



STUDIES ON SUPERABSORBENTS MODIFIED WITH TREHALOSE

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Abstract

Recently, trehalose becomes more and more popular compound. Increasing interest in this disaccharide results from the role as it starts to play in water economy of plants. In conditions of water shortage mentioned organic substance prevents their dehydration. Therefore, application of such interesting additive can contribute to the preparation of materials that can be applied in such areas as agriculture or cultivation of plants. It is also worth mentioning that properties of trehalose make this substance interesting from the medical point of view. Presented additive can have an impact on maintaining organs for transplantation in good condition. In presented research series of polymer superabsorbents modified with described disaccharide have been prepared. Obtained by means of photopolymerization materials differed in an amount of the additive. In the further step, physicochemical properties of superabsorbents have been determined. Following studies have been carried out: swelling studies and incubation studies in simulated body fluids aimed at determination of behavior of prepared materials in solutions similar to those one occurring in human body. Additionally, chemical structure of the hydrogels have been defined using spectroscopic technique. Based on the research it can be concluded that proposed materials are characterized by low sorption capacity and did not have significant impact on simulated body fluids. What is more, prepared hydrogels did not degrade significantly in the mentioned fluids – such conclusion is possible due to the fact that spectra of the tested materials before and after incubation studies obtained

using FT-IR spectroscopy differed slightly from each other. Presented superabsorbents seem to be interesting materials that can be subjected to more advanced studies in view of their future medical application.

Keywords: Superabsorbents, trehalose, agriculture, water economy of plant, swelling

INTRODUCTION

The first industrial-scale hydrogels were obtained by Wichterle and Lim in 1960. The mentioned scientists received the contact lenses using hydroxyethyl-methacrylate. Since then, hydrogel materials have enjoyed a great deal of interest. These polymers are most commonly used in realms such as medicine, pharmacy and increasingly in tissue engineering. In addition to medical applications, above-described materials are also widely used in agriculture and horticulture. In these realms of industry hydrogels are useful in preparation of special water or fertilizer release systems. However, in recent times, the most focused application of hydrogels is their use in the production of tissues as well as in the future in the preparation of the artificial organs, which will lead to a significant development of transplantology (Pluta and Karolewicz 2005).

The wide use of hydrogels in medicine is determined by their unique properties such as biocompatibility, biodegradability, non-toxicity and mechanical strength. What is also important, these materials are characterized by a capability of release of molecules from their interior that allows them to be used in drug delivery systems (Tylińczak and Pielichowski 2007). Trehalose is a naturally occurring disaccharide, which has a variety of properties (Feofilovaa et al. 2014). This organic compound protects against dampness which may be useful in many industries including food industry. Furthermore, trehalose is a substance that helps to survive the extreme conditions of plants and animals living in a demanding climate by providing them a suitable living environment. Introduction of this disaccharide into the hydrogel matrix can result in a very interesting combination of materials that can be used in many realms such as transplantology or agriculture (Thakur et al. 2017; Richards et al. 2002; Ohtake and Wang 2011; Stefanello et al. 2018; Burek et al. 2017; Thambi et al. 2017; Zheng et al. 2017).

Main purpose of presented research is preparation and characterization of hydrogels based on chitosan and modified with trehalose. Research involves studies on sorption capacity of obtained hydrogels, incubation studies in simulated body fluids and characterization of chemical structure of the prepared materials using spectroscopic technique.

MATERIAL AND METHODS

The first step involved a preparation of a solution of gelatin and chitosan in acetic acid – such mixture was subsequently defined as ‘base solution’. Adequate quantities of the previously prepared aqueous solution of trehalose were then introduced into the appropriate volume of the base solution. Then, crosslinking agent and photoinitiator were introduced, and the resulting solution was poured down into a previously prepared Petri dish. Such prepared mixture was then subjected to the process of photopolymerization. The source of the UV radiation was the following lamp: EMITA VP-60. Reaction time was about 2 minutes. After this time, the resulting material was removed from the radiation source, dried at room temperature, and its samples were targeted for further investigation. Compositions of synthesized polymers are presented in **Table 1**.

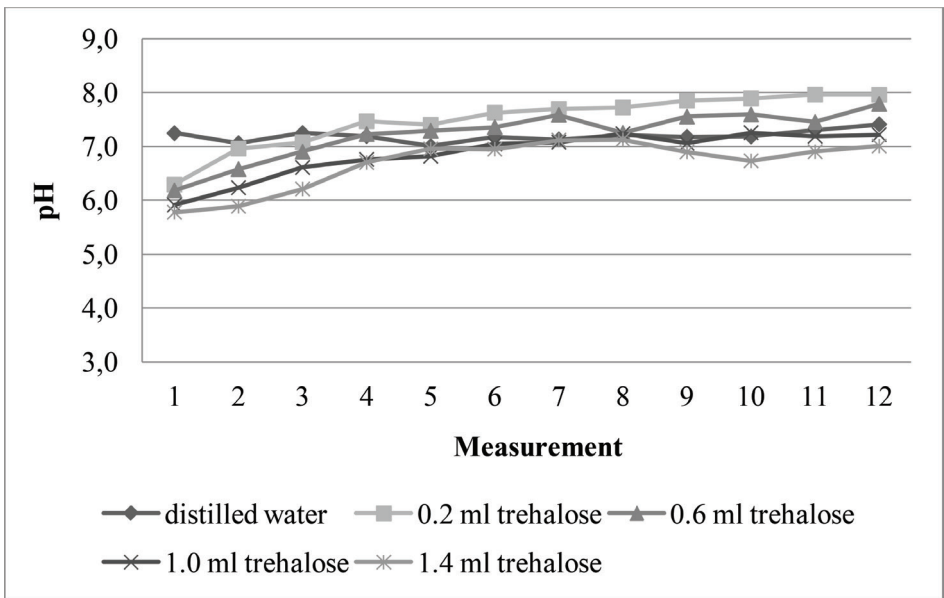
Table 1. Compositions of hydrogels modified with trehalose.

Sample	Base solution [ml]	3% trehalose solution [ml]	Crosslinking agent [ml]	Photoinitiator [ml]
1.	10	0.0	1.6	0.25
2.	10	0.2	1.6	0.25
3.	10	0.6	1.6	0.25
4.	10	1.0	1.6	0.25
5.	10	1.4	1.6	0.25

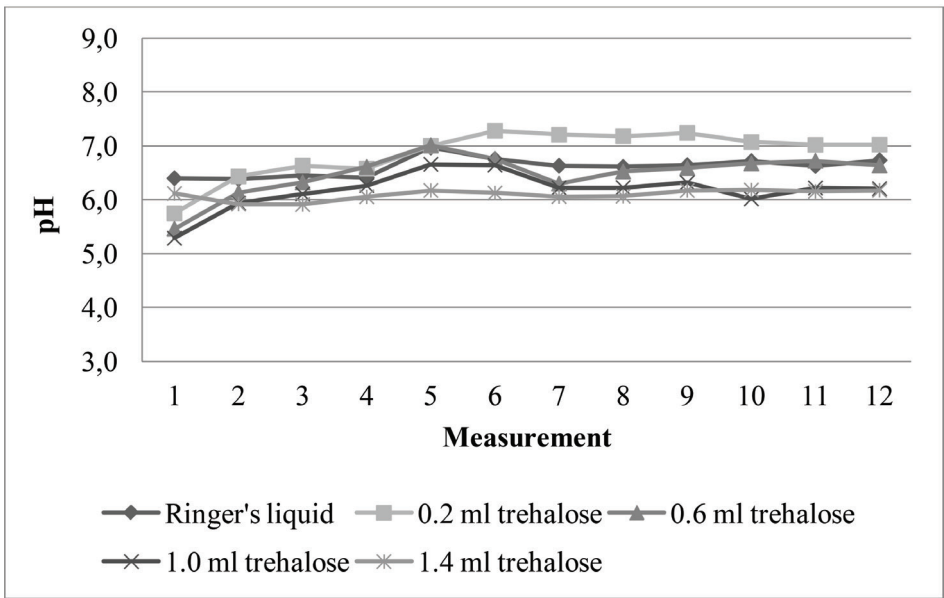
RESULTS AND DISCUSSION

Incubation studies

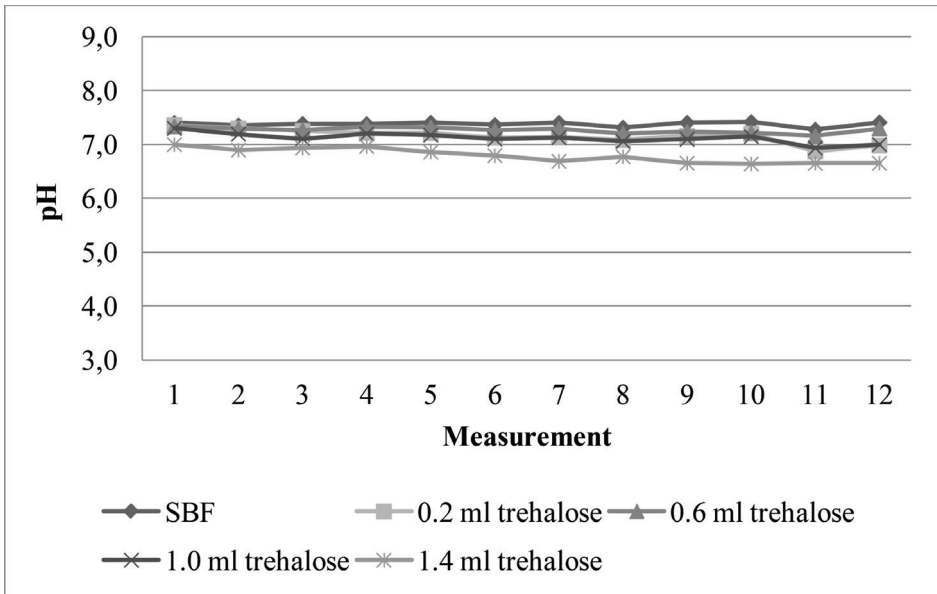
Due to the potential use of prepared materials for biomedical purposes it is necessary to define their impact on simulated body fluids therefore main purpose of incubation studies is to determine the behavior of modified hydrogels in such fluids. Furthermore, it is important to check whether the resulting polymer materials have a tendency to degradation in an environment which composition is similar to the composition of fluids present in the human body. The study was carried out as follows: hydrogels' samples were introduced to the tested fluid for a period of 30 days during which pH of every solution was regularly monitored. Incubation was conducted at a temperature of 37°C. The results of the pH measurements are shown in **Figure 1**.



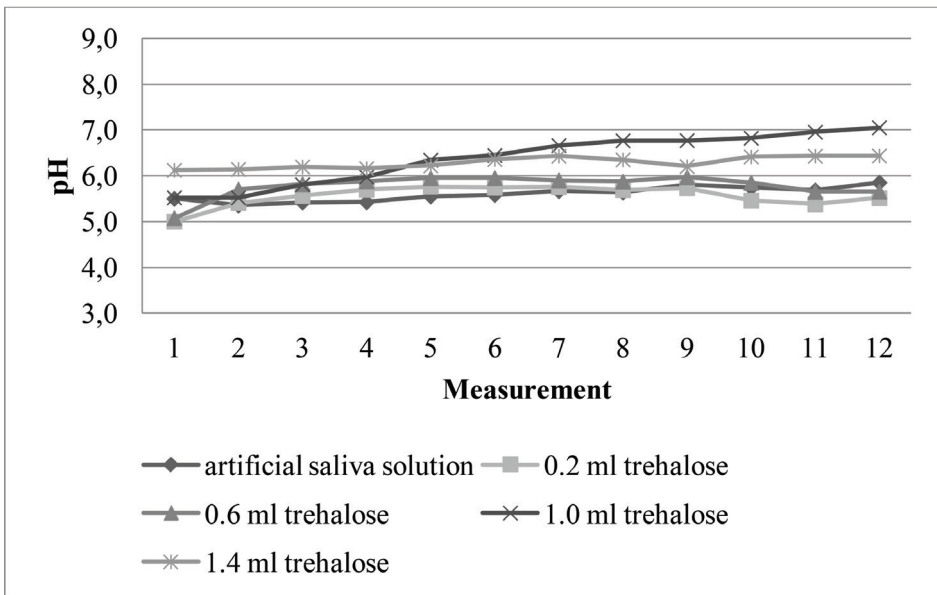
a)



b)



c)



d)

Figure 1. Results of pH measurements in: a) distilled water, b) Ringer's liquid, c) artificial saliva solution and d) simulated body fluid (SBF).

Based on the results of incubation tests it can be stated that prepared hydrogels are not degraded. Moreover, probably hydrogel materials introduced into the selected liquids do not negatively affect these simulated fluids, which can be confirmed by the fact that pH of the solutions maintain at a similar level through all incubation period. It can therefore be concluded that trehalose introduced into the hydrogel polymers sufficiently stabilizes their structure and as a result body fluids have a limited ability to penetrate into the interior of the tested material. Hence, process of elution of unreacted reagents that could affect the rapid changes in the pH of the solutions in which the incubation takes place is not observed. Moreover, it should be noticed that pH values in solution such as SBF and artificial saliva are less divergent in comparison to the values measured for distilled water and in Ringer's fluid. This difference is due to the presence of large amount of ions compared to the first mentioned solutions. This influences the formation of additional cross-links between the polymer chains, which in turn increases the cross-linking degree of the tested material. The high cross-linking degree of the material impedes the diffusion of fluid into its interior and limits a possible elution of unreacted reagents that would contribute to the numerous pH fluctuations. Therefore, lower changes of pH are observed for SBF and artificial saliva compared to the values measured for distilled water and Ringer's fluid.

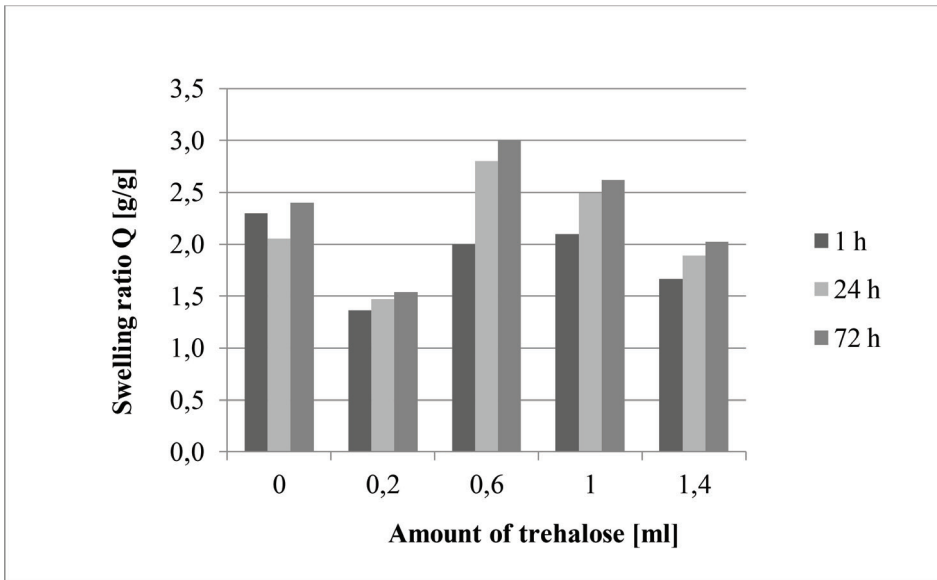
Study of swelling capability

Superabsorbents are well known from their great sorption capacity therefore swelling capability of obtained materials have been characterized. For this purpose, dry samples of hydrogels were weighed and then placed in the following solutions: artificial saliva solution, distilled water, Ringer's fluid and simulated body fluid (SBF). Swelling ability was determined by calculation of the swelling ratios Q using the formula:

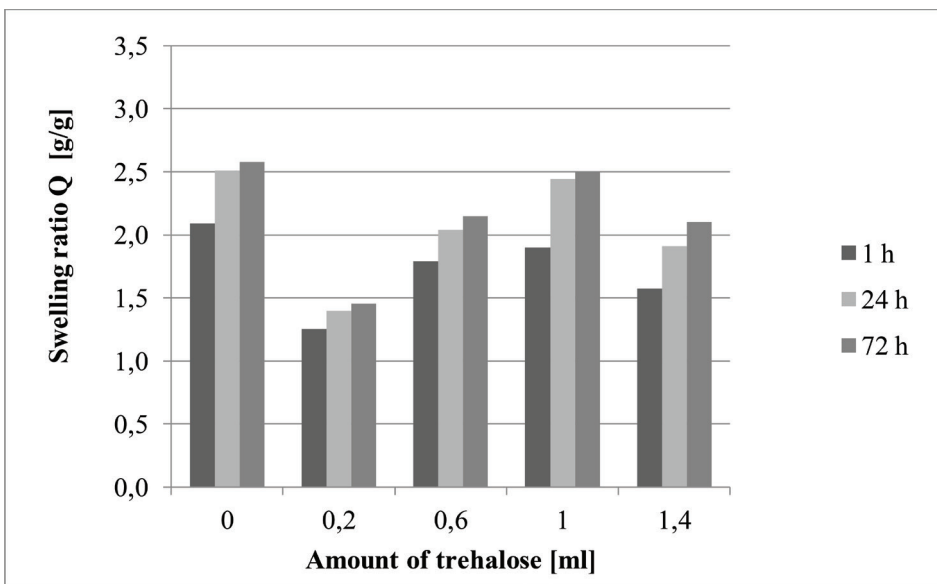
$$Q = \frac{W_1 - W_0}{W_0},$$

where: W_1 – weight of swollen hydrogel [g], W_0 – weight of dry hydrogel [g].

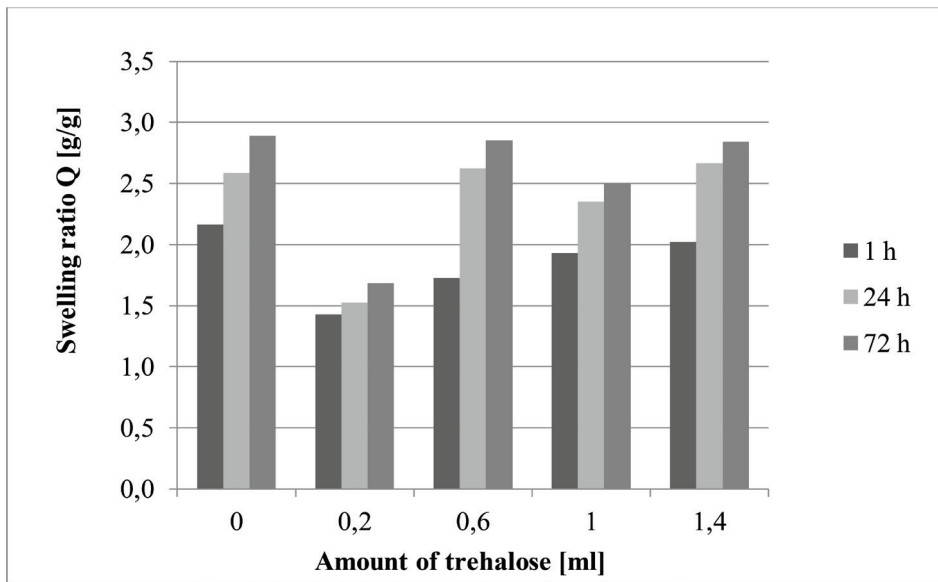
Hydrogels' samples were weighed after 1, 24 and 72 h of swelling. Results presenting sorption capacity of prepared hydrogel materials are shown in **Figure 2**.



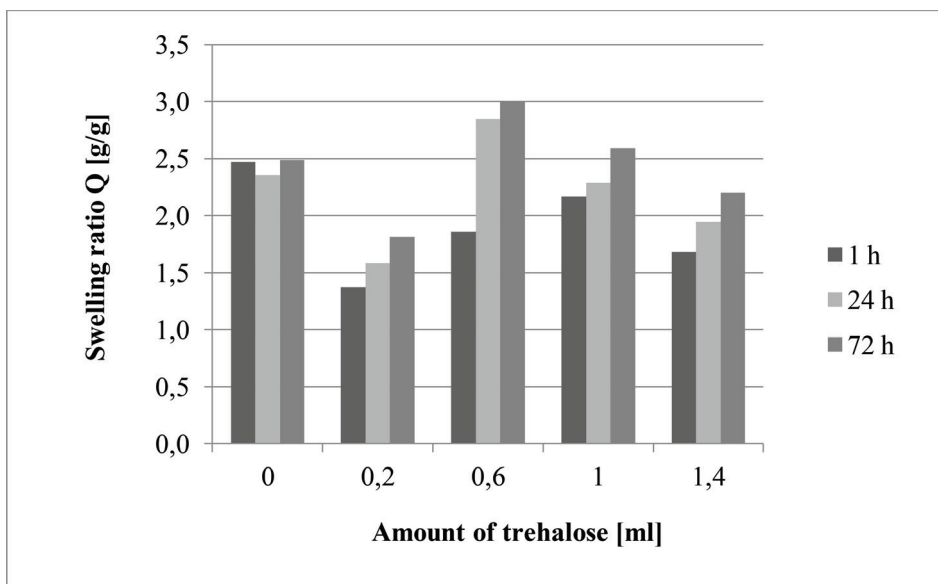
a)



b)



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d)

Figure 2. Results of swelling studies of hydrogels in: a) distilled water, b) Ringer's liquid, c) artificial saliva solution and d) SBF.

By analyzing the results of swelling tests it can be stated that obtained hydrogels are characterized by low sorption capacity. The increase in swelling ratio is on average 0.15 – 0.2 between 24 and 72 h. The highest values of the mentioned ratio are about 3.0 [g/g], which is a very low result, considering the most characteristic feature of hydrogels which undoubtedly is great swelling ability. The type of solution which is absorbed by hydrogel sample has an important impact on its swelling ability. Based on the above-presented graphs it can be concluded that swelling ratios of hydrogels' samples tested in SBF as well as in the artificial saliva solution are characterized by similar values compared to the values of these ratios calculated for materials swelling in two other tested solutions. Such conclusion concerning the similarity in behavior of tested materials in solutions such as artificial saliva and SBF reminds the observation drawn from the results of pH measurements during incubation studies. Mentioned likeness involves the presence of greater amount of ions in the first two solutions. The ions cause an increase the crosslinking degree of hydrogels by formation of additional bonds between polymer chains and thereby limit the penetration of liquids into the interior of the hydrogel. Hence mentioned similar results and very small sorption capacity. Another factor affecting such results is the presence of trehalose in the hydrogel matrix. One of the most interesting feature of this organic compound is the prevention of moisture/water ingress which is useful in many areas including food industry (with special emphasis on confectionery products). Limited sorption ability may also be due to the formation of hydrogen bonds between a disaccharide present in hydrogel matrix and functional groups occurring in polymer chains. Such phenomenon results in the fact that hydrogel three-dimensional network becomes more compact and therefore a very little space is available which could be filled by absorbed fluid. Due to such observations it can be stated that it is possible to modify swelling ability of hydrogels by introduction into them materials such as trehalose. This is an important information because swelling ability of hydrogel is related also with its ability to release active substance from its interior therefore such modification is significant in view of application of proposed materials as drug carriers.

Spectroscopic analysis

Spectroscopic studies were carried out in order to define the chemical structure of the prepared materials as well as to determine the impact of 30 day incubation in simulated body fluids on this structure. The research was also performed to verify whether the samples will degrade in the tested environments. Analysis was conducted using the following apparatus: Spectro-Lab Nicolet iS5. Received spectra are shown in **Figure 3**. and in **Figure 4**.

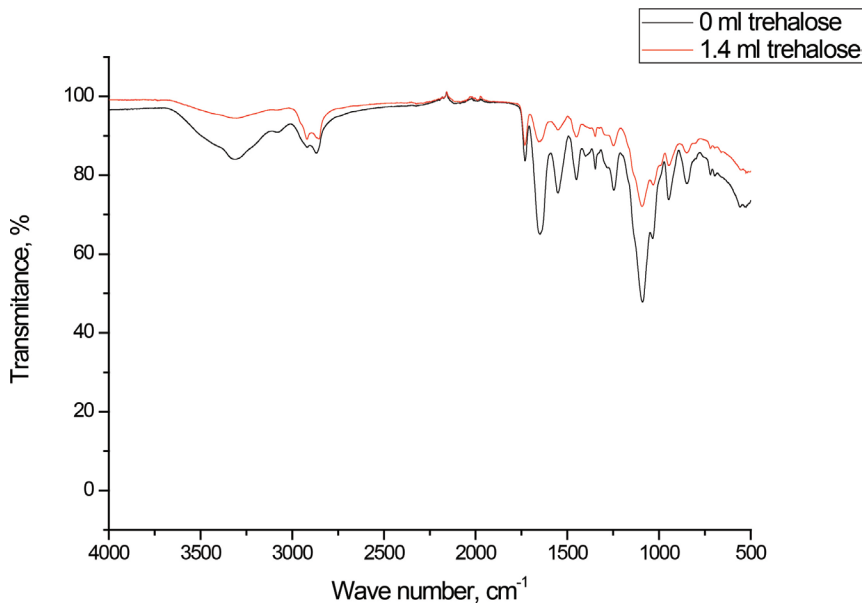


Figure 3. Comparison of the structure of hydrogels with the lowest and the highest amount of trehalose.

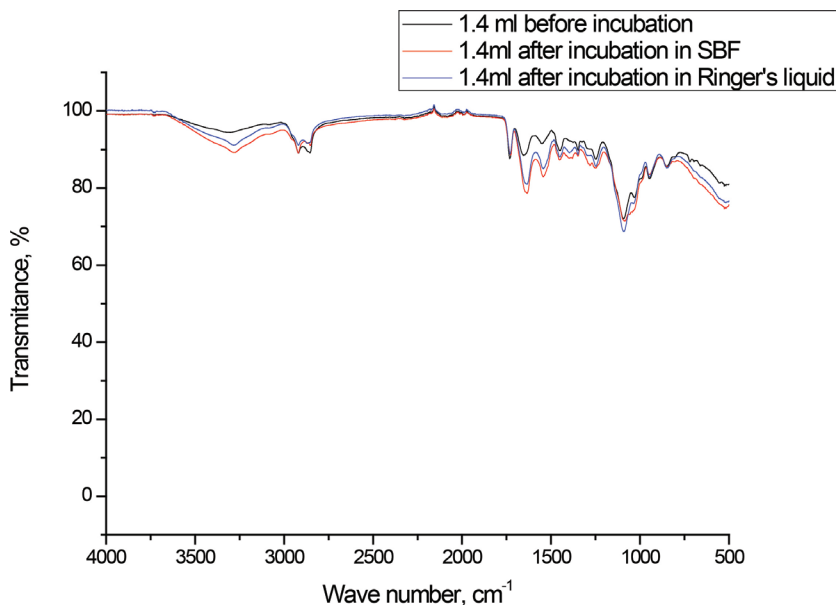


Figure 4. FT-IR spectra of hydrogels presenting an impact of incubation on the structure of the tested materials.

The performed spectroscopic research allowed the identification of the particular functional groups present in the tested hydrogel polymers as well as the defining an impact of incubation studies on their chemical structure. As it can be seen on the obtained spectra all samples are characterized by a presence of a band in the wavelength range 3280-3250 cm deriving from the hydroxyl group and the hydrogen interactions. The bands corresponding to the mentioned groups may derive both from the components of hydrogel matrix as well as from the additive introduced into this matrix – trehalose, which is characterized by the presence of a large number of functional groups in its chemical structure. Moreover, peaks in the range 3000-2980 cm^{-1} that are characteristic for C-H (vinyl or aryl) bonds also can be observed. Subsequently, vibrations characteristic for the ester group (1750-1740 cm^{-1}) and the carbonyl group (1100-1000 cm^{-1}), which may be derived from trehalose but also from chitosan can also be noticed (Silverstein et al. 2005). Comparing the vibrations of the tested samples before and after incubation in selected liquids it can be stated that vibrations deriving from a sample being incubated are more intense. Therefore, it can be concluded that prepared hydrogels did not have a significant tendency to degradation during incubation in the selected fluids. However, in case of bands deriving from samples after incubation slightly less intensity can be noticed that indicates a slight degradation. It is worth mentioning that more intense bands were observed for hydrogels containing greater amount of the additive in the matrix. Based on obtained spectra it can be noticed that the most visible peaks occur for samples modified with 1.4 ml of trehalose solution. Therefore, it can be concluded that trehalose reduces the degradation of the tested hydrogels. The lower degree of degradation of these samples as a result of incubation demonstrates that the addition of trehalose causes an increase of the stability of the hydrogel material. Such information is important in view of the future application of such materials because in many cases rapid degradation is not a desirable phenomenon.

SUMMARY

Series of chitosan based hydrogels which were additionally modified with different amount of trehalose solution was synthesized by means of photopolymerization process. Next part of the research involved studies aimed at determining the physicochemical properties of the obtained materials. For this purpose incubation studies, study of swelling capability as well as spectroscopic analysis were performed. Based on the results of the mentioned studies it can be concluded that the resulting hydrogels exhibit low swelling ability, wherein any significant impact of trehalose on this property was not observed. However, some differences in the sorption capacity of tested polymers in particular fluids were noticed. This is probably related to the chemical composition of these solutions,

because the presence of various types of ions in the fluid in which swelling takes place affects the crosslinking density of the introduced material. Spectroscopic analysis confirmed the presence of functional groups characteristic for both substrates forming the hydrogel matrix as well as for the trehalose in the material being examined. Samples subjected to the incubation tests did not negatively affect the simulated body fluids and did not significantly degrade. However, due to the lower intensity of the bands deriving from the material being incubated, it should be noticed that the tested materials slightly degraded. What is essential, it can be found that hydrogels containing a greater amount of the additive were less affected by the mentioned process. It can therefore be stated that the trehalose introduced into the hydrogel matrix stabilizes the structure of these polymers. Furthermore, such additive limits the penetration of body fluids into the material and hinders elution of unreacted reagents from the interior of the hydrogel.

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