KONTROLISANO OTPUŠTANJE KOFEINA IZ TRODIMENZIONIH MREŽA NA BAZI POLI(METAKRILNE KISELINE) I KAZEINA – ISPITIVANJE UTICAJA KONCENTRACIJE KOFEINA NA PROCES OTPUŠTANJA

CONTROLLED RELEASE OF CAFFEINE FROM THREE DIMENSIONAL NETWORKS BASED ON POLY(METACRYLIC ACID) AND CASEIN - ANALYSIS OF THE EFFECT OF CAFFEINE CONCENTRATION ON RELEASE PROCESS

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Svakodnevne potrebe za bezbednijim i efikasnijim terapijama koje se primenjuju za lečenje mnogih bolesti, naročito težih oboljenja kao što je kancer, dovode modernu nauku pred mnoge izazove. Jedan od njih se ogleda u činjenici da je veliki broj antikancerogenih lekova slabo vodorastvoran zbog čega je njihova inkapsulacija i kontrolisano otpuštanje veliki izazov. U ovom radu smo otišli korak dalje u našem istraživanju nosača na bazi hidrofilne poli(metakrilne kiseline) modifikovane amfifilnim kazeinom, u koji je inkapsuliran kofein (PMAC-Caf hidrogelovi). Ispitano je kako stepen neutralizacije metakrilne kiseline (MAA) i količina inkapsuliranog kofeina utiče na proces bubrenja ovih hidrogelova i otpuštanje kofeina. Predstavljen je jednostavan način sinteze nosača tokom koga je moguće istovremeno i inkapsulirati kofein, a kojim su dobijeni PMAC-Caf hidrogelovi sposobni da se prilagode specifičnim zahtevima ciljane dostave slabo vodorastvornih lekova - da ih zaštite u sredini koja je simulirala ljudski želudac i otpuste u sredini koja je simulirala ljudska creva. Promenom parametara sinteze (stepena neutralizacije MAA i/ili količine inkapsuliranog kofeina) postignuto je kontrolisano otpuštanje kofeina, čime je moguće smanjiti broj potrebnih terapeutskih doza leka i smanjiti neželjene efekte. Rezultati su pokazali da PMAC-Caf hidrogelovi imaju veliki potencijal za kontrolisano otpuštanje slabo vodorastvornih lekova.

Ključne reči: *hidrogelovi; poli(metakrilna kiselina); kazein; kontrolisano otpuštanje; slabovodorastvorni lekovi*

Everyday demands for safer and more efficient therapy for many diseases, especially serious ones such as various types of cancer, put various challenges in front of modern science. One of them lies in the fact that numerous anticancer drugs are poorly-water soluble and therefore their encapsulation and controlled release are quite demanding processes. In the present study, we deepened our research of carrier based on hydrophilic poly(methacrylic acid) modified with amphiphilic casein, in which poorly water-soluble model drug caffeine was encapsulated (PMAC-Caf hydrogels). It was investigated how neutralization degree of methacrylic acid (MAA) and amount of encapsulated caffeine affected swelling behavior of the PMAC-Caf hydrogels and caffeine release. Easy, one pot, simultaneous synthesis of the carrier and the encapsulation of caffeine is presented, obtaining thereby as prepared PMAC-Caf drug delivery system that could respond to the specific demands of the targeted delivery of the poorly water-soluble drug - protecting it in the environment which simulated human stomach and releasing it in the environment which simulated human intestines. Changing the synthesis parameters (neutralization degree of MAA and/or amount of encapsulated caffeine) we

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achieved controlled release of caffeine, indicating that the number of the required doses of the drug in the treatment and its side effects could be reduced. Results showed that the PMAC-Caf drug delivery systems have huge potential for controlled release of poorly water-soluble drugs.

Key words: *hydrogels; poly(methacrylic acid); casein; controlled release; poorly water-soluble drugs*

1 Introduction

Nowadays, global pandemic and other factors which threaten human health are serious problems which are mankind struggle with. Controlled release of drugs can be a solution for these issues and it is widely employed as safe and effective tool for improvement of the therapy of various diseases. Hydrogels are showed to be good chose for delivery of the drugs to the specific place in the human body and control of the kinetics of drug release. Hydrogels are three dimensional polymeric networks able to absorb and retain large amount of water or physiological fluids [1, 2]. One group of these materials, which is investigated for controlled release of drugs, are non-toxic and biocompatible hydrogels based on poly(methacrylic acid) (PMAA) [3]. PMAA hydrogels are hydrogels sensitive to the changes of pH in the surrounding environment. These pH-sensitive hydrogels are able to swell in the environments with pH values that are higher than pKa of PMAA (4.6 [4]). Namely, deprotonation of carboxylic groups of PMAA occurs in such environments, which further leads to the generation of negative charges along the polymer chains and to their repulsion. As a result PMAA hydrogels swell, which is employed for controlled drug release.

However, PMAA application is limited by PMAA lack of good mechanical properties [5-7]. Also, due to the highly PMAA hydrophilic nature only water soluble drugs can be encapsulated in PMAA and delivered to the site of action in the human body [8]. We have overcome these issues in our previous study by modifying PMAA with amphiphilic casein and investigate how different synthesis parameters affect the release of a poorly water-soluble model drug – caffeine (PMAC-Caf hydrogels) [3, 4]. Casein is non-toxic, biocompatible and pH-sensitive protein [3]. The usage of this natural polymer is approved by Food and Drug Administration (FDA) [4]. We demonstrated that casein improved mechanical properties of PMAA and enabled encapsulation of poorly water-soluble caffeine and its controlled release. Namely, hydrogen bonds and hydrophobic interactions were established between casein and caffeine which enabled caffeine encapsulation and its controlled release. In the present study we go step forward and investigate how degree of neutralization of methacrylic acid (MAA) and caffeine concentration affects the PMAC swelling and caffeine release profiles. Namely, adequate concentration of drug and its release rate at the site of action are of great importance. Drug bioavailability and therefore safety and efficacy of a therapy directly depend on the drug amount present at the targeted site in the human body.

Hydrogels based on poly(methacrylic acid) and casein with encapsulated caffeine are synthetized in present study. Swelling behavior of the PMAC-Caf hydrogels and caffeine release are analyzed in two media with pH values similar to the environments in the human stomach and intestines. The influence of degree of neutralization of MAA and caffeine concentration on the swelling process of the PMAC hydrogels and caffeine release profiles are analyzed, as well.

2 Materials and methods

2.1 Materials

Methacrylic acid (99.5%) and caffeine were supplied from Merck (Germany). Sodium caseinate was obtained from Lactoprot Deutschland GmbH (Germany). The crosslinker N,N'-methylenebisacrylamide (p.a.) (MBA) and sodium hydroxide (p.a.) were purchased from Aldrich Chemical Co. (USA). The initiator, 2,2'-azobis-[2-(2-imidazolin-2-yl)propane] dihydrochloride (99.8%) was obtained from Wako Pure Chemical Industries (Japan). Monobasic sodium phosphate (anhydrous) and dibasic sodium phosphate (anhydrous) was supplied from Centrohem (Serbia). Hydrochloric acid (37%) was supplied from Zorka Pharma (Serbia). All chemicals were used as received.

2.2 Preparation of PMAC-Caf hydrogels

The rout of the hydrogels synthesis and characterization are described in details in our previous research [3, 4]. In this study we varied the neutralization degree of MAA and concentration of caffeine. Briefly, four milliliters of MAA and various weights of caffeine were dissolved in distilled water (see Table 1. for feed composition), sodium hydroxide was added in reaction mixture of some samples in order to completely neutralized MAA (Table 1.). Then, the temperature of reaction mixture was elevated to 60 ºC and during vigorously stirring of the reaction mixture four grams of casein was added. After casein dissolution, MBA was added (0.4 mol% with respect to MAA) and dissolved, followed by the addition and dissolution of the initiator (0.9 ml of 1 wt% aqueous solution). Then, the reaction mixture was quickly poured in glass molds and left in the air oven at 60 °C for 5 h after which disc shaped samples were cut and dried at room temperature. Synthetized samples were denoted as PMAC-xN-y, where xN represents neutralization degree of MAA and y represents caffeine weight encapsulated in the hydrogel.

Table 1. Feed composition

Sample	*Neutralization degree of MAA, %*	*Distilled water, ml*	*Caffeine weight, g*
PMAC-0N-0.2	0	8.00	0.2
PMAC-0N-1	0	7.23	1
PMAC-0N-2	0	6.23	2
PMAC-100N-0.2	100	6.20	0.2
PMAC-100N-1	100	5.37	1
PMAC-100N-2	100	4.37	2

2.3 PMAC-Caf hydrogels swelling

The experiments of the hydrogels swelling were performed at 37 ºC in two environments with pH values: 0.1M HCl with pH of 1 (as simulation of human stomach) and phosphate buffer with pH of 6.8 - PB 6.8 (as simulation of human intestines) [4]. The weight of each hydrogel was first measured (m_p, g) and then each hydrogel was immersed in each medium. At previously defined time intervals hydrogel was removed from the medium, its weight was measured (m_t, g) and then the hydrogel was immersed again. The experiment was performed until equilibrium state was reached (i.e. until weight of the hydrogels stay constant). The swelling degree (SD) was calculated according to the following equation:

$$SD = (m_t - m_p)/m_p \quad (1)$$

The equilibrium swelling degree (SDeq) of each hydrogel was calculated by using the same equation (Eq. (1)) in which m_t was replaced with m_e (the weight of hydrogel in equilibrium state).

2.4 Caffeine release from PMAC-Caf hydrogels

Caffeine release process was analyzed in the same media and at the same experimental conditions as was hydrogels swelling. At predetermined time intervals 3 ml of the solution was collected and UV analyzed at 273 nm (caffeine maximum peak value), after which the solution was returned back. Each experiment was conducted three times and mean value of absorbance was used for further determination of concentration of caffeine released in the medium.

3 Results and discussion

The curves of the PMAC-Caf hydrogels swelling in two media are presented in Fig. 1. and obtained values of equilibrium swelling degree of the PMAC-Caf hydrogels are listed in Table 2. The

analysis of the swelling behavior of the hydrogels with non-neutralized MAA in PB 6.8 showed that the PMAC-0N-1 hydrogel swells significantly less than the initial sample - PMAC-0N-0.2 hydrogel (Fig. 1. a)). This could be a consequence of the interactions established between caffeine and MAA due to which diffusion of the medium (PB 6.8) into the hydrogels network was hindered. Further increase in the encapsulated caffeine weight did not affect the hydrogels swelling behavior. Namely, the swelling curves of the initial sample PMAC-0N-0.2 and the PMAC-0N-2 hydrogel were similar (Fig. 1. a)). It is possible that this weight (2 g) of the model drug was too large to be encapsulated into the system and some amount of the drug was very close to the surface of the hydrogels network, so diffusion of the medium into the hydrogels network was not hindered. The same trend of the swelling behavior of the PMAC-Caf hydrogels in 0.1M HCl was observed (Fig. 1. b)).

The PMAC-Caf hydrogels with complete neutralized MAA have higher SDeq values than when they swell in 0.1M HCl. Namely, pH value of PB 6.8 is higher than pKa of MAA and pI of casein (4.6 [4]) which leads to deprotonation of carboxylic groups of PMAA and repulsion of polymer chains due to which the medium diffuses more easily into the polymer network. Increase in encapsulated weight of caffeine in the PMAC-Caf hydrogels with complete neutralized MAA, did not affect the swelling behavior of these hydrogels. Exception was PMAC-100N-2 hydrogel which had higher SDeq than the other samples. As was already explained, some part of the encapsulated amount of caffeine was probably located near or at the surface of the carrier [9], so lower amount of the drug was in the carrier network and did not interfere the path of the medium (PB 6.8) into the polymer network. Also, the repulsion of polymer chains (favored in PB 6.8) led to the easier diffusion of the medium into the carrier network.

Table 2. SDeq values of PMAC-Caf hydrogels in 0.1M HCl i PB 6.8

Sample	SD_{eq}	
	0.1M HCl	*PB 6.8*
PMAC-0N-0.2	2.30	8.80
PMAC-0N-1	0.63	2.98
PMAC-0N-2	2.81	7.22
PMAC-100N-0.2	12.4	23.9
PMAC-100N-1	8.48	24.5
PMAC-100N-2	9.64	43.8

The curves of caffeine release from the PMAC-Caf hydrogels are presented in Fig. 2. a) and b). Increase in the weight of encapsulated caffeine caused higher release rate and release amount of the drug in both media. Burst release of the drug occurred at the start of the release process from the PMAC-0N-2 and PMAC-100N-2 hydrogels (hydrogels with the highest encapsulated amount of drug) in both media. It is more evident in acidic environment (Fig. 2. b)) even though precipitation of casein is favored in acidic environment due to which it is expected that drug carrier would be more compact and caffeine release would be hindered. It is possible that interactions between caffeine and casein became weaker in some parts of the hydrogels network due to the micellarization of casein, so caffeine release was easier. Also, weak interactions between casein and caffeine could be a result of broken proline and calcium phosphate bridges in casein structure which is favored in acidic environment [10]. Burst release of caffeine from the carriers with 0% and 100% neutralized MAA in PB 6.8 can be a consequence of the presence of caffeine near or at the surface of the hydrogels which is first released at the start of the release process.

By compering curves of caffeine release in 0.1M HCl and PB 6.8 it can be concluded that around three times higher amount of caffeine was released in PB 6.8 than in 0.1M HCl (Fig. 2. a)) due to the specific pH dependent swelling of the PMAC-Caf hydrogels.

4 Conclusions

In present study, synthesis of three dimensional polymeric networks based on poly(methacrylic acid) and casein with encapsulated caffeine (PMAC-Caf) were successfully synthetized. It was investigated how the change in the neutralization degree of MAA and encapsulated amount of caffeine affect the swelling process of the PMAC-Caf hydrogels and caffeine release. The swelling behavior of the PMAC-Caf hydrogels and the process of the caffeine release were investigated in two environments which simulated environment in human stomach and intestines (0.1M HCl and phosphate buffer with pH value of 6.8 – PB 6.8).

The analysis of the swelling process of the PMAC-Caf hydrogels showed that these samples swell more in PB 6.8 than in 0.1M HCl. Namely, PB 6.8 have pH value higher than pKa of PMAA and pI of casein, so negative charges along the polymer chains are generated causing their repulsion and the PMAC-Caf hydrogels swelling. Also, neutralized samples have higher values of equilibrium swelling degree due to the polymer chains repulsion and easier diffusion of external medium into the polymer network. The increase in the encapsulated amount of caffeine caused decrease in the SDeq values. This was a result of the increase in the number of interactions established between caffeine and casein which led to the more compact polymer network and diffusion of external medium was hindered. Further increase in the encapsulated amount of caffeine did not affect the swelling behavior of the hydrogels. Namely, during the synthesis process of the hydrogels with the highest amount of caffeine probably some amount of caffeine was located near or at the surface of the hydrogels. This enabled easier diffusion of the medium into the hydrogels network and swelling of the hydrogels. The same trend was observed for the caffeine release from the hydrogels. The increase in the neutralization degree of MAA and encapsulated amount of caffeine led to the increase in the released amount of caffeine. Burst release of caffeine at the start of release process occurred from the PMAC-0N-2 and PMAC-100N-2 hydrogels (hydrogels with the highest encapsulated amount of drug). The reason for this manner of the drug release could be due to the micellarization of casein in some parts of the hydrogels network which caused weakening of the interactions between casein and caffeine, so caffeine was easier released. Also, higher amount of caffeine was released in PB 6.8 than in 0.1M HCl due to the specific pH dependent swelling behavior of the PMAC-Caf hydrogels.

Results obtained in this study showed that the PMAC-Caf hydrogels have huge potential for controlled release of poorly water-soluble active substance and kinetic of its release can be easily tuned by changing one of the synthesis parameter.

5 Figures

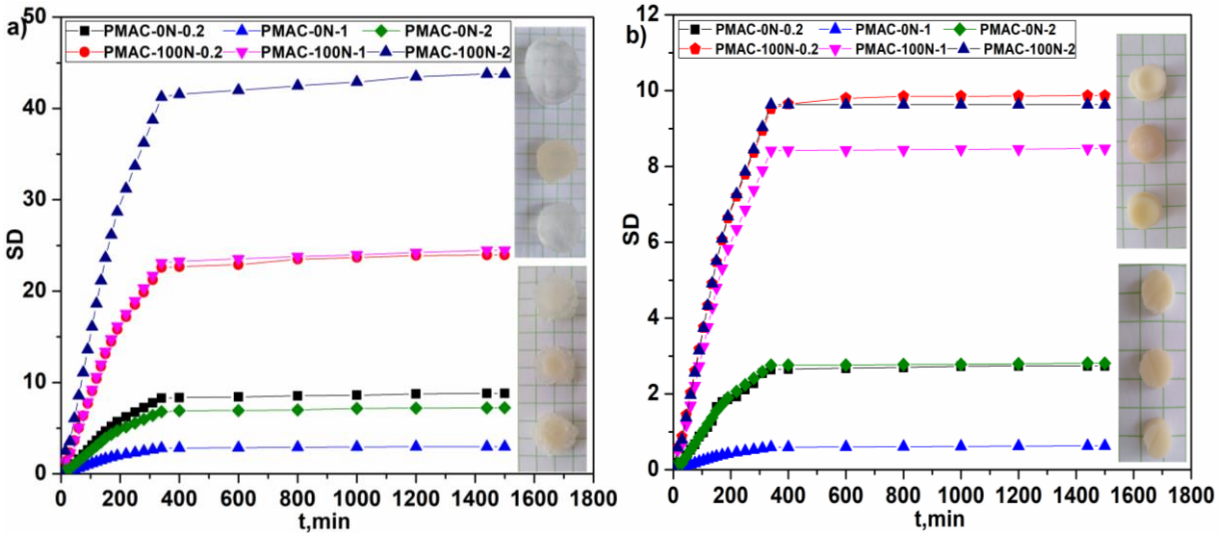

Figure 1. The curves of PMAC -Caf hydrogels swelling in: a) PB 6.8 and b) 0.1M HCl (the pictures present PMAC-Caf hydrogels swollen to equilibrium in investigated medium)

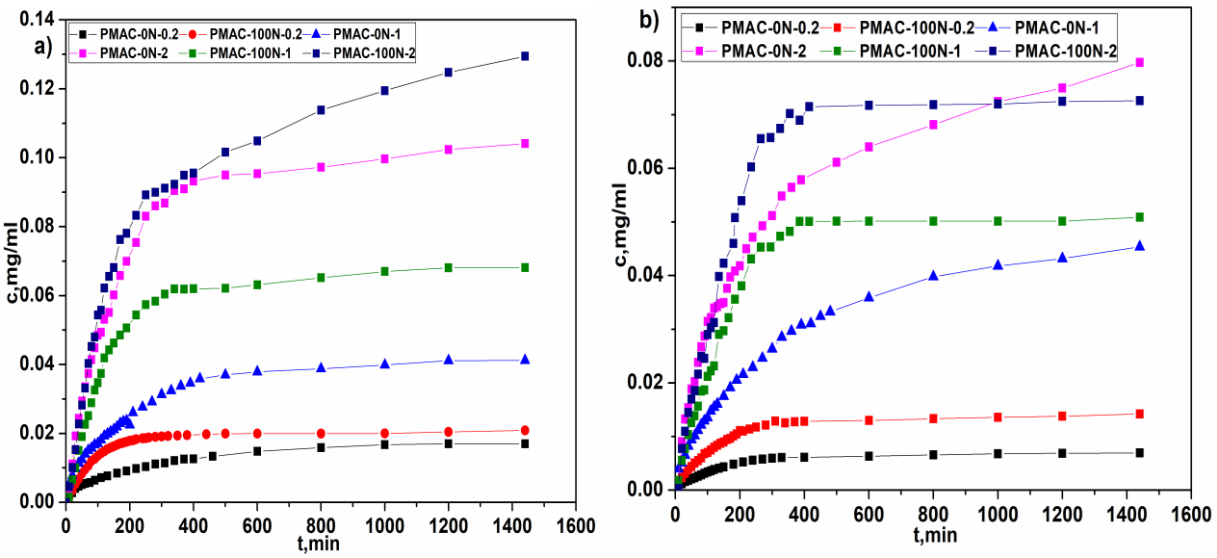

Figure 2. The profiles of caffeine release from PMAC-Caf hydrogels in: a) PB 6.8 and b) 0.1M HCl

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