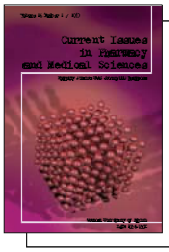

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Evaluation of physicochemical and mechanical properties of polymeric formulations for use in the oral cavity

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ABSTRACT

The aim of this research was to create and investigate a formulation that could be placed within post-extraction alveolus. The main material used in this work is a natural polymer, known as microcrystalline chitosan (MCCh). It was formulated as an aqueous hydrogel with the addition of the cross-linking agent ($CaCl_2$) and various plasticizers (propylene glycol, glycerol and sorbitol). The swelling of the preparations was tested by three different methods under completely different acceptor fluids such as distilled water, phosphate buffer and simulated saliva. The pH was measured on the surfaces of each formulation, as well as the pH of the acceptor fluids; evidently, both of them had a similar pH to that of the oral cavity. Additionally, mechanical properties of the tested materials such as hardness, springiness and cohesiveness were evaluated. Based on research, the best compositions of materials were chosen to obtain preparations of the desired geometry and mechanical characteristics. The results from the study are able to prove that MCCh not only creates a neutral environment within the oral cavity but also, due to its significant absorptive properties, can reduce exudate and potentially be considered as a new carrier for medicinal substances used in dental implantation and management.

Keywords: polymeric formulations, phosphate buffer, simulated saliva, swelling, mechanical properties

INTRODUCTION

Nowadays, there is a seemingly huge growth within the field of controlled drug release and therapy. Modern technology of drug release aims to achieve a complex therapeutic effect while minimizing adverse side effects of the drug especially for those prone to it. The controlled drug release formulation enables to achieve it while maintaining its therapeutic effect and/or prolonging the action of the active substance within. In certain situations, it is really important to limit the effects of systemic exposure of the main substance, and to eliminate the effect of the first-pass metabolism in order to simplify and advance the drug uptake that is beneficial not only for the patient but also for the physician/dentist involved in the procedure.

The oral cavity provides an excellent environment for drug administration. The mucosal lining of buccal tissues offers a perfect location for drug delivery and absorption.

The aim of the research was to create an intra-alveolus formulation to act as a carrier for therapeutic substances, while at the same time having a dressing function by preventing the passage of food debris and microorganisms post-extraction of the tooth. The main material used in this work is a natural polymer-polysaccharide known as microcrystalline chitosan (MCCh) the modified form of chitosan. Its biocompatibility, biodegradability, antibacterial/antifungal activity and lack of allergic reaction with the patient's tissues proved it an excellent material [11, 15].

On a biochemical level, the material is positively charged at physiological pH; enabling it to show its bio-adhesive abilities by increasing the retention within the place of application [4, 10]. Thus, the hydrophilic surface is able to promote cell adhesion, proliferation and differentiation [7]. The main part of the work focuses on creating a therapeutic system designed to minimize pain, reduce inflammation and accelerate tissue regeneration. Based on research, the best compositions of materials were chosen to obtain preparations with the desired geometry and mechanical characteristics. Each formulation was characterized in terms of hardness, cohesiveness and springiness using Texture Profile Analysis [1, 6]. Appropriate pH of the surfaces as well as the aqueous extract help to confirm the justification for the use of the material in the desired locations. The results obtained enable to prove that MCCh not only creates a neutral environment

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within the oral cavity but also, due to its significant absorptive properties, it can reduce exudates formation and potentially be considered as a new carrier for medicinal substances used in dental implantation and management.

MATERIALS AND METHODS

Reagents. Microcrystalline chitosan 1 (MCCh 1) as a 3.88 wt% hydrogel, microcrystalline chitosan 2 (MCCh 2) as a 2.57 wt% hydrogel, Institute of Biopolymers and Chemical Fibres, Łódź, Poland, propylene glycol (PG) (POCh, Gliwice, Poland), glycerol (G), min. 88%, phosphoric acid (POCh, Gliwice, Poland), calcium chloride, sodium chloride, disodium hydrogen phosphate, monopotassium phosphate (Chempur, Piekary Śląskie, Poland), sorbitol (Merck, Darmstadt, Germany), hydroxyapatite (HA) (Fluka), diclofenac sodium (DNa), sodium hyaluronate (Hial), sodium alginate (Alg), amoxicillin (Am) (Sigma-Aldrich Co., USA), lidocaine hydrochloride (Lid_{HCl}) (Amara, Poland), distilled water. All used reagents were of analytical grade.

Preparation of polymeric formulations. All formulations were prepared from sterilized microcrystalline chitosan as a hydrogel with two different polymer content (3.88 and 2.57 wt%), average molecular weight M_w (340 and 150 kDa), degree of deacetylation DD (81.0 and 79.8%) and pH 6.8 & 7.2. All the preparations were made utilizing the same methods. MCCh hydrogel (acc. 600 mg) was mixed well using a mechanical stirrer with cross-linking agent ($CaCl_2$, 1.11 mg), plasticizers (Table 1) and 2 drops of distilled water. Then the materials were transferred into a special-shaped tube (shape cone) and later, placed in an incubator for 24 hours. All samples (Table 1) were stored at 4°C prior to further analysis.

Preparation of polymeric formulations for mechanical properties. Formulations prepared for mechanical properties were made analogical as above. After adding excipients, micronized active substances: DNa (50.0 mg), Am (50.0 mg) or HA (300 mg) were placed in MCCh hydrogel which consisted of one layer preparation. For bilayer formulations, external layer was prepared of hydrogel (acc. 400 mg) with Alg (15.0 wt%) or Hial (2.0 wt%) containing 200 mg Lid_{HCl}. Internal layer was made like all preparations with MCCh hydrogel containing 50.0 mg DNa. Different shapes of formulations were obtained by two kinds of tubes (cylinder and shape cone).

Evaluation of formulations

Organoleptic properties. All formulations selected were uniformly symmetric cone-shaped, plastic, cream-colored and without specific odor.

Weight variation. The minimal weights ranged from the smallest being 36.0 mg and the largest 91.0 mg. Almost all formulations had mostly regular shape. The composition of formulations, which showed the best swelling properties in water, was checked thrice within six time intervals in a simulated saliva solution.

Swelling studies. The ability of swelling is an essential characteristic to be analyzed for this kind of formulations. It was carried out in three different fluids acceptors.

They are as follows:

- swelling studies in simulated saliva solution, which was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl in 1000 ml of distilled water adjusted with phosphoric acid to pH 6.75 [8],
- swelling studies in distilled water [13],
- swelling studies in phosphate buffer, pH 7.43 [12].

The experiment with simulated saliva solution was made to depict the hydration and matrix erosion (DS) percentage of studied preparations.

Six selected dried preparations were weighted (w_1) and kept in 50 ml of simulated saliva solution at pH 6.75 for 0.5, 1, 2, 4, 8 and 24 hours at ambient temperature. After soaking, the formulations were wiped off and dried with filter paper and weighted (w_2) again. These formulations were then dried in an incubator for 24 h at 60 ± 2 °C and kept in desiccators for 48 h; afterwards, were weighted again (w_3). The research was conducted for 3 samples repeated 3 times, and the average shows the results. The composition of all formulations, which had been tested in this experiment, was analogical to the F1 formulation. All formulations were weighted with an accuracy of 0.0001g.

Table 1. Composition of preparations with different amount and kind of plasticizer

Formulation	Weight formulation mg	Base	Plasticizer			
			Propylene glycol mg	Glycerol mg	Sorbitol, mg	
					Solution*	Powder
F1	42.0	MCCh 1	25.0	-	-	-
F2	62.0		50.0	-	-	-
F3	69.0		75.0	-	-	-
F4	36.0	MCCh 2	25.0	-	-	-
F5	44.0		50.0	-	-	-
F6	59.0		75.0	-	-	-
F7	57.0	MCCh 1	-	50.0	-	-
F8	81.0		-	100.0	-	-
F9	58.0	MCCh 2	-	50.0	-	-
F10	91.0		-	100.0	-	-
F11	41.0	MCCh 1	-	-	20.0	-
F12	47.0		-	-	-	20.0

* 20 wt%

Hydration and matrix erosion (DS) percentage were calculated according to the following formulas [9]:

$$\text{Hydration} = (w_2 - w_1)/w_2 \times 100\% \quad (1)$$

$$\text{DS} = (w_1 - w_3)/w_1 \times 100\% \quad (2)$$

The next step of the experiment was to check the swelling properties in distilled water and phosphate buffer, pH 7.43. The research was made in duplicate for formulations with 3 different plasticizers and microcrystalline chitosan as a hydrogel with two different polymer contents. The weighted (w_0) formulations with accuracy of 0.0001 g were kept in 50 ml of distilled water or phosphate buffer, pH 7.43 at ambient temperatures. Measurements were made after 5, 10, 15, 30 min, 1, 2, 3, 4, 5, 6, 7 h (w_t). The swelling index (SI) was calculated according to the formula below:

$$SI = (w_t - w_0)/w_0 \ \ 100\% \quad (3)$$

w_t – weight of the formulation after time (mg)
w_0 – weight of the formulation before swelling (mg).

The swelling characteristic of formulations was evaluated by determination of swelling percentage index (SI). Increase in weight of formulations was determined at present time intervals until a constant weight was observed. The SI, which was checked in distilled water and phosphate buffer, pH 7.43, is analogical to hydration percentage used for simulated saliva.

Surface pH. The pH of surface of formulations [2] was measured with the combined glass electrode which was in contact with previous preparations kept in 50 ml of distilled water (pH = 6.00 ± 0.05) for at least 2 hours at ambient temperatures. The time of the measurement was 2 minutes per formulation. The method developed by Battenberg *et al* was used [3].

pH of the acceptor fluid. After removal of the formulation from distilled water or phosphate buffer, the pH of these fluids was checked with the calibrated electrode at 20 ± 0.5 °C. This experiment was made to check the influence of the formulation on the acceptor fluid.

Mechanical properties. The mechanical properties of chosen formulations were examined by texture profile analysis (TPA) using a Stable Micro Systems Texture Analyzer TA-TX2. The analytical probe was compressed twice into each sample with trigger force 5 g at a rate of 5.00 mm s^{-1}. A delay period of 5 s was allowed between the end of the first and the beginning of the second compression. All tests were performed at ambient temperature.

The parameters that may be derived from TPA are as follows:

– *Hardness* – force required to attain a given deformation.
– *Springiness* – the ratio of the time required to achieve maximum structural deformation in the second compression cycle to that of the first compression cycle, where successive compressions are separated by a defined recovery period.
– *Cohesiveness* – the ratio of the area under the force-time curve produced on the second compression cycle to that produced on the first compression cycle, where successive compressions are separated by a defined recovery period [5].

RESULTS AND DISCUSSION

Organoleptic properties. The chosen polymeric material and individual components enable to obtain the correct shape and homogeneous structure. The plasticity of the preparations could be controlled by changing the time of drying. The required hydration can be maintained by proper storage.

Weight variation. Formulations with the smallest weight had the best swelling properties. However, in general there is not much difference noticed within the swelling properties of the smallest weight acc. 30 mg and the average weight acc. 60 mg. Whereas, a significant decrease in swelling capacity was noticed within these formulations having masses greater than 90 mg.

Swelling studies. Formulations with the best swelling profiles as F1 in water were checked in simulated saliva solution. All formulations are characterized by fast hydration. Figure 1 shows the average of the results. The hydration percentage of formulations within each time frame was characterized by different hydration. The highest hydration percentage (70%) was obtained after 24 h, due to the quick absorption of water without dissolution of formulation. The DS data [9] derive from the comparison between the initial and final weight of formulations after immersion in water as shown in Figure 1. This material has a high ability to absorb water, while a large capacity to undergo to matrix erosion - after 24 hours comes to 84%. It is also related to eluting of excipients, which are not permanently bound with hydrogel. Also, a high degree of erosion of the matrix (DS) is related to the removal of water that at the beginning was inside the formulation giving it the flexibility.

The swelling abilities were checked in three aqueous media. Figures 2 - 5 show the swelling profiles in water and phosphate buffer (pH 7.43) with different amount of plasticizer, which are included in Table 1. As observed, the higher amount of plasticizer the lower swelling ability related to propylene glycol, which is due to the rigidity of the structure causing fewer possibilities for water penetration. Figure 3 depicts that the formulation F1, which contains the smallest amount of propylene glycol, has the best swelling profile. Formulation F3, which has the largest amount of plasticizer (propylene glycol), has the lowest swelling. When comparing the swelling in used acceptor fluids, there is a slight difference between swelling

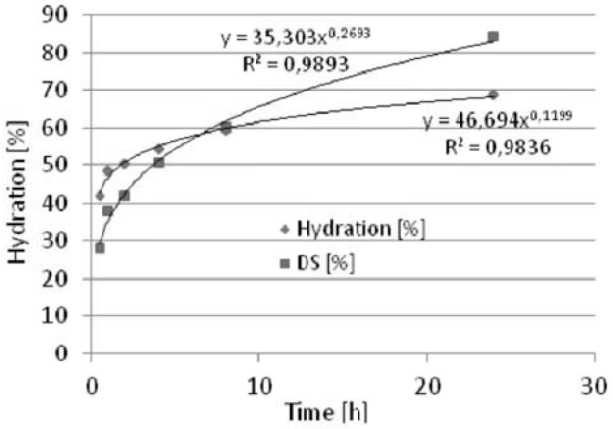

Fig. 1. Hydration and the degree of erosion matrix (DS) percentage of F1 formulation

in water and phosphate buffer, pH 7.43 in favor of water. Formulations which contain MCCh 2 with 2.57 wt% polymer in hydrogel show worse swelling properties than the MCCh 1 with 3.88 wt% (as seen in Fig. 2 and 3).

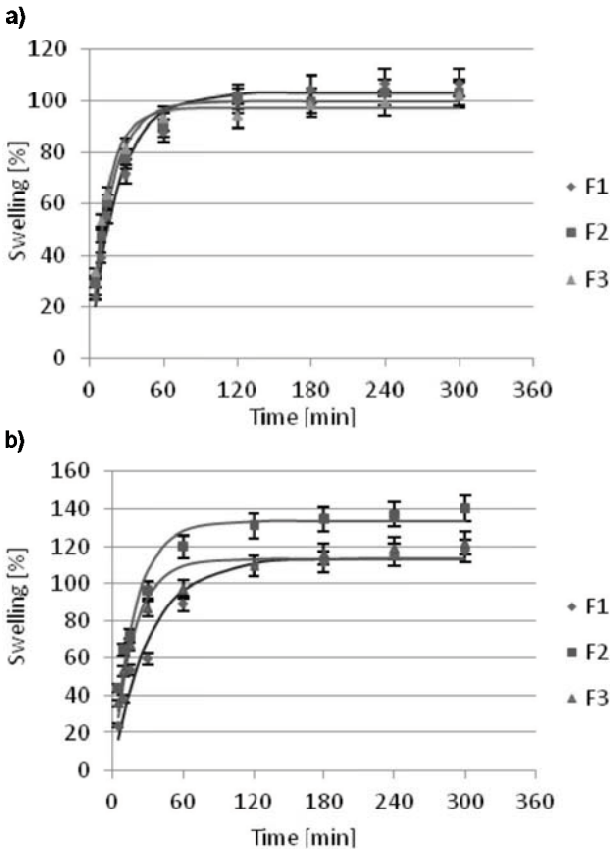

Fig. 2. Swelling percentage of MCCh 1 formulations (F1, F2, F3) with propylene glycol in: a) distilled water and b) phosphate buffer, pH 7.43

The proper selection of the appropriate type and amount of plasticizer, as well as the percentage of polymer in the hydrogel has a significant impact on the profile of swelling. This is visible in Figure 4. Formulation with MCCh 1 (F7, F8) showed values similar to each other at approximately 50%. The sample (F10) with the best swelling profile contains MCCh 2 and the largest amount of glycerol in comparison to formulations with propylene glycol (Fig. 3). An exception is the formulation (F10). It reached 164% of swelling in water but in phosphate buffer 123%. As in the case of formulations with propylene glycol, there is no significant difference between the one with water and phosphate buffer. The formulations with the same amount of different plasticizer as F5 and F9 (Table 1), depict absolutely different profiles (Fig. 3 and 4) of swelling. It can be inferred from the importance of the appropriate plasticizer choice.

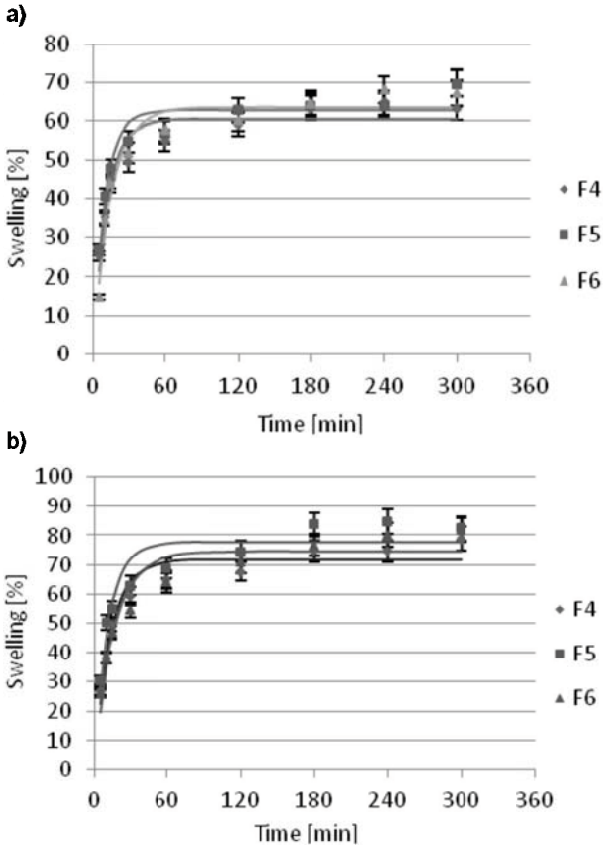

Fig. 3. Swelling percentage of MCCh 2 formulations (F4, F5, F6) with propylene glycol in: a) distilled water and b) phosphate buffer, pH 7.43

Additionally, MCCh 1 preparations with sorbitol were also able to reach good swelling points. Samples that were added as a solution of sorbitol (F11, F12), showed slightly better properties than the ones added as a powder (F11a, F12a). As mentioned above, there were no significant differences between formulation in water (F11, F11a) and phosphate buffer (F12, F12a).

Surface pH. Due to the need for intra-alveolus application, it is essential to check pH on the surface of each material. The surface pH of formulations was examined and presented in Table 2. These results show that preparations have the appropriate pH between 6.70–6.83; this is due to its neutrality within the pocket and gingival mucosa allows it to have minimum to no influence on saliva and the oral cavity. The salivary pH usually varies from 6.2 to 7.4 (from low to high flow rates) [14].

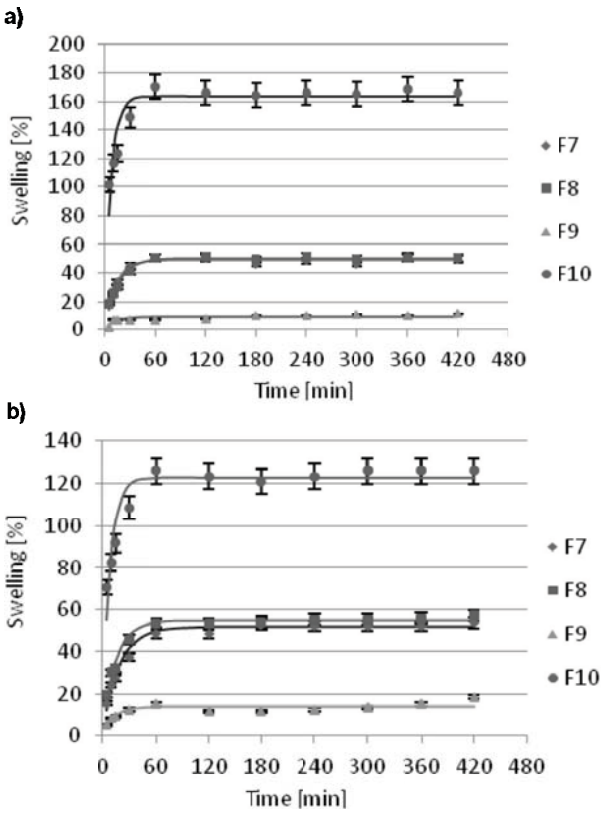

Fig. 4. Swelling percentage of MCCh 1 and MCCh 2 formulations (F7, F8 and F9, F10) with glycerol in: a) distilled water and b) phosphate buffer, pH 7.43

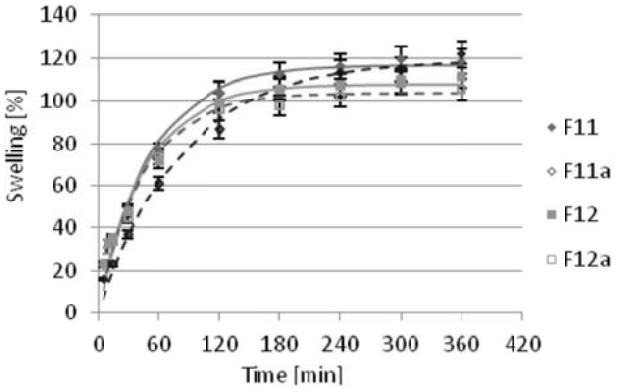

Fig. 5. Swelling percentage of MCCh 1 formulations with sorbitol in distilled water (F11, F12) and phosphate buffer (F11a, F12a)

pH of the acceptor fluids. This measurement was conducted to check the influence of formulations on the acceptor fluids. The study enabled to show that formulations seldom have a slight impact on the change in the aqueous medium that is presented in Table 2.

Mechanical properties. None of the formulations were destroyed or crushed during the experiment; this is a perfect example of their elastic properties, highlighting their importance within the application process. The preparations with the best swelling properties were chosen for their mechanical properties. Compositions of formulations, which are shown in Table 3, are analogical to F1 formulation. Also, differences between the shape – cone and cylinder, along with the difference in the force required to attain a given deformation were measured.

Dried cylinder (No 2) formulation showed greater hardness than dried cone (No 1) but when the preparation had been swollen, not much difference was noted in the hardness between the cone and cylinder shapes (No 3 & No 4). The ones with MCCh 1 exhibited greater hardness than the ones with MCCh 2 (No 1 and No 10). Interestingly, the shape of the formulations had no influence on its springiness and cohesiveness.

Table 2. pH on the surface and acceptor fluids of each formulations

Sample number	pH acceptor fluids		Surface pH
	distilled water	phosphate buffer	
F1	7.05	7.49	6.83
F2	6.86	7.48	6.76
F3	6.87	7.44	6.72
F4	6.86	7.48	6.73
F5	6.88	7.49	6.73
F6	6.87	7.48	6.72
F7	6.72	7.48	6.73
F8	6.94	7.46	6.71
F9	6.83	7.43	6.70
F10	6.93	7.46	6.70
F11	7.35	7.48	6.73
F12	7.01	7.48	6.70

Formulations with active substances showed lower springiness and cohesiveness which is related to their influence on the structure by their ability to brace it. The highest cohesiveness is exhibited by materials in the shape of a cylinder without the active substance, which may be explained by a more regular arrangement of particles in the formulation. The lowest cohesiveness of formulations with hydroxyapatite (HA) No 7 confirms the poor mechanical properties of hydroxyapatite.

Table 3. Effects of MCCh on the hardness, springiness and cohesiveness as determined by texture profile analysis (TPA)

No.	Base	Addition	Condition	Form	Hardness [kN]	Springiness [-]	Cohesiveness [-]
1	MCCh 1	–	dry	cone	78.0 ± 1.44	0.470 ± 0.022	0.562 ± 0.024
2		–	dry	cylinder	182.2 ± 0.52	0.570 ± 0.037	0.633 ± 0.020
3		–	swollen	cylinder	22.1 ± 0.16	0.778 ± 0.025	0.723 ± 0.010
4		–	swollen	cone	9.7 ± 0.21	0.788 ± 0.039	0.713 ± 0.018
5		DNa	dry	cone	11.0 ± 0.93	0.529 ± 0.043	0.432 ± 0.042
6		Am	dry	cone	71.1 ± 1.11	0.389 ± 0.207	0.423 ± 0.005
7	MCCh 1 + Alg	DNa + Lid_{HCl}	dry	cone	169.7 ± 1.57	0.504 ± 0.039	0.501 ± 0.081
8	MCCh 1 + Hial	DNa + Lid_{HCl}	dry	cone	18.2 ± 0.62	0.520 ± 0.078	0.492 ± 0.040
9	MCCh 2	–	dry	cone	50.4 ± 2.05	0.464 ± 0.051	0.534 ± 0.043
10		HA	dry	cone	22.1 ± 1.28	0.415 ± 0.160	0.170 ± 0.057

CONCLUSIONS

In this study, it was demonstrated that formulations based on microcrystalline chitosan with addition plasticizer possess different physical and mechanical properties.

1. The proper selection of the plasticizer has a significant impact on the swelling properties.
2. It was found that the formulations were able to absorb water and increase up to triple their volume and double their mass.
3. Swelling of the preparations decreased when the amount of plasticizer was increased.
4. Formulations containing glycerol showed the worst swelling properties.
5. The best swelling properties are attributed to formulation with solution of sorbitol as plasticizer.
6. The differences between acceptor fluids - distilled water or phosphate buffer, pH 7.43 do not have any significant influence on swelling ability. The two kinds of acceptor fluids (phosphate buffer, pH 7.43 and simulated saliva, pH 6.75) were chosen because of their similarity to human saliva and blood and due to the place of their application. Distilled water was a standard medium in this study.

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