



SYNTHESIS OF BEES' CHITOSAN BASED HYDROGELS CONTAINING ALOE VERA

Sonia Kudlacik-Kramarczyk, Anna Drabczyk, Bożena Tylińczak
Cracow University of Technology

Abstract

Chitosan based hydrogels due to their specific properties and structure belong to the group of materials widely used in medicine. They are used as drug carriers with controlled release of active substances and in the preparation of innovative wound dressings. The main aim of the conducted research was the choice of the most favorable parameters for carrying the cross-linking reaction by means of UV radiation and microwaves. Subsequently, an adequate composition of the mixture containing chitosan and gelatin for further modification of such prepared the polymer matrix with *Aloe vera* extract was selected. Subsequently, an impact of introduction of *Aloe vera* extract into hydrogel on its physicochemical properties and structure was determined. The scope of the research included the measurement of swelling abilities of hydrogels in distilled water and selected simulated body fluids as well as incubation studies aimed at determination of tendency of prepared materials to degradation in previously mentioned fluids and their compatibility in relation to such environments. In order to characterize the chemical structure of the polymers and impact of incubation on this structure spectroscopic analysis was performed. Microscopic analysis was used to define the surface morphology of the hydrogels. Moreover, materials containing *Aloe vera* extract were subjected to the studies of release of additive from hydrogel matrix. Research was conducted both in acid and alkaline environment.

Keywords: Beetosan, chitosan, hydrogels, Aloe vera, bees, waste management

INTRODUCTION

Polymers of natural origin such as polysaccharides or proteins are increasingly used as components in the preparation of hydrogels. This is mainly due to their properties, that are desirable in such areas as medicine and pharmacy. Described materials are biocompatible in relation to the human body, non-toxic and susceptible to enzymatic degradation. Chitosan belongs to the group of natural polymers. Mentioned polysaccharide becomes more and more popular in the production of hydrogels.

Hydrogels on the basis of this organic compound are characterized by many favorable properties that make them useful in cosmetics. These polymers firm and moisturize the skin as well as stimulate its regeneration by a process of release the active substances from the polymer matrix. Moreover, they are microbiologically stable up to 6 months without the need for introduction of additives such as preservatives. Hydrogels based on chitosan exhibit antimicrobial activity and are compatible with most active substances used in the pharmaceutical and cosmetic industry. Furthermore, they are used primarily for the preparation of controlled drug delivery systems and dressing materials.

Aloe vera juice, which can be used as a modifier for hydrogel materials is not only characterized by healing properties, but also anti-aging, so it is widely used in cosmetology. It is also used as a component of cosmetic preparations used mainly for sensitive skin, acne as well as for the care of all types of hair. Extract from *Aloe vera* has anti-inflammatory and disinfecting properties. It maintains skin moisture, makes it firmer and, moreover, protects it from harmful sunlight.

MATERIAL AND METHODS

Research included preparation of Beetosan® and commercial chitosan based hydrogel polymers modified with extract from *Aloe vera*. The main objective was to determine impact of Beetosan® on the structure and physicochemical properties of such prepared materials. Beetosan® was obtained from naturally died honeybees that do not survive difficult weather conditions and constituted a waste. Precise compositions of prepared polymers are presented in **Table 1**.

Prepared mixtures were subjected to the cross – linking reaction using UV radiation for 2 min (by means of the following lamp: EMITA VP 60).

Subsequently, the following characteristics of obtained materials were determined:

- sorption capacity
- surface morphology using Scanning Electron Microscopy technique (SEM)

- chemical structure and the presence of characteristic functional groups by means of Fourier Transform Infrared (FT-IR) spectroscopy
- possibility of release of modifying agent from the material's interior.

Table 1. Compositions of prepared hydrogels modified with extract from *Aloe vera*.

Próbka	Chitosan/ Beetosan® 3% v/v [ml]	Chitosan 3% v/v [ml]	Gelatine 2% v/v [ml]	Photoinitiator* [ml]	Crosslinking agent** [ml]	Extract from <i>Aloe vera</i> [ml]
1.	37.5	-	12.5	0.25	12	-
2.	37.5	-	12.5	0.25	12	10
3.	-	37.5	12.5	0.25	12	-
4.	-	37.5	12.5	0.25	12	10

* (2-hydroxy-2-methylpropiophenone (Darocur 1173))

** (diacrylate poly(ethylene glycol); $M_w=575$)

RESULTS AND DISCUSSION

Studies on swelling sorption

Studies on swelling ability of hydrogels modified with extract from *Aloe vera* were carried out in distilled water, acetate buffer (pH = 4.65), phosphate buffer (pH = 7.4) and in hemoglobin of animal origin. Swelling sorption was defined by means of swelling ratio Q that was calculated from the formula:

$$Q = \frac{m_2 - m_1}{m_1}$$

where: Q - swelling ratio [g/g], m_1 - weight before study [g], m_2 - weight after study [g].

Results of the research are shown in **Figure 1**.

Based on the above-presented graphs it can be stated that the obtained hydrogels exhibit swelling ability in each of the tested solutions. The highest degree of sorption can be observed for samples tested in an acetate buffer. Such phenomenon results from the fact that in acidic environment a strong protonation of amino groups present in the structure of chitosan occurs and causes the formation of NH_3^+ ions. Then, the mentioned ions repel each other and the structure of hydrogel polymers becomes less compact. This, in turn, results in better sorption capacity of the tested materials. What is also important, results of conducted studies clearly show that the greatest jump in absorption capacity is visible after first 24 h of the research. On the other hand, after 72 h of studies only minor changes of swelling ratios in comparison to their values calculated after 1 h were

observed. On the basis of the research it can also be concluded that the addition of extract from *Aloe Vera* has a certain impact on sorption capacity of the tested materials – slight improvement of swelling ability is observed. This can be caused by the formation of hydrogen bonds between compounds within the extract from *Aloe vera* and the tested solution that results in increased swelling sorption. Based on the results it can be stated that introduction of such additive as *Aloe vera* juice did not affect in a very significant way the swelling ability of the tested hydrogels.

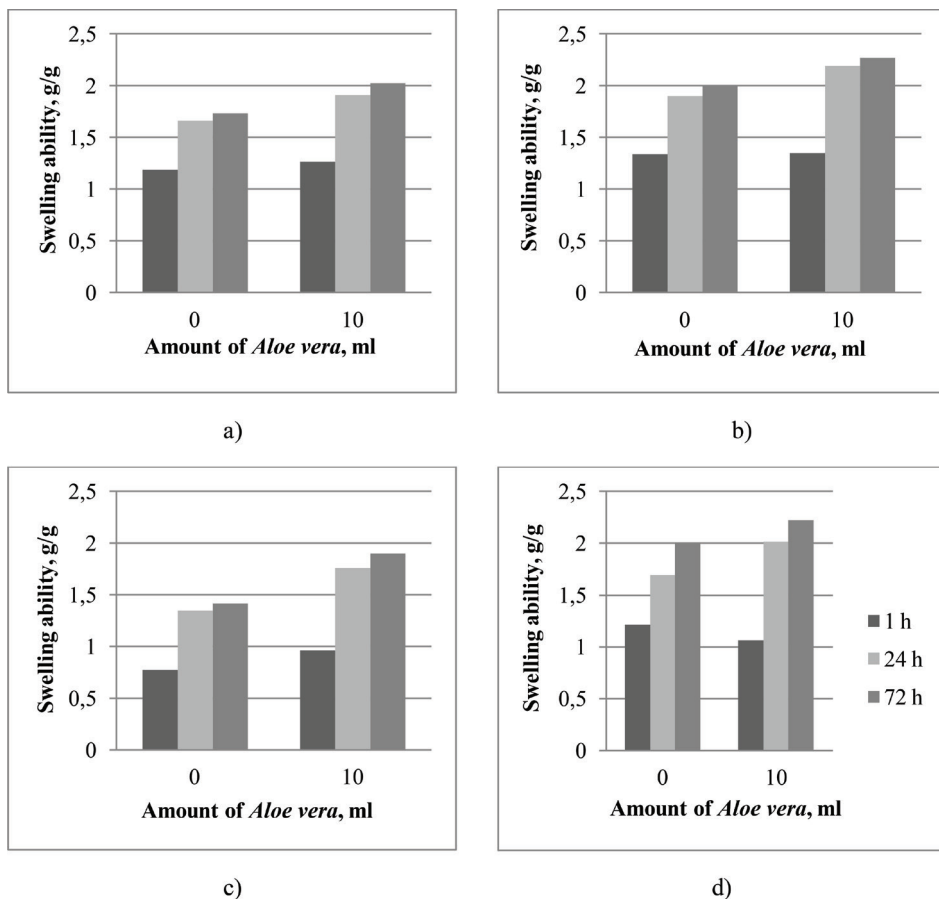


Figure 1. Swelling abilities of tested hydrogel materials in: a) distilled water; b) acetate buffer; c) phosphate buffer; d) hemoglobin after 1, 24 and 72 h.

Study of surface morphology using SEM analysis

The impact of application of Beetosan® on the surface morphology of the tested hydrogels was determined by means of Scanning Electron Microscopy. Obtained microphotographs of the polymers are shown in **Figure 2.** and **Figure 3.** Research was carried out using the following microscope FEI Helios NanoLab H50HP. SEM images were recorded at an accelerating voltage of 5 kV.

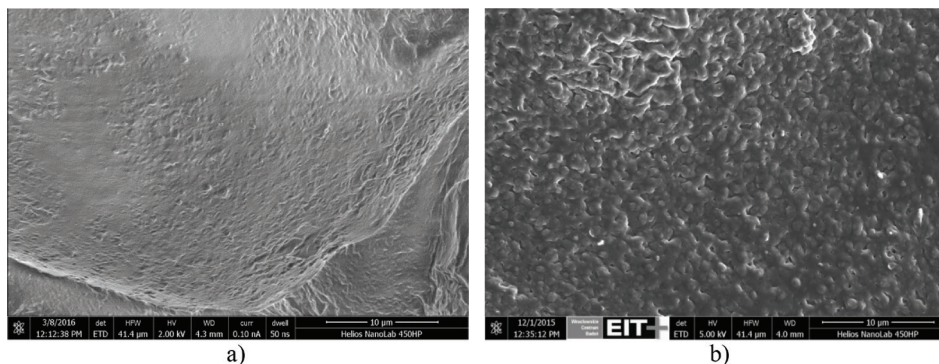


Figure 2. SEM images of hydrogels without extract from *Aloe vera* and based on: a) Beetosan® and b) commercial chitosan.

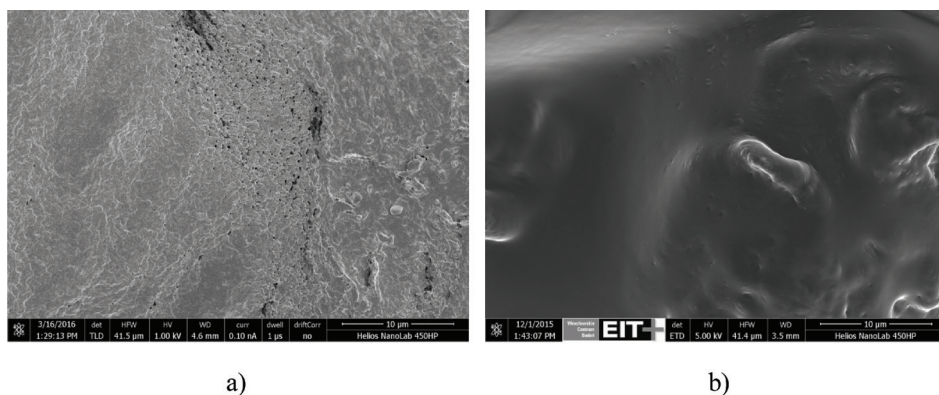


Figure 3. SEM images of hydrogels modified with 10 ml of extract from *Aloe vera* and based on: a) Beetosan® and b) commercial chitosan.

Based on the resulted SEM microphotographs it can be stated that the sample of Beetosan® based hydrogel is characterized by a lower porosity in comparison to the sample of hydrogel on the basis of commercial chitosan. Probably

this is due to the presence of a certain amount of chitin in Beetosan®. It should be mentioned that Beetosan® derives from the multistage chemical processing of died honeybees. Unlike chitosan, chitin is an insoluble compound and can penetrate into the pores of the hydrogel thus reducing its porosity.

By analyzing the SEM microphotographs it is possible to notice that *Aloe vera* penetrates the pores of the hydrogel on the basis of the commercial chitosan resulting in a decrease in its porosity. In case of sample of hydrogel based on Beetosan® the mentioned additive probably entered the pores of the tested hydrogel. As a result, unreacted chitin occurring in the hydrogel's pores deposited on the surface of the tested material, which can be visible on the SEM micrographs showing uneven surface of the hydrogels.

FT-IR spectroscopy

Prepared hydrogel materials based on Beetosan® and modified with *Aloe vera* extract were subsequently subjected to the structural analysis by means of the following apparatus: Nicolet iS5 Spectro-Lab. FT-IR spectra of the tested hydrogels are presented in **Figure 4**.

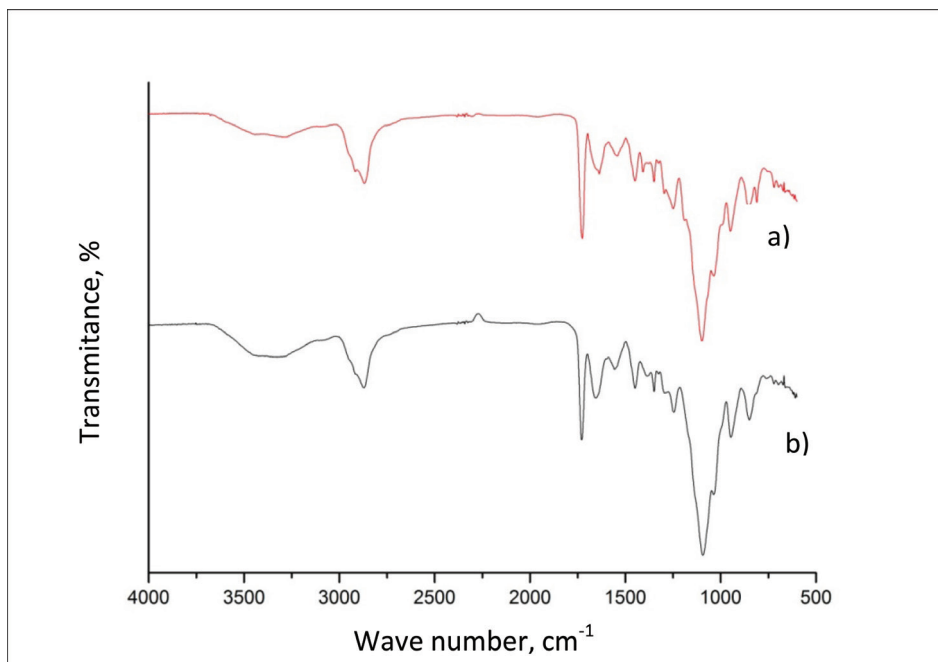


Figure 4. FT-IR spectra of Beetosan® based hydrogels: a) unmodified and b) containing 10 ml of *Aloe vera* extract.

Based on the correlation tables, analysis of obtained FT-IR spectra was performed. Analyzing the above-presented figure it can be said that obtained spectra are very similar. Furthermore, all occurring bands indicate the presence of functional groups characteristic for chitosan, gelatine and compounds present in *Aloe vera* juice.

In **Table 2.** and **Table 3.** types of characteristic vibrations occurring in tested hydrogels are summarized.

Table 2. Compilation of characteristic vibrations present in a sample of hydrogel without *Aloe vera* extract.

Vibration [cm ⁻¹]	Type of vibration	Functional group
3327	stretching	-OH, -NH ₂
2870	stretching	-CH ₂
1726	stretching	-C=O
1653	deformation	-N-H
1249	stretching	-C-O
1098	stretching	-C-N
948, 853	bending	-N-H

Table 3. Compilation of characteristic vibration occurring in a sample of hydrogel modified with *Aloe vera* extract.

Vibration [cm ⁻¹]	Type of vibration	Functional group
3328	stretching	-OH, -NH ₂
2874	stretching	-CH ₂
1728	stretching	-C=O
1653	deformation	-N-H
1246	stretching	-C-O
1092	stretching	-C-N
945, 849	bending	-N-H

In order to determine an impact of Beetosan® on the structure of hydrogel matrix FT-IR spectrum of hydrogel based on Beetosan® as well as this on deriving from the polymer on the basis of commercial chitosan were compared. Results are visible in **Figure 5.** and **Figure 6.**

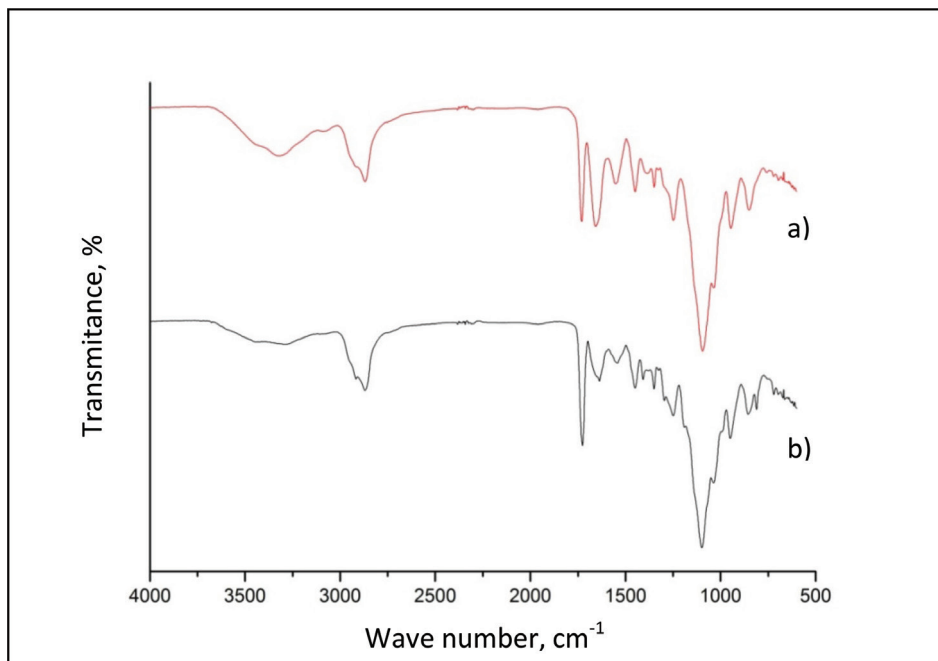


Figure 5. FT-IR spectra of materials unmodified with *Aloe vera* extract and based on: a) commercial chitosan and b) chitosan deriving from bees (Beetosan®).

On the basis of above-presented results of FT-IR spectroscopy of the hydrogel materials it can be noticed obtained spectra they look very similar regardless of the type of chitosan used for the synthesis of the polymers being tested.

In each case, bands indicating the presence of chitosan-derived groups are observed. Within the range of 1098-1092 cm^{-1} clear bands deriving from the stretching vibrations of C-N group occur. What is more, the spectrum of absorption at the wave number of 1653 cm^{-1} showing a presence of -N-H bond also can be noticed. In case of the spectrum representing the Beetosan® based hydrogel this band is characterized by a lower intensity, which may be due to the greater amount of chitin present in Beetosan®. On each of the resulted spectra, there are also bands corresponding to the bending vibrations of the N-linkage of the amino group in the range of 948-849 cm^{-1} . Their intensity is slightly higher in comparison to the same bands occurring on the spectrum deriving from the material based on the commercial chitosan. This is related to the lower content of chitin in the polysaccharide of commercial origin that results in a greater number of amino groups. Based on all observations it can be concluded that Beetosan® deriving from naturally dead honeybees does not significantly affect

the structure of the resulting hydrogel matrix. However, such prepared polysaccharide may contain more chitin.

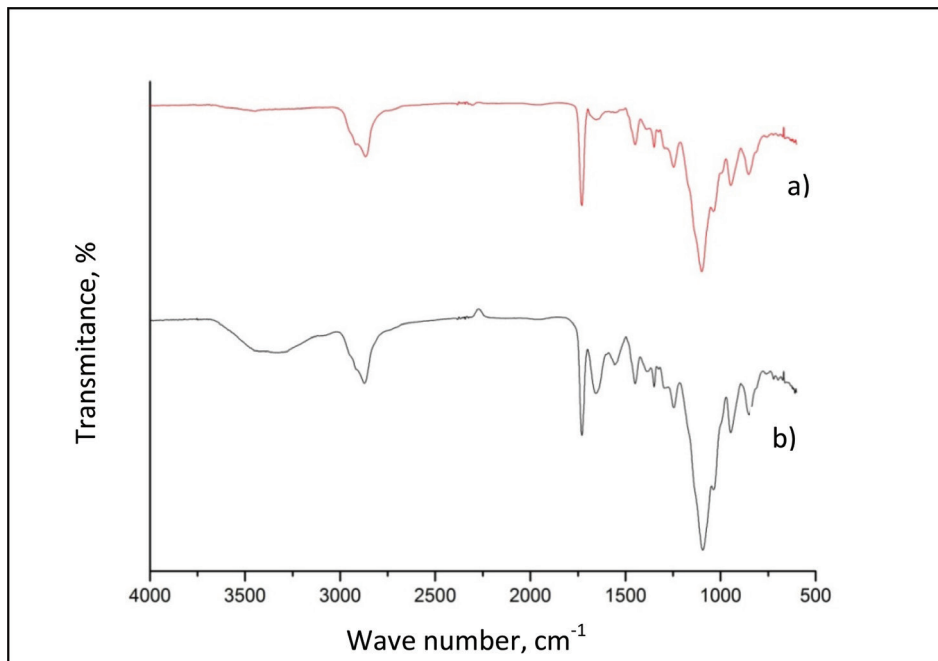


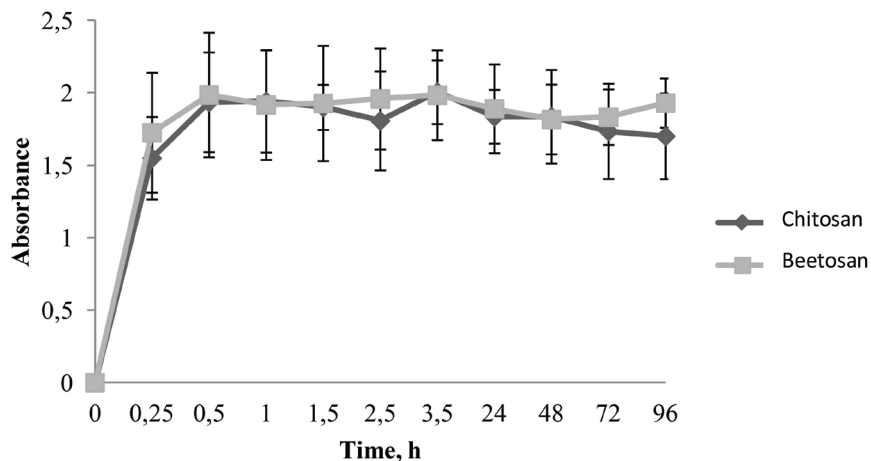
Figure 6. FT-IR spectra of hydrogels containing 10 ml of *Aloe vera* extract and based on: a) commercial chitosan and b) Beetosan®.

Determination of the release profile of *Aloe vera* from hydrogel matrix

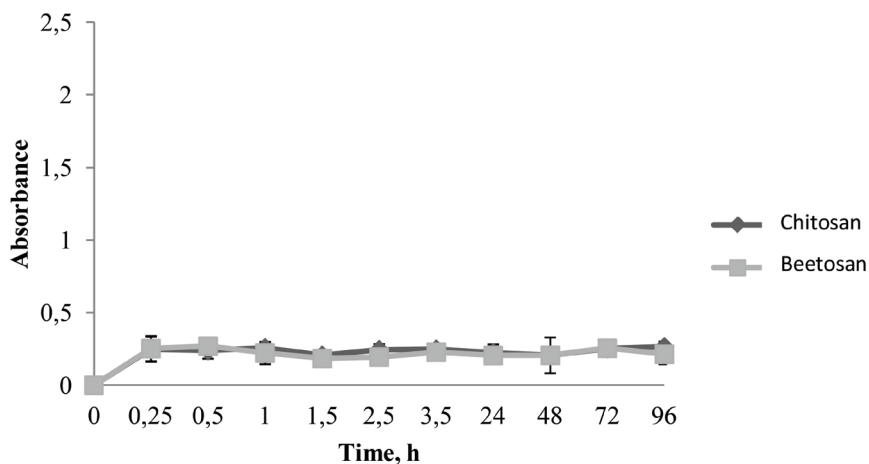
Studies on the release of the active substance (extract from *Aloe vera*) from hydrogel matrix based on Beetosan® and commercial chitosan were conducted in order to determine an impact of Beetosan® on the release profile of the mentioned additive. Research was carried out at a temperature of 37°C. Released active substance was determined qualitatively using a UV-Vis spectrophotometer. Maximum absorbance was determined at a wavelength $\lambda = 350$ nm. Obtained results are presented in **Figure 7**.

The release profile of active substance from a hydrogel matrix based on Beetosan® is very similar to that one deriving from commercial chitosan, which was observed in both acidic and basic environments. It can be stated that Beetosan® obtained from honeybees has a similar structure and properties to the commercial chitosan and therefore does not interfere with the process of release of the active substance from the hydrogel matrix. What is also important, process

of release of active substance from hydrogels is more effective in acidic environment. It is an essential information in view of application of presented materials as drug carriers. Tested solutions were selected in view of environments with different pH occurring in human body (acidic environment in stomach and alkaline environment in duodenum). Therefore it is possible to conclude that proposed modified hydrogels can be used as effective drug carriers in acidic environments.



a)



b)

Figure 7. Release profile of *Aloe vera* from hydrogel matrix based on Beetosan® and commercial chitosan in: a) citric acid (pH = 1.8), b) phosphate buffer (pH= 7.4).

SUMMARY

The conducted research shows that honeybees can be an alternative source of chitosan, which is now extracted on an industrial scale mainly from crustaceans. Synthesized bee-derived chitosan-based materials are characterized by a sorption capacity in fluids simulating liquids occurring in the human body. They are materials with hydrophilic properties and porous structure. What is also important, hydrogels can be modified with active substances of different origin that may enhance their functionality by giving them new properties. These materials have also a capability of the release of an active substance from their interior, which suggests the possibility of their potential biomedical use. In future, it is planned to carry out cell line studies in order to determine the cytotoxicity of such materials.

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Corresponding Author

Sonia Kudłacik-Kramarczyk, MSc.

Institute of Inorganic Chemistry and Technology,

Warszawska 24 St. 31-155 Cracow,

e-mail: skudlacik@chemia.pk.edu.pl,

Ph.: 628-27-23

Anna Drabczyk, MSc.

Institute of Inorganic Chemistry and Technology,

Warszawska 24 St. 31-155 Cracow,

e-mail: adrabczyk@chemia.pk.edu.pl,

Ph: 628-27-23

Bożena Tylińczak, PhD,

Department of Chemistry and Technology of Polymers,

Warszawska 24 St. 31-155 Cracow,

btyluszczak@chemia.pk.edu.pl,

Ph: 628-25-72

Cracow University of Technology

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