraction of collagen above 0.2, especially

Table 6. Values of interaction parameters for the CTS/HA (50/50) blend with different addition of collagen, ($w_{CTS/HA}$ – weight fraction of 50/50 blend; w_{COL} – weight fraction of collagen).

w_{COL}	$w_{CTS/HA}$	$[\eta]_m^{exp}$ [dl/g]	$[\eta]_m^{id}$ [dl/g]	$\Delta[\eta]$	b_m^{exp} $[dl/g]^2$	b_m^{id} $[dl/g]^2$	Δb_m
0.0	1.0	2.95	—	—	3.97	—	—
0.1	0.9	3.54	4.30	– 0.76	12.98	5.29	7.69
0.2	0.8	3.89	5.64	– 1.74	14.18	10.85	3.33
0.3	0.7	4.11	6.98	– 2.86	12.27	20.65	– 8.38
0.4	0.6	6.13	8.32	– 2.19	29.73	34.69	– 4.96
0.5	0.5	7.11	9.66	– 2.55	48.87	52.97	– 4.10
0.6	0.4	7.45	11.00	– 3.55	47.90	75.48	– 27.58
0.8	0.2	9.23	13.68	– 4.45	82.53	133.21	– 50.68
1.0	0	16.37	—	—	207.90	—	—

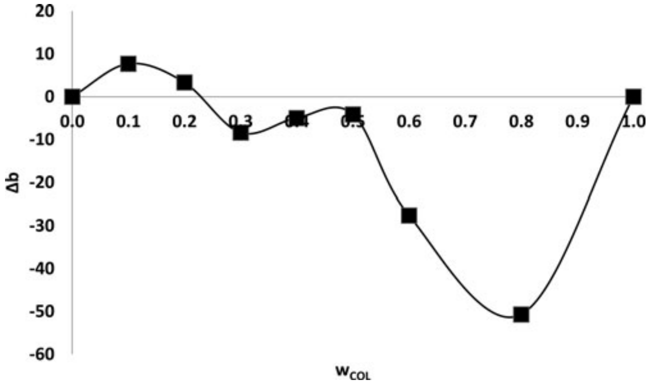

Figure 3. Values of viscosity interaction parameters, Δb_m, versus weight fraction of collagen (w_{COL}) in three component blends.

for the blends with the high content of collagen. So, according to the used criteria of polymer–polymer miscibility, the ternary blends are partially miscible at the low content of collagen.

4. Conclusion

Films prepared by blending chitosan with hyaluronic acid and collagen in different compositions were used to study the wettability and surface morphologies. Contact angle measurements and surface free energy determinations reveal that chitosan films are more polar when hyaluronic acid and collagen were added. Surface properties examined by tapping-mode atomic force microscopy (AFM) showed that the surface roughness of investigated blends increases with the increasingcontent of chitosan and collagen in the blend. For the blends of chitosan with hyaluronic acid and collagen in solution with different compositions, the miscibility was determined by viscometric method and it was found that the blends are partially miscible. Thus, the obtained results suggest that the modification of several properties of binary and ternary blends is a consequence of the interaction between chitosan and hyaluronic acid and collagen.

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References

[1] Jayakumar, R., Menon, D., Manzoor, K., Nair, S.V., & Tamura, H. (2010). *Carbohydr. Polym.*, *82*, 227.
[2] Tian, H., Tang, Z., Zhuang, X., Chen, X., & Jing, X. (2012). *Prog. Polym. Sci.*, *37*, 237.
[3] Sionkowska, A., Lewandowska, K., Michalska, M., & Walczak, M. (2016). *J. Mol. Liq.*, *215*, 323.
[4] Sionkowska, A. (2011). *Prog. Polym. Sci.*, *36*, 1254–1276.
[5] Nair, L.S, & Laurencin, C.T. (2007). *Prog. Polym. Sci.*, *32*, 762.
[6] Armentano, I., Dottori, M., Fortunati, E., Mattioli, S., & Kenny, J.M. (2010). *Polym. Degrad. Stabil.*, *95*, 2126.
[7] Sionkowska, A., Wiśniewski, M., Skopińska, J., Kennedy, C.J., & Wess, T.J. (2004). *Biomaterials*, *25*, 795.
[8] Chandra, R., & Rustgi, R. (1998). *Prog. Polym. Sci.*, *23*, 1273.

[9] Agboh, O.C, & Qin, Y. (1997). *Polym. Advan. Technol.*, *8*, 355.

[10] Jang, M., Kong, B., Jeong, Y., Lee, C.H., & Nah, J. (2004). *J. Polym. Sci.: Part A: Polym. Chem.*, *42*, 3423.

[11] Dutta, P.K., Dutta, J., &Tripathi, V.S. (2004). *J. Sci. Ind. Res.*, *63*, 20.

[12] Rinaudo M. (2006). *Prog. Polym. Sci.*, *31*, 603.

[13] Segura, T., Anderson, B.C., Chung, P.H., Webber, R.E., Shull, K.R., & Shea, L.D. (2005), *Biomaterials*, *26*, 359.

[14] Schante, C.E., Zuber, G., Herlin, C., & Vandamme, T.F. (2011). *Carbohyd. Polym.*, *85*, 469.

[15] Kenne, L., Gohil, S., Nilsson, E.M., Karlsson, A., Ericsson, D., Kenne, A.H., & Nord, L.I. (2013). *Carbohyd. Polym.*,*91*, 410.

[16] Collins. M.N., & Birkinshaw, C. (2013). *Carbohyd. Polym.*, *92*, 1262.

[17] Fakhari, A., & Berkland, C. (2013). *ActaBiomater.*, *9*, 7081.

[18] Price, R.D., Berry, M.G., & Navsaria, H.A. (2007). *J.Plast.Reconstr.Aesthet.Surg.*, *60*, 1110.

[19] Kakehi, K., Kinoshita, M., & Yasueda, S. (2003). *J. Chromatogr.: B*, *797*, 347.

[20] Monheit, G.D., & Coleman, K.M. (2006). *Dermatol. Ther.*, *19*, 141.

[21] Parenteau-Bareil, R., Gauvin, R., & Berthod, F. (2010). *Materials*, *3*, 1863.

[22] Silvipriya, K.S., Krishna Kumar, K., Bhat, A.R., Dinesh Kumar, B., John, A., & Lakshmanan, P. (2015). *J. Appl. Pharm. Sci.*, *5*, 123.

[23] Meyers, M.A., Chen, P., Lin, A.Y., & Seki, Y. (2008). *Prog.Mat. Sci.*, *53*, 1.

[24] Ferreira, A.M., Gentile, P., Chiono, V., & Ciardelli, G. (2012). *Acta Biomater.*, *8*, 3191.

[25] Lewandowska, K., Sionkowska, A., & Grabska, S. (2015). *J. Mol. Liq.*, *212*, 879.

[26] Lewandowska, K. (2013). *J. Solut. Chem. 42*, 1654.

[27] Sionkowska, A., & Płanecka, A. (2013). *J. Mol. Liq.*, *186*, 157.

[28] Huggins, M.H. (1942). *J. Am. Chem. Soc.*, *64*, 2716.

[29] Sionkowska, A., Lewandowska, K., & Płanecka, A. (2014). *J. Mol. Liq.*, *189*, 354.

[30] Lewandowska, K., Sionkowska, A., & Krasińska, K. (2014). *Progress on Chemistry and Applications of Chitin and Its Derivatives*, *19*, 73.

[31] Owens, D.K., & Wendt, R.C., (1969). *J. Appl. Polym. Sci.*, *13*, 1741.

[32] Krigbaum, W.R., & Wall, F.T. (1950).*J. Polym. Sci.*, *5*, 505.

[33] Garcia, R., Melad, O., Gómez, C.M., Figueruelo, J.E., & Campos, A. (1999). *Eur. Polym.J.*, *47*, 35.

[34] Catsiff, E.H., & Hewett, W.A.(1962). *J. Appl. Polym. Sci.*, *6*, S30.

[35] Chee, K.K. (1990).*Eur. Polym. J.*, *26*, 423.

[36] Pingping, Z., Haiyang, Y., & Shiqiang, W. (1998). *Eur. Polym. J.*, *34*, 91.