Beetosan®-Based Hydrogels Modified with Natural Substances

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ABSTRACT: In this study a series of hydrogels based on Beetosan® and containing natural substances, such as bee pollen and sage (*Salvia officinalis*), have been prepared. Beetosan is a type of chitosan that is obtained from the external skeletons of naturally occurring dead honeybees. In multistage chemical treatment, dead insect waste is converted into chitosan that is used in research as a hydrogel matrix. Prepared materials were synthesized using photopolymerization. Studies on the obtained hydrogels included determining their swelling ability and behavior in a simulated body fluid environment, as well as determining their chemical structure by Fourier transform infrared spectroscopy (FTIR). Based on the research it can be stated that all synthesized modified hydrogels are characterized by swelling ability and do not tend to degrade after immersing in solutions with a composition similar to body fluids.

KEYWORDS: Bee pollen, chitosan, hydrogels, *Salvia officinalis* (sage)

1 INTRODUCTION

Recently, substances of natural origin have been growing in popularity both in the food and medical science industries. Many plants have a diversified chemical composition, which makes them a valuable component of various formulations desirable in pharmaceutical medicine.

Undoubtedly, *Salvia officinalis* (sage) belongs to the group of such plants. Sage is one of the species from the Lamiaceae family. Its name is derived from the Latin word "salvus," which means "health." It should be noted that an essential oil contained in the leaves of this species is unique in terms of its nutrients. Furthermore, sage also includes substances such as organic acids, flavonoids, carotene, tannins and many vitamins (e.g., niacin, A, B_1, C). Due to having such a diverse composition, *Salvia officinalis* is applied as a component of formulations used in diseases of the digestive and respiratory systems and also in cases of skin diseases. Additionally, recent research indicates that sage is characterized by an antimicrobial and anticancer action [1–3]. The next noteworthy ingredient of natural origin is definitely bee pollen. It is a substance collected by bees from flowering plants and processed by these insects. Due to the numerous beneficial nutritional properties of this material it is a useful substance in medicine as well as cosmetics. Bee pollen contains a series of desired substances, including minerals, vitamins (retinol, niacin, tocopherols, ascorbic acid and many others), essential oils, enzymes or even growth promoters [4–6].

In the present study, bee pollen and *Salvia officinalis* constitute an additive to the hydrogel matrix. Undoubtedly, one of the interesting characteristics of hydrogel materials is the possibility of introducing diverse substances into their interior, which results in enrichment of the material with new features and/or new properties. These polymers are known for their nontoxicity, biocompatibility and other features, which affect the possibility of applying these materials for medical purposes [7–9]. Beetosan is a compound constituting hydrogel matrix. It is a chitosan whose name comes from its acquired source, i.e., naturally occurring dead honeybees. It is currently a highly popular biopolymer that requires a multistep process, including removal of all unwanted substances present in the bee's body [10–13]. Synthesis has been conducted at the Cracow University of Technology. A process consisting of several stages is required because of the necessity of removing substances, such as waxes, mineral salts,

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proteins or dyes, from the dead insects. Each step requires the selection of appropriate parameters (including temperature and pH) in order to obtain the desired material.

2 MATERIALS

Diacrylate poly(ethylene glycol) at a molecular weight equal to 700 g/mol (PEGDA 700) and 2-hydroxy-2-methylpropiophenone were supplied from Sigma-Aldrich SA. Gelatin was purchased from POCH SA (Poland). Reagents used in the research were analytically pure. Additives introduced into the hydrogel matrix, i.e., *Salvia officinalis* and bee pollen, were delivered from Herbapol Lublin SA. Beetosan was obtained in a multistep chemical process, in which naturally occurring dead honeybees were used as raw material. Synthesis was carried out at the Cracow University of Technology.

3 EXPERIMENTAL

3.1 Synthesis of Hydrogels

In the first stage, a solution of gelatin and Beetosan (both in acetic acid) were prepared. Then, appropriate amounts of bee pollen solution or sage infusion were added to the mixture. Subsequently, reagents such as crosslinking agent (diacrylate poly(ethylene glycol) at a molecular weight equal to 700 g/mol; PEGDA 700) and photoinitiator (2-hydroxy-2-methylpropiophenone) were also introduced and the thus created mixture was poured into a Petri dish and subjected to the polymerization process under UV radiation. Samples were exposed to radiation for 1–2 min. As its source, an Emita VP-60 quartz lamp was applied (characterized by the following parameters: power –120 W; wavelength λ = 320 nm). In Table 1, compositions of particular hydrogel materials are presented.

3.2 Studies on Sorption Capacity

Due to the characteristic feature of hydrogels, i.e., ability of reversible liquid absorbing, the obtained hydrogel materials were investigated to determine their sorption capacity. Studies were conducted as follows: dry and accurately weighed samples were immersed into the appropriate solution for a suitable period of time after which solution was separated from the material. The same procedure was repeated after every immersion period, i.e., 1 h, 24 h and 72 h. Then, the swollen hydrogel was weighed and based on both samples the mass swelling ratio (Q; g/g) was calculated as follows:

$$Q = \frac{w - w_0}{w_0} \tag{1}$$

where w was the weight of swollen sample, and w_0 was the weight of dry sample.

3.3 Incubation Studies

Due to the potential use of materials prepared, it is important to determine their behavior in an environment resembling body fluids. For this purpose, samples of hydrogels modified with natural substances have been immersed into some selected liquids and incubated for a defined period of time at a temperature of 37 °C. During all periods of incubation, pH values of liquid containing immersed sample were monitored.

3.4 Determining Chemical Structure of Hydrogels Using FTIR Spectroscopy

The chemical structure of attained materials has been determined using Fourier transform infrared spectroscopy. Research was carried out using an FTIR spectrometer with an ATR diamond/ZnSe (PerkinElmer Spectrum 65).

Table 1 Compositions of synthesized hydrogels.

No.	Gelatin (2%) [ml]	Beetosan® (3%) [ml]	Bee pollen solution [ml]	Sage infusion [ml]	Crosslinking agent [ml]	Photoinitiator [ml]
1.	30	20	1	–	8	0.25
2.	30	20	3	–	8	0.25
3.	30	20	5	–	8	0.25
4.	30	20	–	1	8	0.25
5.	30	20	–	3	8	0.25
6.	30	20	–	5	8	0.25

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4 RESULTS AND DISCUSSION

4.1 Results of Swelling Studies

The results of conducted studies on the swelling ability of modified Beetosan-based hydrogels are shown in Figures 1 and 2.

On the basis of conducted studies it can be concluded that prepared materials are characterized by relatively high swelling ability in every tested solution. The highest values of swelling ratios were observed in the case of studies in distilled water. In other solutions, immersed samples were characterized by lower sorption capacity. This is a result of the presence of many chemical

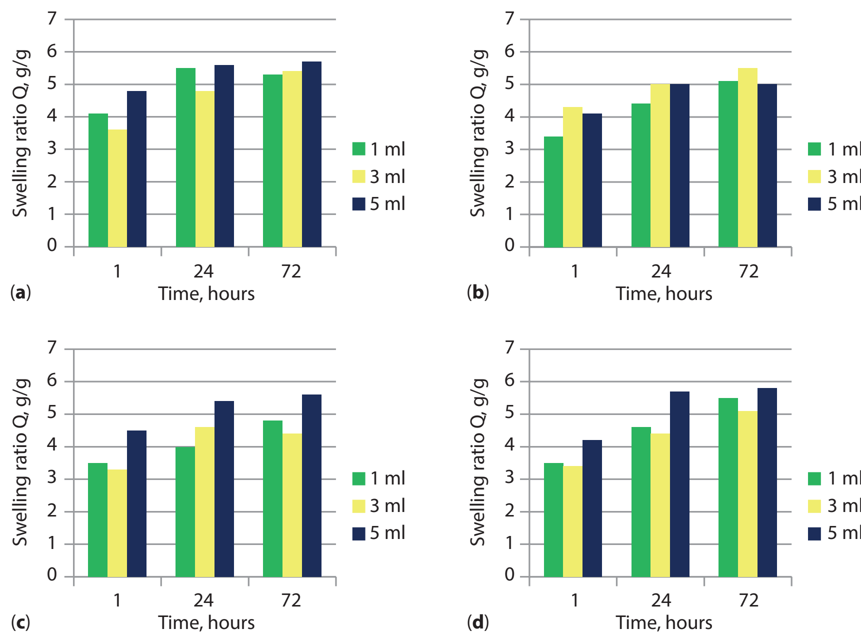

Figure 1 Results of swelling studies of Beetosan-based hydrogels modified with *Salvia officinalis* in: (**a**) distilled water; (**b**) Ringer's liquid; (**c**) artificial saliva solution; (**d**) SBF.

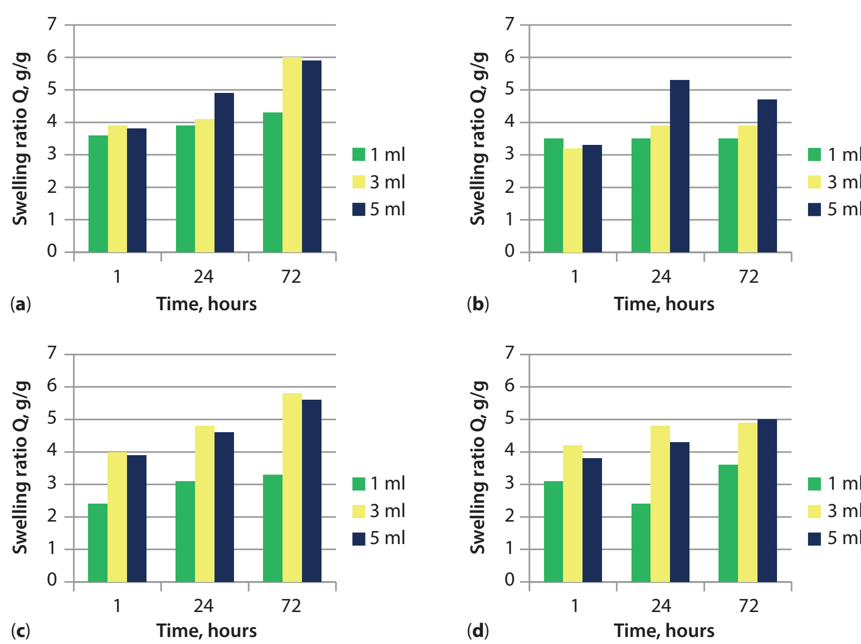

Figure 2 Results of swelling studies of Beetosan-based hydrogels modified with bee pollen in: (**a**) distilled water; (**b**) Ringer's liquid; (**c**) artificial saliva solution; (**d**) SBF.

compounds comprising the tested solutions. Swelling ability is affected by many actors, including the chemical structure of tested material, but also the composition of solution in which the research is carried out. Solutions such as simulated body fluid (SBF), artificial saliva solution or Ringer's liquid are characterized by diversified chemicals, including a number of mono- and divalent ions. Such elements tend to connect to the functional groups present in the structure of the material that is subjected to the swelling studies. As a result, an increase in the degree of crosslinking is observed. The formation of additional crosslinks between the polymer chains results in the creation of a more compact structure, thereby reducing the space available for absorbed fluid.

It is also worth noting that an increase of additive content (i.e., a solution of bee pollen or infusion of *Salvia officinalis*) causes a slight improvement of the sorption capacity. *Salvia officinalis* and bee pollen are materials containing a large number of chemical compounds and some of them are hydrophilic, which increases the affinity for water and can contribute to a slight increase of swelling ability.

4.2 Results of Incubation Studies

The results of conducted studies on the swelling ability of hydrogels modified with *Salvia officinalis* are presented in Figures 3 and 4.

In Figures 5 and 6, the results of conducted studies on the swelling ability of hydrogels modified with bee pollen are shown.

Considering the potential application of the tested materials for medical purposes, it is important to determine their behavior after placing a hydrogel sample in a composition of simulated body fluid. In the course of incubation studies it was found that pH values of solutions in which the study takes place are maintained at very similar levels, which may indicate the biocompatibility of the synthesized modified hydrogel in the body fluid environment. Moreover, tested materials were not degraded and retained their structure. It is worth mentioning that any impact of the amount of additive present in the polymer matrix on the pH value of tested solutions was not observed. At the beginning, after placing the sample in a solution it starts to absorb liquid, which involves eluting from its interior unreacted reactants. Then it is observed that a balance is achieved and hence pH fluctuations are negligible.

4.3 FTIR Spectroscopy

Spectra obtained as a result of spectroscopy are shown in Figures 7 and 8.

On the basis of the presented results it is possible to specify functional groups present in the

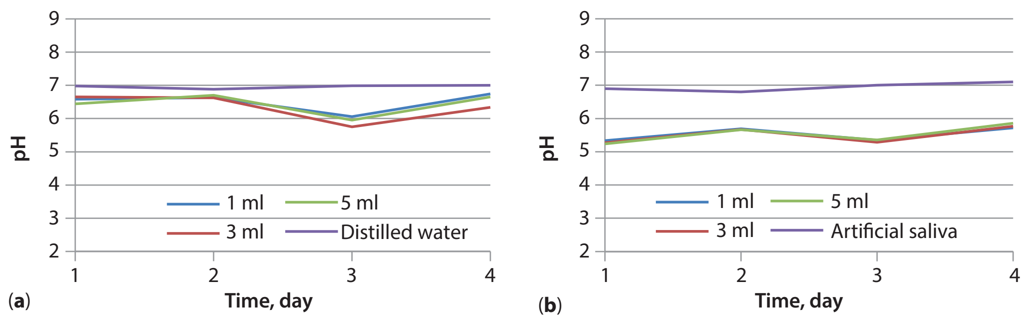

Figure 3 Incubation of Beetosan hydrogels modified with *Salvia officinalis* in distilled water (**a**) and in artificial saliva solution (**b**).

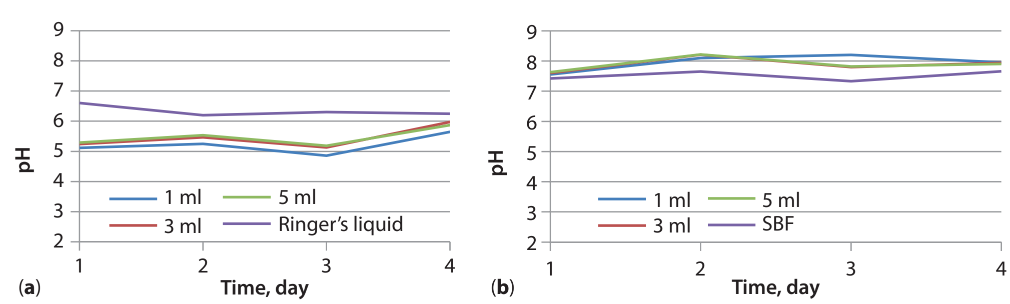

Figure 4 Incubation of Beetosan hydrogels modified with *Salvia officinalis* in Ringer's liquid (**a**) and in SBF (**b**).

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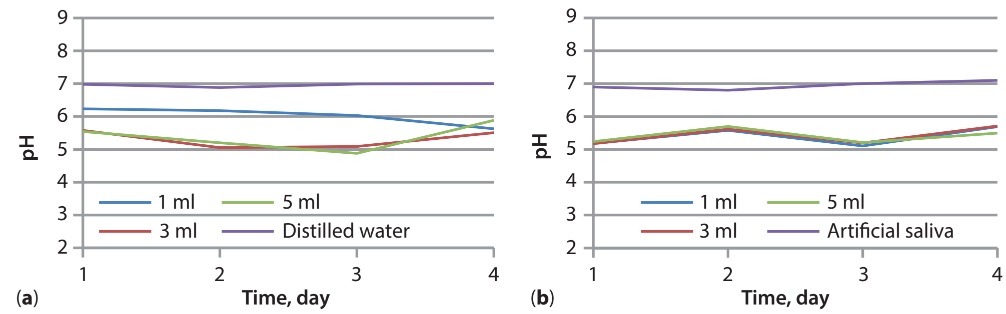

Figure 5 Incubation of Beetosan hydrogels modified with bee pollen in distilled water (**a**) and in artificial saliva solution (**b**).

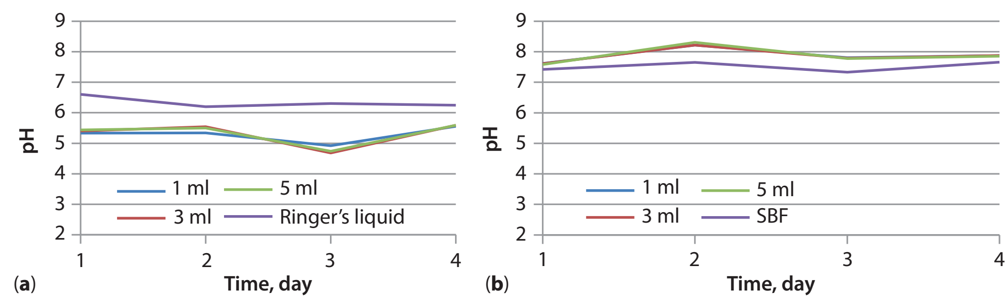

Figure 6 Incubation of Beetosan hydrogels modified with bee pollen in Ringer's liquid (**a**) and in SBF (**b**).

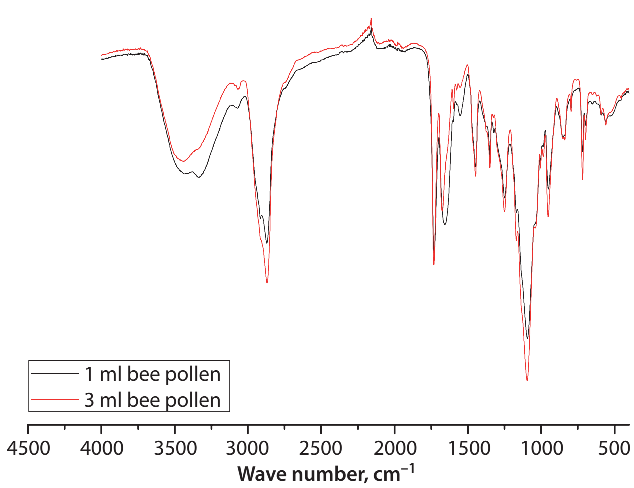

Figure 7 FTIR spectra of hydrogels modified with bee pollen.

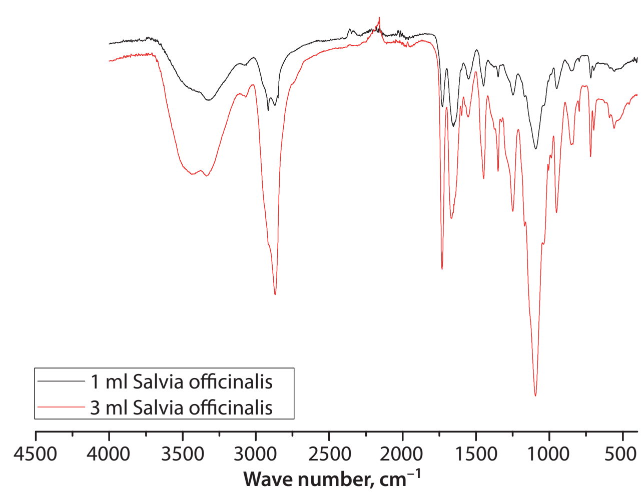

Figure 8 FTIR spectra of hydrogels modified with *Salvia officinalis*.

material. A list of the vibrations characteristic for particular groups identified on the basis of an appropriate wavelength is presented in Table 2. Based on the spectroscopic analysis it can be found that any peaks derived from bee pollen or *Salvia officinalis* are not observed. This is probably due to the fact that the concentration of these substances in tested materials was too small. Such a conclusion stems from the fact that any differences in the intensity of the peaks originating from samples containing different amounts of bee pollen were not determined.

On the other hand, analysis of the results appearing in Figure 8 leads to the conclusion that a fairly significant difference in the intensity of peaks derived from the samples is observed. It most likely results from the difference in quantity of additive introduced into the hydrogel matrix between the two samples.

Table 2 Vibrations of groups presented in tested hydrogels.

Range of vibrations [cm^{-1}]	Functional groups	Type of variations
3500	gr. –OH, gr. –NH_2	stretching
2900	C-H aromat.	stretching
1650	gr. –N-H	deformation
1420	gr. –OH	valence
1370	C-H	deformation
1300	C-N	valence
1260	gr. –C-O	stretching
1020	gr. –C-N	stretching
675	C-H aromat	deformation

Probably during the synthesis of a hydrogel sample containing less amount of *Salvia officinalis* an undesirable effect occurred that in turn adversely affected the balance of the system and as a consequence less intensity of peaks is observed. Perhaps a sufficient degree of polymerization was not achieved or incorrect measurement was conducted (i.e., pin of the spectroscope could not adhere completely to the surface of the sample).

5 CONCLUSION

Hydrogels based on Beetosan and modified with different amounts of sage infusion or bee pollen solution have been synthesized as a result of photopolymerization. Prepared materials were characterized by their swelling ability, which was slightly higher when the amount of additive introduced into the hydrogel matrix was greater. On the basis of the incubation studies it can be stated that synthesized materials do not degrade in simulated body fluids, as evidenced by the slight variations in the pH values of the solutions in which the incubation takes place. Furthermore, based on the spectroscopic analysis it can be concluded that any peaks derived from introduced additives were not observed. This was probably due to their small amount inside the hydrogel material. It is also worth mentioning that chitosan derived from naturally occurring dead honeybees (Beetosan) constitutes an interesting material used as a hydrogel matrix.

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REFERENCES

1. B. Pavlic, S. Vidovic, J. Vladic, R. Radosavljevic, M. Cindric, and Z. Zekovic, Subcritical water extraction of sage (*Salvia officinalis* L.) by-products: Process optimization by response surface methodology. *J. Supercrit. Fluids* **116**, 36–45 (2016).
2. A. Sallam, A. Mira, A. Ashour, and K. Shimizu, Acetylcholine esterase inhibitors and melanin synthesis inhibitors from *Salvia officinalis*. *Phytomedicine* **23**(10), 1005–1011 (2016).
3. P. Hasanein, Z. Feleghan, and A. Emamjomeh, Preventive effects of *Salvia officinalis* L. against learning and memory deficit induced by diabetes in rats: Possible hypoglycaemic and antioxidant mechanisms. *Neurosci Lett.* **622**, 72–77 (2016).
4. R.C. de Oliveira, S.C. Queiroz, C.F. da Luz, R.S. Porto, and S. Rath, Bee pollen as a bioindicator of environmental pesticide contamination. *Chemosphere* **163**, 525–534 (2016).
5. C. Zuluaga, A. Martinez, J. Fernandez, J. Lopez-Baldo, A. Quiles, and D. Rodrigo, Effect of high pressure processing on carotenoid and phenolic compounds, antioxidant capacity and microbial counts of bee-pollen paste and bee-pollen-based beverage. *Innov. Food Emerg.* **37**(A), 10–17 (2016).
6. A. Ragno, E. Cavallaro, D. Marsili, M. Apa, L. D'Erasmo, and L.S. Martin, Honey, bee pollen and vegetable oil unsaponifiables in wound healing. *J. Tissue Viability* **25**(3), 189 (2016).
7. E.M. Ahmed, Hydrogel: Preparation, characterization, and applications: A review. *IJAR* **6**, 105–121 (2015).
8. B. Tyliszczak and K. Pielichowski, Characteristics of hydrogel matrices - biomedical applications of polymer superabsorbents. *Czas. Techn.* **104**, 159–167 (2007).
9. A.S. Hoffman, Hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **54**, 3–12 (2002).
10. S. Nemtsev, O.Y. Zueva, M.R. Khismatullin, A.I. Albulov, and V.P. Varlamoc, Isolation of chitin and chitosan from honeybees. *Appl. Biochem. Microbiol.* **40**(1), 39–43 (2004).
11. Z. Draczyński, Honeybee Corpses as an available source of chitin. *J. Appl. Polym. Sci.* **109**, 1974–1981 (2008).
12. B. Tyliszczak, K. Bialik-Wąs, A. Drabczyk, S. Kudłacik, and A. Sobczak-Kupiec, Animal-derived chitosans. Characteristics, comparison, application. *Przem. Chem.* **95**(10), 2059–2062 (2016).
13. M. Mucha, Chitosan - a versatile polymer deriving from renewable sources, WNT (2010).

