Effect of neutralization degree of methacrylic acid on hydrogel swelling and drug release

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ISSN 2712-1267
UDC: 544.773.432:676.017.66
https://doi.org/10.7251/JCTE2203001P
Original scientific paper

Paper received: 21 Oct 2022
Paper accepted: 24 Dec 2022
Paper available from 30 Dec 2022
at https://glasnik.tf.unibl.org/

Keywords:
hydrogels,
casein,
liposomes,
neutralization degree,
release kinetics.

Drug delivery system is an amazing tool which is widely used for drug protection and its controlled release in order to enhance drug bioavailability, reduce side effects and therefore to improve overall therapy. Hydrogels have been attracted great attention as drug carriers due to their great physicochemical properties, similarity to the living tissues and biocompatibility. One group of pH sensitive hydrogels are based on poly(methacrylic acid) (PMAA). These non-toxic hydrogels are used as drug delivery system because they swell as a response to the change in pH of external environment and drug is being released during the process. In present study, in order to improve the control of drug release rate, caffeine was encapsulated in liposomes which were further embedded into PMC hydrogel (PMCL). It was investigated how the change in neutralization degree of methacrylic acid affect the swelling degree of PMCL hydrogels and caffeine release in two environments at 37 °C for 24 h: 0.1 M hydrochloride acid (pH 1) and phosphate buffer with pH value of 6.8 (pH 6.8), as a simulation of pH environment in human stomach and intestines, respectively. Obtained results show that PMCL hydrogels have great potential for controlled release of poorly water-soluble drugs in human intestines.

INTRODUCTION

The researchers are faced with a lot of challenges in their efforts to develop new drugs and improve present ones in order to achieve safer and more efficient therapy of various diseases. Drug delivery systems represent promising tool for drug protection and its controlled release for prolonged period of time at the site of action, due to which drug bioavailability can be improved and side effects can be reduced. Hydrogels are non-toxic and biocompatible polymer materials able to absorb and retain large amount of water of physiologic fluids due to which they are widely applied as drug delivery systems (Markovic et al., 2022). The external medium diffuses into polymer network during the process of hydrogels swelling. At the same time encapsulated drug is released through the pores of the network into the medium. One group of these materials are pH sensitive hydrogels based on poly(methacrylic acid) (PMAA). These hydrogels are able to swell in the environment with pH value which is higher than pKa of methacrylic acid. This is a consequence of the deionisation of carboxylic groups presented along the polymer chains which leads further to the repulsion of the chains and hydrogel swelling. Highly hydrophilic nature of PMAA hydrogels limits their usage only for delivery of hydrophilic drugs. Poor mechanical properties are also one of the factors which affect the PMAA hydrogel application. In our previous research we overcome these limitations by modifying PMAA hydrogels with amphiphilic casein (PMC) (Markovic et al., 2019; Markovic et al., 2020). Casein is non-toxic, biocompatible and pH sensitive natural polymer which enabled the encapsulation of poorly water-soluble model drug - caffeine into PMAA hydrogel and its controlled release (Markovic et al., 2020). Mechanical properties of PMAA hydrogels were also improved by the addition of casein. In order to prolong the caffeine release and to improve the control of its release rate, in present study caffeine was first encapsulated into the liposomes, which were further embedded into the PMC hydrogels (PMCL). It was investigated how the neutralization degree of MAA affected the swelling behaviour of PMCL hydrogels and release rate of caffeine in two environments with different pH values, which simulated human stomach and intestines.

MATERIALS AND METODS

Methacrylic acid (MAA) (99.5%) was chosen as monomer and was supplied from Merck, Germany. Sodium caseinate (CSNa) powder (88.9 wt% of protein)

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was purchased from Lactoprot Deutschland GmbH, Germany. NATIPIDE® containing phospholipids from soybean >20% (with 3-sn-phosphatidylcholine 76+3%) was purchased from Lipoid, Germany. The initiator, 2, 2'-azobis-[2-(2-imidazolin-2- yl)propane] dihydrochloride (VA-044) (99.8%) was purchased from Wako Pure Chemical Industries, Japan. Caffeine (poorly water-soluble model drug) was purchased from Merck, Germany. The crosslinker - N, N'-methylenebisacrylamide (MBA) (p.a.) and sodium hydroxide (p.a.) (NaOH) were supplied from Aldrich Chemical Co, USA. Hydrochloride acid (37%) was obtained from Zorka Pharma, Serbia. Monobasic sodium phosphate (anhydrous) (NaH_2PO_4) and dibasic sodium phosphate (anhydrous) (Na_2HPO_4) were supplied from Centrohem, Serbia. All chemicals were used as received.

Preparation of liposomes suspension with encapsulated caffeine was carried out via following procedure. The aqueous solution of caffeine (20 mg/mL) was gradually added to the NATIPIDE® (10 wt% with respect to final liposomes suspension) under continuous stirring. The mixture was stirred for additional 20 min. Obtained liposomes suspension was further used for synthesis of the PMCL samples.

The PMCL samples were prepared in following manner. Total volume of reaction mixture for each sample was 20 mL. Four milliliters of MAA were dissolved in distilled water and liposomes suspension were added dropwise under continuous stirring at 25 °C. The volume ratio of liposomes suspension and distilled water were 50:50. The certain amount of sodium hydroxide was dissolved in reaction mixture of the samples with certain neutralization degree of MAA (50 % and 100 %). Then, the temperature of reaction mixture was elevated to 40 °C followed by the addition and dissolution of four grams of casein. Further, MBA (0.4 mol% with respect to the monomer) was dissolved and VA-044 (0.9 mL of 1 wt% aqueous solution) was poured. The reaction mixture was stirred for additional 15 minutes and instantly poured into the glass moulds (plates, 15 x 15 cm, separated by a 3 mm thick PVC hose) which were left in the air oven at 60 °C for 5 h. After polymerization and crosslinking were finished, the disc-shaped samples (7 mm in diameter) were cut and dried at room temperature. The samples were denoted as PMCL-xN, where xN represented the neutralization degree of MAA (0 %, 50 % i 100 %).

The degree of caffeine encapsulation into liposomes (%) (used for preparation of PMCL samples) was determined using the UV-Vis Shimadzu UV-1800 spectrophotometer. The supernatant, which was obtained after the liposomes suspension with caffeine was centrifuged, was used for determination of encapsulation degree of caffeine in liposomes (*E*):

$$E = ((mct - mcs)/mct) \times 100 \quad (1)$$

where mct (g) is the total caffeine weight in the liposomes suspension and mcs is the caffeine weight in the supernatant determined by using the UV-Vis spectrophotometer (g).

The analysis of the influence of MAA neutralization degree on swelling behavior of PMCL was performed in two media for 24 h: 0.1 M hydrochloric acid (pH 1) and phosphate buffer with pH value of 6.8 (pH 6.8), as a simulation of pH environment in human stomach and intestines, respectively (Markovic et al., 2021). The temperature of the media was set on 37 °C as simulation of physiological temperature. PMCL xerogels were weighted (ms, g), immersed into each medium and the process of their swelling was followed: the each sample was removed from the medium at specified time moment, its mass was determined (mtp, g) and the sample was then returned into the medium. This procedure was repeated until equilibrium state was reached (me, g). The swelling degree of the PMCL samples (SD) was calculated according to the following equation:

$$SD = (mtp - ms)/ms \quad (2)$$

The equilibrium swelling degree (SDeq) of the PMCL samples was determined by replacing the mtp with me in Eq. (2).

The analysis of caffeine release from the PMCL hydrogels was carried out under the same experimental conditions as the analysis of swelling behavior of PMCL. The volume of each medium in which sample was immersed was 100 mL. At predefined time intervals, 3 mL of the medium was withdrawn, analyzed by UV-Vis spectrophotometer and then returned into the glass where release experiment was conducted. The absorbances were measured at 273 nm due to the maximal absorption which caffeine showed at this wave length.

RESULTS AND DISCUSSION

The encapsulation degree of caffeine in liposomes suspension (used for the preparation of PMCL samples) was 84.3 %. This value was high taking into account reported values of the encapsulation degree of various drugs into liposomes (Pentak, Maciążek-Jurczyk, & Zawada, 2017).

The swelling curves of the PMCL samples in pH 1 and pH 6.8 are presented in Fig. 1. a) and b), respectively. The equilibrium swelling degree of the PMCL samples in PH 6.8 was around 10 times higher than in pH 1. pH value of the pH 6.8 medium is higher than pKa of MAA

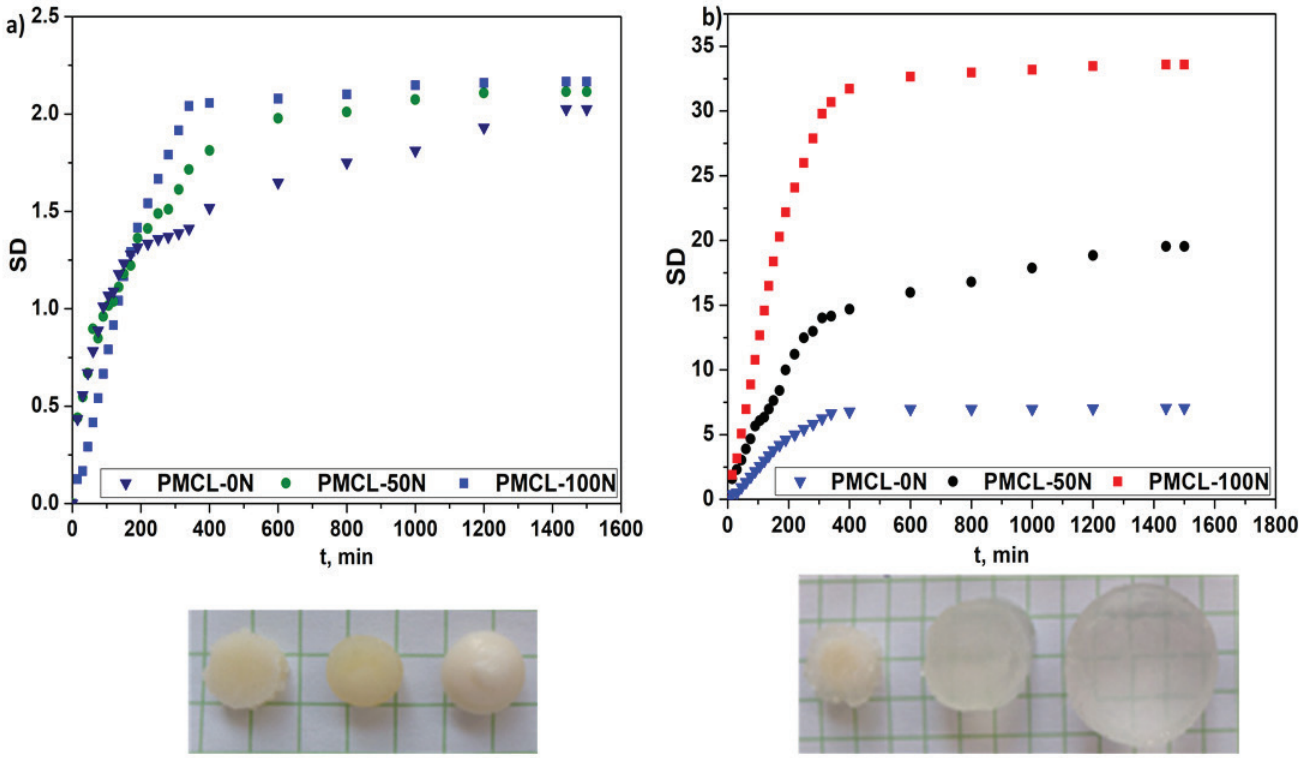

Figure 1. Swelling curves of PMCL hydrogels in: a) pH 1 and b) pH 6.8 (the pictures illustrate swollen PMCL samples in equlibrium state; at each picture from left to right: PMCL-0N, PMCL-50N and PMCL-100N)

and casein (4.6) (Markovic et al., 2020), so deionization of the carboxylic groups presented along the polymer chains occurs. This further lead to the generation of negative charge and repulsion of the polymer chains due to which hydrogel network expands and hydrogel swells. The increase in the neutralization degree of MAA led to the increase in the number of deionized species. Consequently, hydrophilicity of the hydrogels network increased and hydrogel swelled. The addition of the liposomes into the hydrogels led to the decrease in the swelling degree of the samples. Namely, in our previous research the samples of the same composition but without the liposomes, were prepared and their SDeq values were around 1.5 times higher than those of the PMCL samples. This can be attributed to the hindered diffusion of the external medium into the hydrogels because the liposomes occupied the pores of the polymer network (Markovic et al., 2020).

The curves of caffeine release from the PMCL samples in pH 1 and pH 6.8 are shown in the Fig. 2. a) and b), respectively. It can be concluded that the amount of caffeine released in PH 6.8 was higher than in pH 1. This was a consequence of the pH dependent swelling of the PMCL samples. The deionization of the carboxylic groups and repulsion of the polymer chains led to the higher SDeq values in pH 6.8 than in pH 1. This further led to the faster release of caffeine from the hydrogels in pH 6.8. It can be also concluded that the liposomes facilitated better control of the release rate of caffeine (Marković et al., 2019).

Obtained results show that PMCL samples due to its nontoxicity, biocompatibility, pH sensitivity and easily tunable properties have potential for controlled release of poorly water-soluble model drug - caffeine in the human intestines. The PMCL samples can protect poorly water-soluble drug and release it in the intestines where its absorption is the highest providing in that manner more efficient and safer therapy.

CONCLUSIONS

In present study hydrogels based on poly(methacrylic acid), casein and liposomes with encapsulated poorly water-soluble model drug – caffeine (PMCL hydrogels) were successfully synthesized via free radical polymerization. Swelling behavior of the PMCL hydrogels and caffeine release were investigated in two media with different pH values at 37 °C for 24 h: 0.1 M hydrochloride acid (pH 1) and phosphate buffer with pH value of 6.8 (PH 6.8), as a simulation of pH environment in human stomach and intestines, respectively. The pH dependent swelling of the PMCL hydrogels was confirmed: equilibrium swelling degree of the samples was around 10 times higher in pH 6.8 than in PH 1. As

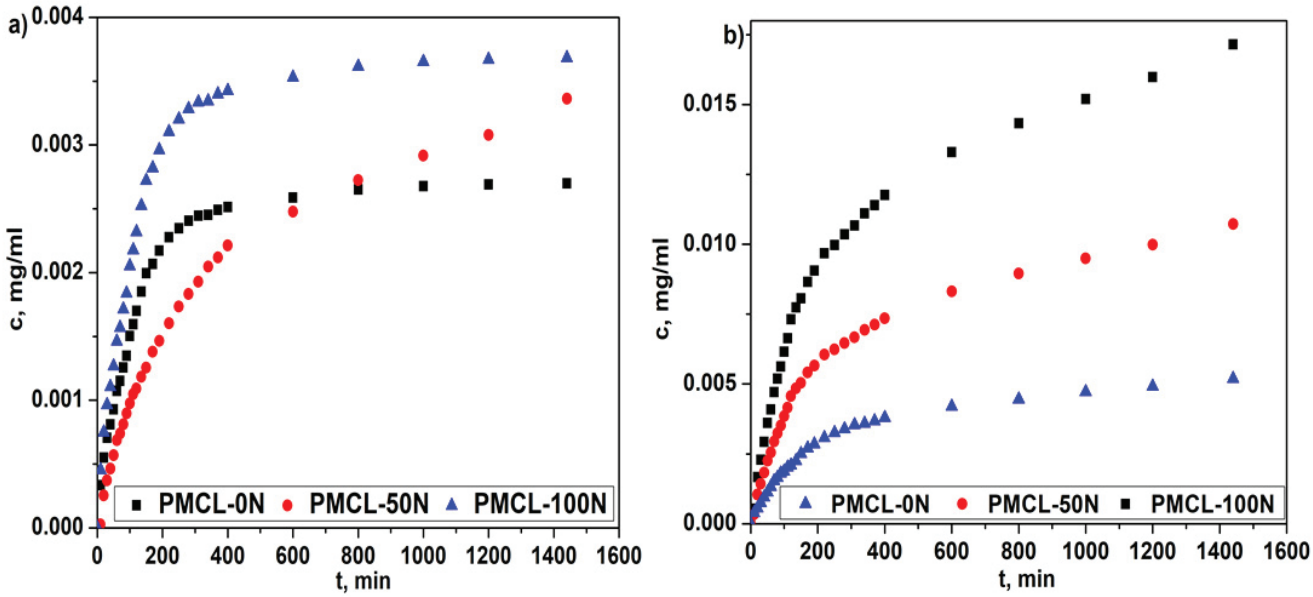

Figure 2. Caffeine release from PMCL hydrogels in: a) PH 1 and b) PH 6.8

a consequence, the amount of released caffeine was 1.5 times higher in pH 6.8 than in PH 1. The increase in the neutralization degree of MAA led to the increase in the hydrophilicity of hydrogels network and therefore to the increase in the equilibrium swelling degree of the samples. Consequently, the amount of released caffeine increased, as well. The SDeq of the PMCL samples were slightly lower than the SDeq of the same samples which did not have embedded liposomes. This could be a consequence of the presence of the liposomes in the pores, which hindered diffusion of external medium into the hydrogels network. Also, the addition of the liposomes improved the control of caffeine release rate. Controlled release of caffeine was achieved for 24 h. Obtained results show that PMCL hydrogels have potential for controlled release of poorly water-soluble active substance in human intestines.

Acknowledgement: *This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200287).*

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