Poly(methacrylic acid) hydrogels crosslinked by poly(ethylene glycol) diacrylate as pH-responsive systems for drug delivery applications

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Abstract

Hydrogels are attractive materials for drug delivery applications due to biocompatible, porous structure with the possibility to load and deliver drugs in a controllable manner. In this paper, poly(methacrylic acid) (PMAA) hydrogels are described, which are synthesized by free-radical polymerization, using poly(ethylene glycol) diacrylate (PEGDA) as a crosslinker. Influence of the PEGDA content on hydrogel properties was investigated and compared to commonly used crosslinker - *N,N*'-methylenebisacrylamide (MBA). The increasing concentration of crosslinkers led to a higher degree of crosslinking, which was demonstrated by a higher degree of conversion, lower swelling capacity, and improved thermal stability and mechanical properties. Also, the PEGDA-crosslinked hydrogels demonstrated a higher degree of crosslinking than the corresponding MBA-crosslinked hydrogels. Potential application of the synthesized hydrogels for controlled drug delivery was investigated by using two model drugs - oxaprozin and ciprofloxacin. In vitro drug release tests indicated that the interactions between drug, polymer and medium have a key influence on the drug release behavior, rather than the swelling rate. Drug release tests in simulated gastrointestinal conditions indicated that PEGDA-crosslinked PMAA hydrogels are suitable for colon-targeted delivery of oxaprozin.

Keywords: smart materials; colon-specific delivery; ciprofloxacin; oxaprozin.

Available on-line at the Journal web address: http://www.ache.org.rs/HI/

ORIGINAL SCIENTIFIC PAPER

UDC: 544.773.432+77.018.62:615.012

Hem. Ind. **77(4)** 235-249 (2023)

1. INTRODUCTION

Hydrogels are three-dimensional crosslinked networks based on hydrophilic polymers, which have been extensively applied in a wide range of different fields. Due to their unique properties such as high porosity, permeability and ability to absorb high amounts of water without being dissolved, hydrogels have found practical application in cosmetics and personal care [1], drug delivery [2,3], enzyme immobilization [4,5], but also in the food industry [6], agriculture [7,8], water treatment [9,10], sensors and actuators [11], *etc.* Due to the structure resembling living tissues, biocompatibility and the possibility to incorporate cells and drugs, hydrogels have been largely explored for biomedical applications [12-15]. Development of stimuli-responsive hydrogels has opened the door to new approaches and strategies in controlled drug delivery. Stimuli-responsive hydrogels can be designed to react in response to different external stimuli which include, but are not limited to changes in pH [16-18], temperature [19,20], and ionic strength [21], as well as exposure to light [22], magnetic and electric fields [23]. Among the family of stimuli-responsive hydrogels, pH-sensitive ones have been particularly interesting for applications as self-regulatory drug delivery systems (DDS) [24]. Due to the presence of ionizable side groups, properties of pH-sensitive hydrogels can be rapidly changed in response to changes in the pH of the environment.

Poly(methacrylic acid) (PMAA) is an ionizable, hydrophilic polymer that is frequently used for fabrication of pH-sensitive materials as it imparts remarkable swelling/deswelling properties due to the protonation/deprotonation effect of carboxylic groups present in the polymer structure [25]. This effect causes low swelling of PMAA hydrogels in acidic environments and remarkably higher swelling in neutral/basic conditions, which can be effectively used for gastro-

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Paper received: 28 December 2022; Paper accepted: 18 July 2023; Paper published: 18 September 2023.
https://doi.org/10.2298/HEMIND221228018U

intestinal drugs to reduce the effect of the harsh gastric conditions and increase the bioavailability of the drug [26]. However, due to the soft nature and rapid degradation of physically crosslinked hydrogels in the physiological environment, hydrogels have been frequently crosslinked by using chemical crosslinkers, which may compromise biocompatibility of the hydrogels. Specifically, PMAA hydrogels, as well as different vinyl-based polymers applied in biomedicine, such as poly(acrylic acid) and poly(acrylamide) have been typically crosslinked by using *N*,*N*'-methylenebisacrylamide (MBA), ethylene glycol diacrylate and ethylene glycol dimethacrylate, which are known to be toxic [27-31]. MBA has been proven to be an effective crosslinker for PMAA hydrogels, however, unreacted molecules of MBA may cause toxicity when applied *in vivo*. Subsequent rinsing of the hydrogels to remove the unreacted molecules prevents the possibility of pre-polymerization drug loading, as the drug will leak out of the hydrogel during the rinsing. Therefore, one of the simplest solutions for this problem is to use crosslinkers which are non-toxic and safe for *in vivo* applications.

Poly(ethylene glycol) diacrylate (PEGDA) is a derivative of poly(ethylene glycol) which is one of the most applied materials in pharmacy and biomedicine due to its biocompatibility, non-toxicity, hydrophilicity and relatively good mechanical properties [32]. Terminal acrylate groups of PEGDA provide creation of bifunctional radicals during the free radical polymerization process, used to crosslink vinyl-based hydrogels [33-35]. Nevertheless, PEGDA-crosslinked vinyl-based hydrogels have been scarcely reported in literature. For example, there has been only one study reporting the PEGDA-crosslinked PMAA hydrogels [36]. In this study, PEGDA-crosslinked PMAA nanoparticles were synthesized and investigated for controlled release of 5-fluorouracil. Even though the obtained PMAA nano-gels demonstrated a pH-sensitive drug release, a stable, three-dimensional structure could not be formed, which may narrow the possible range of biomedical applications. Therefore, synthesis of three-dimensional PMAA hydrogels crosslinked by PEGDA, and investigation of the PEGDA influence on the properties of such hydrogels would be highly beneficial for these applications.

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) is a synthetic fluoroquinolone-based antibiotic, which has a good antibacterial activity against gram-positive and gram-negative bacteria. It has been widely used to treat various infections such as skin and bone infections, gastrointestinal tract (GIT) infections caused by multiresistant pathogens, sexually transmitted diseases, complicated urinary infections, lower respiratory tract diseases, pneumonia, *etc.* [37] However, the use of ciprofloxacin has been associated with serious adverse effects, while traditional administration methods are characterized by low bioavailability, and do not provide prolonged drug release. In addition, most of the ciprofloxacin is degraded by lysosomes before killing bacteria, which usually results in low intracellular drug concentration [38]. Therefore, development of a DDS that can provide sustained and controlled delivery of ciprofloxacin is of great practical significance.

On the other hand, oxaprozin is a propanoic acid non-steroidal anti-inflammatory drug (NSAID) that has intrinsic advantages over similar NSAIDs widely applied for the treatment of musculoskeletal inflammatory diseases [39]. It is as effective as aspirin but applied at significantly lower doses [40]. However, oral administration of NSAIDs could impose serious adverse effects on the gastrointestinal system. Most of the complications are related to the gastroduodenal area and include gastroduodenal subepithelial hemorrhages, erosions and ulcerations. These pathological conditions may lead to death, and impose substantial health risks and economic burden [41]. DDS which can provide controlled drug delivery to a lower GIT may reduce the risks related to NSAIDs oral administration.

The aim of this work was to synthesize PEGDA-crosslinked PMAA hydrogels (PMAA-P) and to investigate their potential application for controlled release of ciprofloxacin and oxaprozin. Hydrogels were synthesized by thermally induced free-radical polymerization and crosslinked by different amounts of PEGDA. In parallel, hydrogels with the same amounts of commonly applied MBA were also synthesized (PMAA-M), for comparison with the novel PMAA-P. The final aim was to investigate the selected optimal PMAA-P hydrogel for controlled ciprofloxacin and oxaprozin release.

2. MATERIALS AND METHODS

2. 1. Materials

Methacrylic acid (MAA) (99.5 %) was supplied from Merck KgaA (Germany). PEGDA (M_n = 700), MBA and ciprofloxacin were obtained from Sigma Aldrich (USA). The initiator, 2,2'-azobis[2-(2-imidazoline-2-yl)propane]

dihydrochloride (VA-044) (99.8 %) was supplied by Wako Pure Chemical Industries (Japan). All chemicals were used as received.

2. 2. Synthesis of hydrogels, oxaprozin and drug-loaded composite hydrogels

PMAA hydrogels were synthesized *via* thermally induced free radical polymerization. 0.4 cm^3 of methacrylic acid (MAA), and desired amount of crosslinker were dissolved in 1 cm^3 of distilled water, and the solution was stirred for 5 min. In the last step, 2 mg of initiator (VA-044) was added, and the solution was stirred for additional 3 min before it was poured into a Teflon mold and placed in an oven at 65 °C for 5 h to complete the reaction. The amount of crosslinker (PEGDA or MBA) added corresponded to the crosslinker/polymer molar ratio of 0.1, 0.2, 0.5 and 1.0 mol.% and were denoted as PMAA-XY, where X = M for MBA or P for PEGDA, and Y = 0.1, 0.2, 0.5 or 1.0 corresponding to the mol.% of the crosslinker added.

Oxaprozin was synthesized by the previously described procedure [42,43].

Drug-loaded hydrogels were synthesized by the same procedure with the addition of 4 mg of the drug (ciprofloxacin or oxaprozin). The obtained hydrogels were cut into equal cylinder-shaped samples and further prepared according to the testing method requirements.

2. 3. Characterization

2. 3. 1. Degree of conversion

The degree of monomer conversion (DC) into the gel phase was determined by using the equation:

$$\mathrm{DC} = \frac{m_0}{m_\mathrm{p}} \tag{1}$$

where m_0 is the mass of the xerogel obtained by drying hydrogels to constant mass after 24 h of swelling in phosphate buffered saline (PBS) at pH 7.4 and t = 37 °C, and m_p is the mass of the xerogel obtained by drying hydrogels to constant mass right after the polymerization process.

2. 3. 2. Determination of the swelling degree and water content

Dynamic swelling measurements were carried out in alternating acidic (pH 1.2) and basic conditions (pH 7.4), as well as in simulated GIT conditions. Acidic conditions were simulated by HCl/KCl buffer, while phosphate buffer solution (PBS) (pH 7.4) was used for basic conditions. During the swelling in GIT, hydrogels were initially swollen in HCl/KCl buffer which simulated stomach conditions (pH 1.2), and after 2 h were transferred to PBS (pH 6.8), which simulated the small intestine conditions where they were kept for 2 h. Subsequently, they were transferred to PBS (pH 7.4), which mimicked colon- conditions for 6 h [44]. The experiments were performed by immersing three samples from different batches of the same type of hydrogel (approximately 3×0.1 g of dry hydrogel in 10 cm^3 of the appropriate liquid) to obtain an average value for at least three batches of the same hydrogel. At regular time intervals, samples were removed from the swelling medium and weighed, after which the samples were returned to the medium again The swelling ratio (SR) of hydrogels was calculated using the equation [28]:

$$\mathrm{SR} = \frac{m}{m_0} \tag{2}$$

where m was the mass of the swollen hydrogel at a specific time. The equilibrium swelling ratio (ESR) was calculated using the same equation except that m_{eq} (mass of the hydrogel swollen at equilibrium *i.e.* after not changing significantly during three consecutive measurements) was used instead of m. ESR measurements were carried out in HCl/KCl buffer (pH 1.2), acetate buffer (pH 4.0) and PBS (pH 7.4). The point of ESR was established.

2. 3. 3. Differential scanning calorimetry measurements

Thermal analysis of the PMAA-M0.1, PMAA-M1.0, PMAA-P0.1 and PMAA-P1.0 xerogels was performed by using a differential scanning calorimeter - DSC (DSC-60Plus differential scanning calorimeter, Shimadzu, Japan). Before the DSC

analysis, the xerogels were ground into powder. The weight of the samples was limited to 3±0.2 mg and all DSC measurements were carried out using hermetic aluminum pans. All the samples were first heated from -50 to 0 °C at 10 °C min^{-1} under a nitrogen purge gas flow of 30 cm^3 min^{-1}. After this treatment, the thermal analysis of the samples was conducted by heating samples from 25 to 300 °C at 10 °C min^{-1} under the same nitrogen purge gas flow rate. The DSC analysis of the M1, M4, P1 and P4 xerogels was conducted according to the procedure reported in the literature [45]. This procedure was adopted for the analysis of the effect of the change in the crosslinker amount on the thermal stability of the hydrogels based on PMAA.

2. 3. 4. Mechanical properties

Mechanical properties of the obtained hydrogels were evaluated by using the Universal Testing Machine AG-Xplus (Shimadzu, Japan), equipped with a 1000 N force load cell (force range from 0.01 to 1000 N). Before testing, cylinder-shaped samples (8.0±1.0 mm in diameter and 8.0±1.0 mm in height) were swollen to equilibrium in PBS at 37 °C. Compression was performed until the sample failure, at a compression rate of 10 mm min^{-1}. Automatic detection of the contact between the plate and hydrogel was performed by setting a contact force of 0.05 N. The values of compressive strength were determined at the point of failure, while the compressive modulus was calculated by measuring the slope of the stress-strain curve within 0-10 % of deformation. At least three specimens were tested for each hydrogel type and the mean values are presented.

2. 3. 5. Drug release

Drug release behavior was examined in HCl/KCl buffer (pH 1.2) and PBS (pH 7.4) at 37 °C, as well as in simulated GIT conditions as described in 2.3.2. The obtained drug-loaded hydrogel samples were cut into four cyllinder-shaped samples (R = 1 cm, H = 2 cm, $m \approx 1$ g) and immersed in 20 cm^3 of buffer. The aliquots of 2 cm^3 were taken out at specific time intervals and analyzed for drug using Shimadzu UV-1800 UV/Vis spectrophotometer (Shimadzu, Japan), after which they were returned to the medium. The obtained drug concentrations at certain intervals were converted into masses, and curves of cumulative drug release *vs* time were ploted.

3. RESULTS AND DISCUSSION

3. 1. Synthesis and chemical structure of PMAA hydrogels

PMAA hydrogels were synthesized *via* thermally induced free-radical polymerization using MAA as a functional monomer, VA-044 as the initiator and MBA or PEGDA as crosslinking agents. Both MBA and PEGDA are bifunctional crosslinkers with terminal acrylate groups on both sides of their molecules. During free-radical polymerization, when the acrylate group of MBA or PEGDA is attacked by a growing macromer radical, it is incorporated into the macromolecular chain and covalently bonds to monomer units. When both ends of crosslinker groups are incorporated in two independent macromolecular chains, they become crosslink points and the obtained hydrogel is covalently crosslinked. As the number of crosslinking points increases, the network becomes more rigid and mechanically stronger. On the other hand, the unreacted crosslinker molecules may not only affect mechanical properties, but they can also reduce the biocompatibility of the hydrogels. However, PEGDA is a non-toxic compound and its potential leaking from a hydrogel would not cause harmful effects on human health [46]. Structures of MBA and PEGDA, as well as the schematic of the free-radical polymerization reaction are presented in Figure 1.

3. 2. Degree of conversion

The degree of conversion (DC) of monomers into a polymer network directly affects the physical and mechanical properties of the synthesized hydrogels, as it shows how much MAA monomer was incorporated into the structure of the polymer. To determine the DC, masses of xerogels obtained after the synthesis were measured and compared with the xerogel masses obtained after 24 h of rinsing of the hydrogels in PBS at 37 °C.

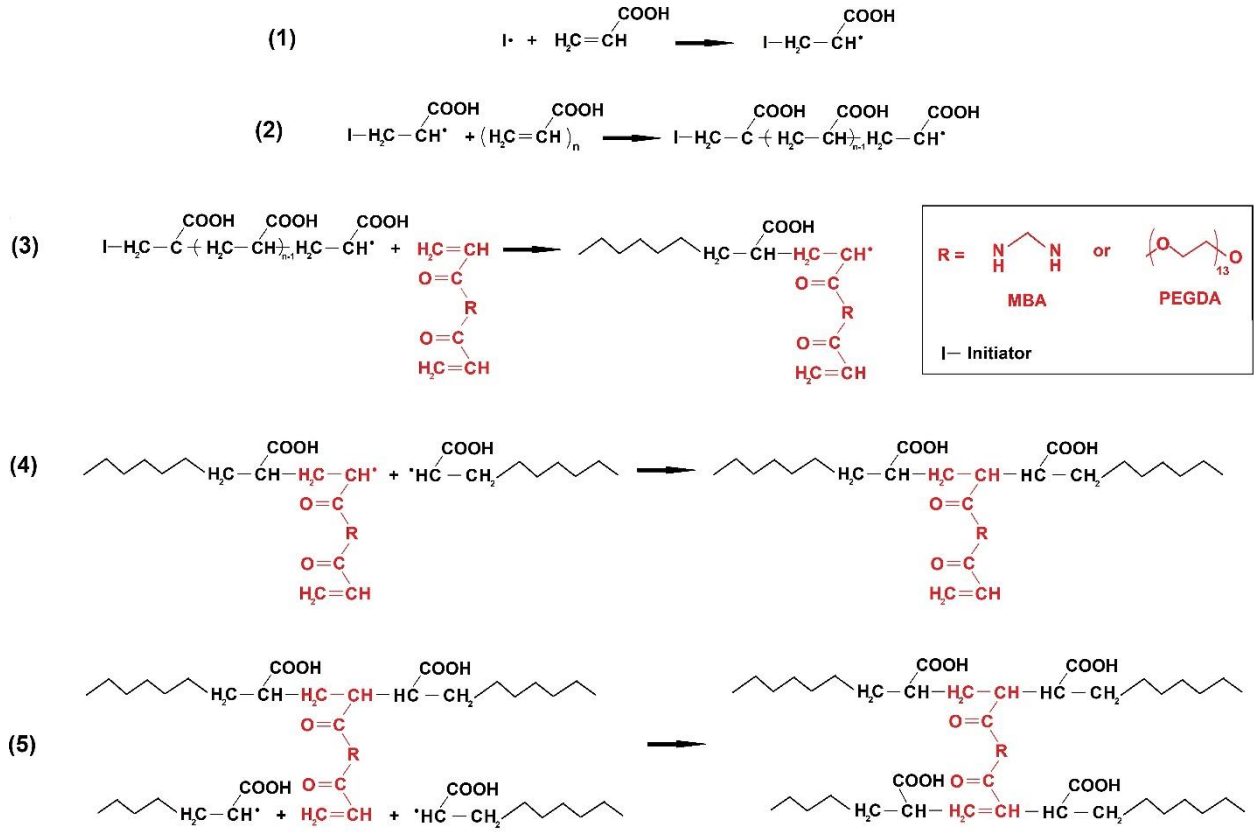

Figure 1. *Simplified schematic of free-radical polymerization of MAA and creation of three-dimensional crosslinked hydrogels: (**1**) initiation -creation of MAA radicals; (**2**) propagation -addition of MAA monomers to a growing chain; (**3**) incorporation of a vinyl group of the crosslinker to the growing chain; (**4**) termination - the reaction of the growing chain with another growing chain; (**5**) reaction of the second vinyl group of the crosslinker with another growing chain -creation of crosslinking points*

The obtained results indicated high polymerization degrees of all hydrogels, as the masses of xerogels after synthesis were similar to the theoretical ones (the total mass of monomer, crosslinker and initiator). On the other hand, the masses of xerogels after rinsing were lower and were highly dependent on the crosslinker type and content. Figure 2 demonstrates the dependence of DC on MBA and PEGDA content.

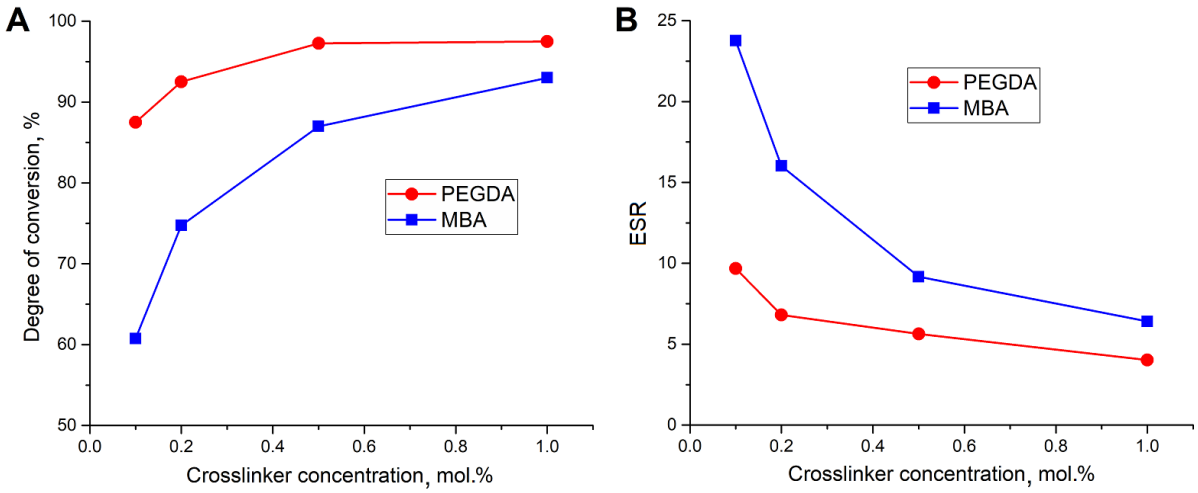

Figure 2. *Influence of the crosslinker content on DC (A) and ESR (B)*

It can be observed that the increase in crosslinker content yielded hydrogels with higher DC. Moreover, the influence of PEGDA on DC was significantly higher compared to that of MBA, at the same molar concentrations. Knowing the fact that all hydrogels had a high degree of polymerization, *i.e*. that practically negligible amounts of unreacted monomer molecules were left after the polymerization reaction, the mass loss after rinsing could be the consequence of smaller and less crosslinked polymer chains, which could easily diffuse out of the structure. Therefore, the increase in crosslinker content resulted in a more interconnected network and a lower amount of soluble phase (unreacted monomer and crosslinker, non-crosslinked oligomeric and polymeric chains) [47]. As higher DC of PMAA-P hydrogels were obtained compared to those of PMAA-M, we assume that PEGDA molecules had a higher tendency to react with free radicals and eventually get incorporated in the growing chains as explained in Section 3. 1., which resulted in efficiently crosslinked hydrogel networks. This can be explained by the lower reactivity of acrylamides compared to methacrylates [48].

3. 3. Swelling studies

Swelling ability is one of the most important characteristics of hydrogels aimed for controlled DD since this property could affect the delivery profiles of active compounds. It is also an indication of the crosslinking density and can be used for evaluation of crosslinking degree of hydrogels. Swelling ability of hydrogels was determined after 24 h of swelling in PBS at 37 °C. In the case of both crosslinkers, the increase in crosslinker content led to decreased ESR of the hydrogels, indicating a higher degree of crosslinking (Fig. 2B) [25]. The ESR of PMAA-P hydrogels was lower than those of PMAA-M opposite to our initial hypothesis that using PEGDA as a crosslinker would yield hydrogels with higher ESR due to longer, flexible crosslinking molecules. However, these results were in agreement with DC results, and the reason for this behavior could be that not all molecules of MBA were incorporated into the hydrogel network, which led to a lower crosslinking degree than it would be initially assumed [47]. To confirm the efficacy of the PEGDA as a crosslinking agent, PMAA hydrogel without any crosslinker added was also synthesized, which completely dissolved under the applied conditions, as it was only physically crosslinked through hydrogen bonds. This confirmed that PEGDA can be effectively applied to crosslink the PMAA network.

3. 4. DSC analysis

DSC thermograms of the synthesized hydrogels, demonstrated in Figure 3, exhibited two endothermic peaks, the sharp one in the range from 193 to 207 °C (T_1) and the wide one, around 240°C.

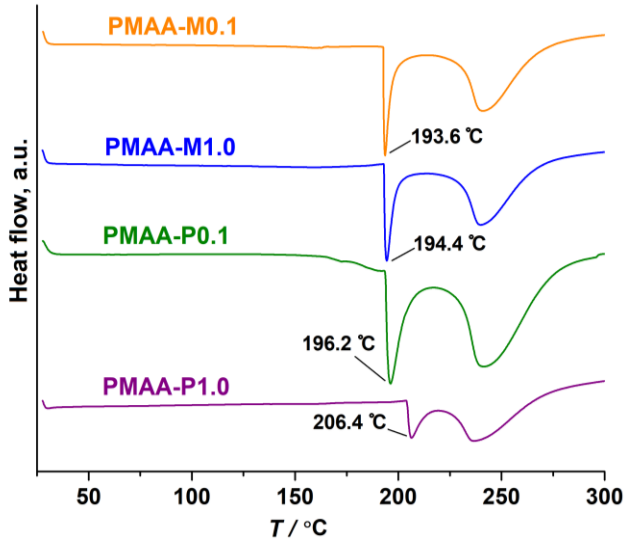

Figure 3. *DSC curves for PMAA hydrogels crosslinked with different amounts of MBA and PEGDA*

Both peaks were ascribed to different degradation phases of the hydrogel networks. As it is well-known from the thermogravimetric analysis (TGA) of PMAA, the first peak refers to dehydration of carboxyl groups and formation of cyclic polyanhydride [49], while the second is related to further degradation of the main PMAA chains, including

decarboxylation, depolymerization, and "decrosslinking" processes [45,46]. The increase in crosslinker content shifted the first degradation stage towards higher temperatures indicating the increase in thermal stability of more crosslinked hydrogels. Additionally, if the effects of the crosslinker type are compared, the samples crosslinked with PEGDA exhibited higher thermal stability than the corresponding analogs crosslinked with MBA. For example, when the MBA content increased 10-fold, the T_1 increased for 0.8 °C, while the 10-fold increase in the PEGDA content led to the T_1 increase of more than 10.4 °C. The increase in the thermal stability of hydrogels was another evidence of successful and efficient crosslinking.

3. 5. Mechanical properties

Compressive mechanical properties of the hydrogels were investigated by using the unconfined compression test. Stress-strain curves of hydrogels are plotted in Figures 4A and B, while the trends of compressive modulus (E_c) and compressive strength (σ_c) *vs*. PEGDA and MBA concentrations are presented in Figures 4C and D.

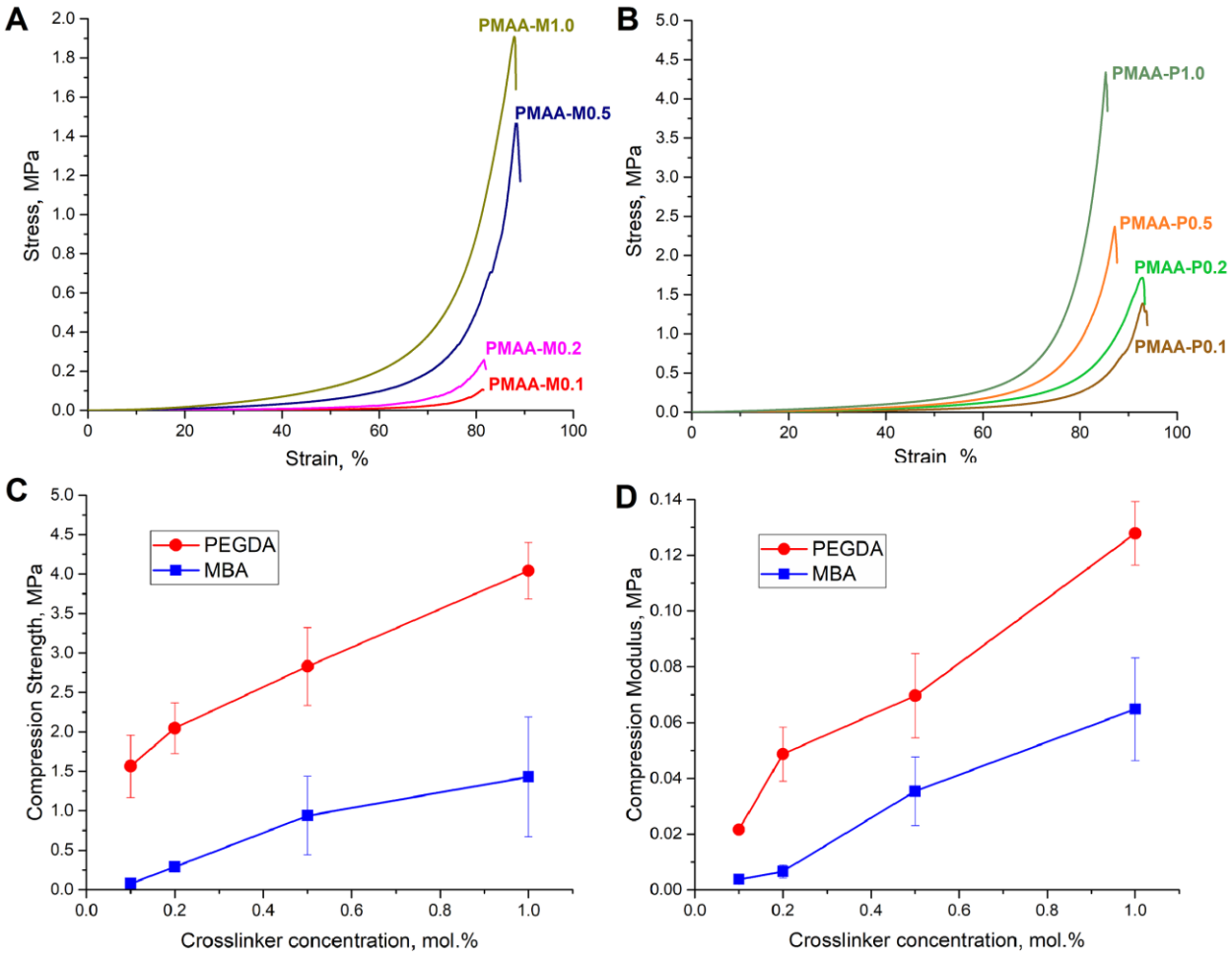

Figure 4. *Stress-strain curves of hydrogels with different contents of MBA (A) and PEGDA (B). The influence of crosslinkers content on the compression strength (C) and compression modulus (D) of the hydrogels*

Compressive strength and compressive modulus significantly increased as the crosslinker concentration increased in both cases. For example, the values of σ_c and E_c of PMAA-M1.0 were 20-fold greater than the corresponding values of PMAA-M0.1, while the σ_c and E_c of PMAA-P1.0 were 2.5- and 6-fold greater than the corresponding values of PMAA-P0.1. In both cases, compression strength and modulus values had almost linear dependences on the crosslinker content. If the influence of different crosslinkers is compared, it could be noticed that using PEGDA yielded hydrogels with significantly higher compression strengths and moduli, which agrees with the DC and swelling results. A hydrogel

crosslinked to a lower degree with higher water content is characterized by lower mechanical properties as compared to the same type of hydrogel crosslinked at a higher degree of croslinking with less water in the structure. Compression strength values for the obtained PMAA-P hydrogels were of the same order of magnitude as the value of the peak stress exerted on a human knee (a human weighing 90 kg exerts 2.5 MPa pressure on the knee during walking [51]), which confirmed the mechanical competence of these materials for biomedical applications.

3. 6. pH sensitivity

In this study, pH-sensitive swelling behavior of hydrogels was investigated in the physiologically relevant pH range of 1.2 to 7.4, at 37 °C, as well as in simulated GIT conditions. The hydrogels were also subjected to an oscillatory swelling-deswelling study to investigate the reversibility and response rate of the swelling process in acidic and basic conditions. The ESR as a function of the pH, for hydrogels with different types and concentrations of crosslinker, is presented in Figures 5A and 5B.

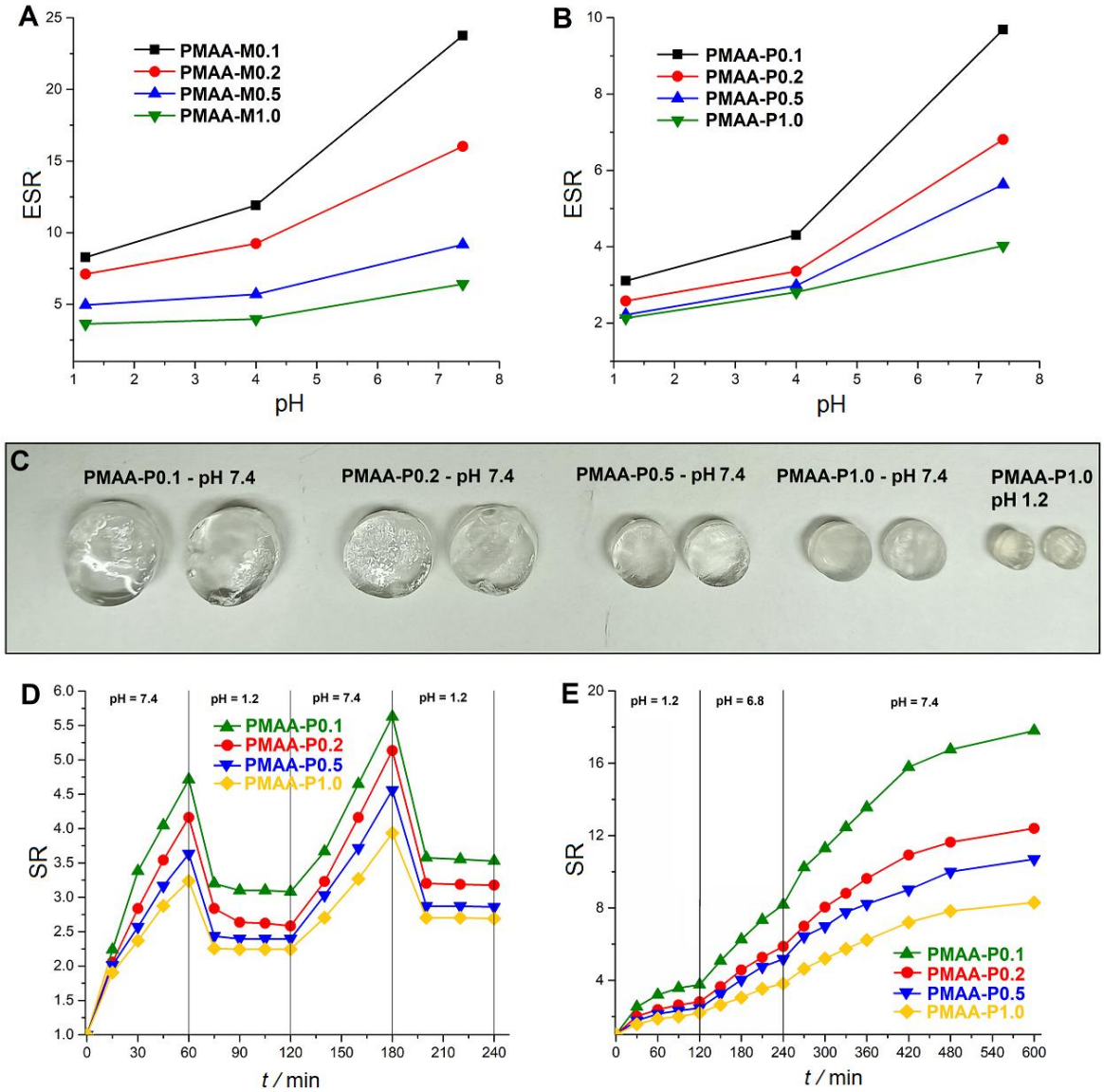

Figure 5. *Dependence of ESR on pH for hydrogels crosslinked by different amounts of MBA (**A**) and PEGDA (**B**). Photographs of PMAA-P hydrogels after swelling in pH 7.4 and pH 1.2 (**C**). Swelling of PMAA-P hydrogels under alternating basic-acidic conditions (**D**) and simulated GIT conditions (**E**)*

All hydrogels demonstrated pH-sensitive behavior during swelling. At pH 1.2, which is below the pK_a value of PMAA (≈4.8), the ESR was low for all hydrogels. As the pH value of the environment exceeded the pK_a value of PMAA, carboxylic

groups became ionized. This caused repulsive forces between negatively charged carboxylate ions and contributed to a higher ionic density, which led to higher ESR. The effect was more pronounced as the concentration of crosslinker decreased. In general, the MBA-crosslinked hydrogels demonstrated markedly higher ESR compared to PEGDA, due to a structure crosslinked to a lower extent. Nevertheless, using PEGDA as a crosslinker did not affect the pH-sensitive nature of the PMAA hydrogels. Figure 5C presents photographs of PMAA-P hydrogels after attaining the swelling equilibrium at pH 7.4, as well as PMAA-P1.0 hydrogel after swelling at pH 1.2.

The oscillatory swelling-deswelling study on PMAA-P hydrogels demonstrated rapid swelling in basic conditions, followed by rapid deswelling in acidic conditions (Fig. 5D). The reversibility of hydrogel swelling slightly decreased with a decrease in the crosslinker content. This was due to higher stretching of macromolecules and formation of new physical bonds in the hydrogel structure under basic conditions, preventing full relaxation under the applied acidic conditions. This effect was more pronounced when the molecular stretching was higher as was the case with less crosslinked hydrogels.

Swelling results obtained in simulated GIT conditions are presented in Figure 5E. The values of SR were relatively low at pH 1.2, but they significantly increased when the samples were transferred to buffers with higher pH values. Lower swelling capacity of PMAA-P hydrogels in acidic pH, and higher swelling capacity in slightly alkaline pH are very advantageous for design of colon-specific drug delivery systems. Such systems could thus protect the drug from the harsh gastric environment and enable controlled delivery in the small intestine and colon. These drug release profiles are also very beneficial for delivery of drugs which can impose serious negative effects on the upper GIT.

3. 7. Drug release studies

Applicability of the obtained PMAA-P hydrogels as DDS for controlled delivery of ciprofloxacin and oxaprozin was investigated *in vitro* under different pH conditions, and the results are presented in Figure 6. The *in vitro* ciprofloxacin release from the hydrogels at pH 7.4 indicated that the crosslinker content had a major influence on the ciprofloxacin release kinetics (Fig. 6A). Increase in the crosslinker content led to a slower ciprofloxacin release, due to the limited diffusion through the more crosslinked network. On the other hand, the crosslinker content and correspondingly the crosslinking degree, did not have a significant influence on the oxaprozin release (Fig. 6B). Hydrogels crosslinked to a lesser degree (*i.e.* PMAA-P0.1 and PMAA-P0.2) had a more pronounced burst effect, however, after 30 h they released a slightly higher amount of drug than the PMAA-P0.5 and PMAA-P1.0 hydrogels. The pH value influenced the ciprofloxacin release rate, so that the total released amount of this drug was almost 5-fold higher at pH 1.2 compared to pH 7.4 (Fig. 6C), even though the swelling tests demonstrated almost 2-fold lower ESR at pH 1.2 compared to pH 7.4. Obviously, the swelling capacity did not have a major influence on the ciprofloxacin release in this case, and diffusion was not the rate-determining step.

It can be assumed that the drug solubility had the main influence on the drug release based on the acid/base equilibrium, as well as the interactions between the drug, medium and the polymer. Ciprofloxacin has two main pK_a values corresponding to two main proton binding sites - the carboxyl group (pK_1 = 5.89) and the nitrogen atom in the piperazine ring (pK_2 = 8.61). Thus according to the pH of the environment, ciprofloxacin can be in the form of cation, anion, or zwitterion [52]. On the other hand, the pK_a of methacrylic acid is 4.65. As shown in Figure 7, when the pH value of the environment was 1.2, which is lower than the pK_a of carboxyl groups on the polymer chains, these carboxyl groups were protonated and in a neutral state, while the molecules of ciprofloxacin were in cationic form, which prevented electrostatic interactions between molecules. At the same time, due to the high solubility of ciprofloxacin in the acidic environment, weak physical bonds between the molecules of ciprofloxacin and hydrogel matrix were relatively easily broken, and ciprofloxacin passed into the medium. In this case diffusion was the only limiting step for the drug to reach the outer solvent, and diffusion rate was dependent on the swelling rate. On the other hand, when the pH of the medium was 7.4, most of the polymer carboxyl groups were ionized and in the anionic form, while ciprofloxacin was in a zwitterionic state, which allowed electrostatic interactions between positively charged groups of ciprofloxacin and negatively charged groups of the hydrogel polymer. Taken into account also the low solubility of ciprofloxacin in basic conditions, this resulted in a low drug release rate, as the drug molecules preferred interactions with the hydrogel, instead of the medium [36].

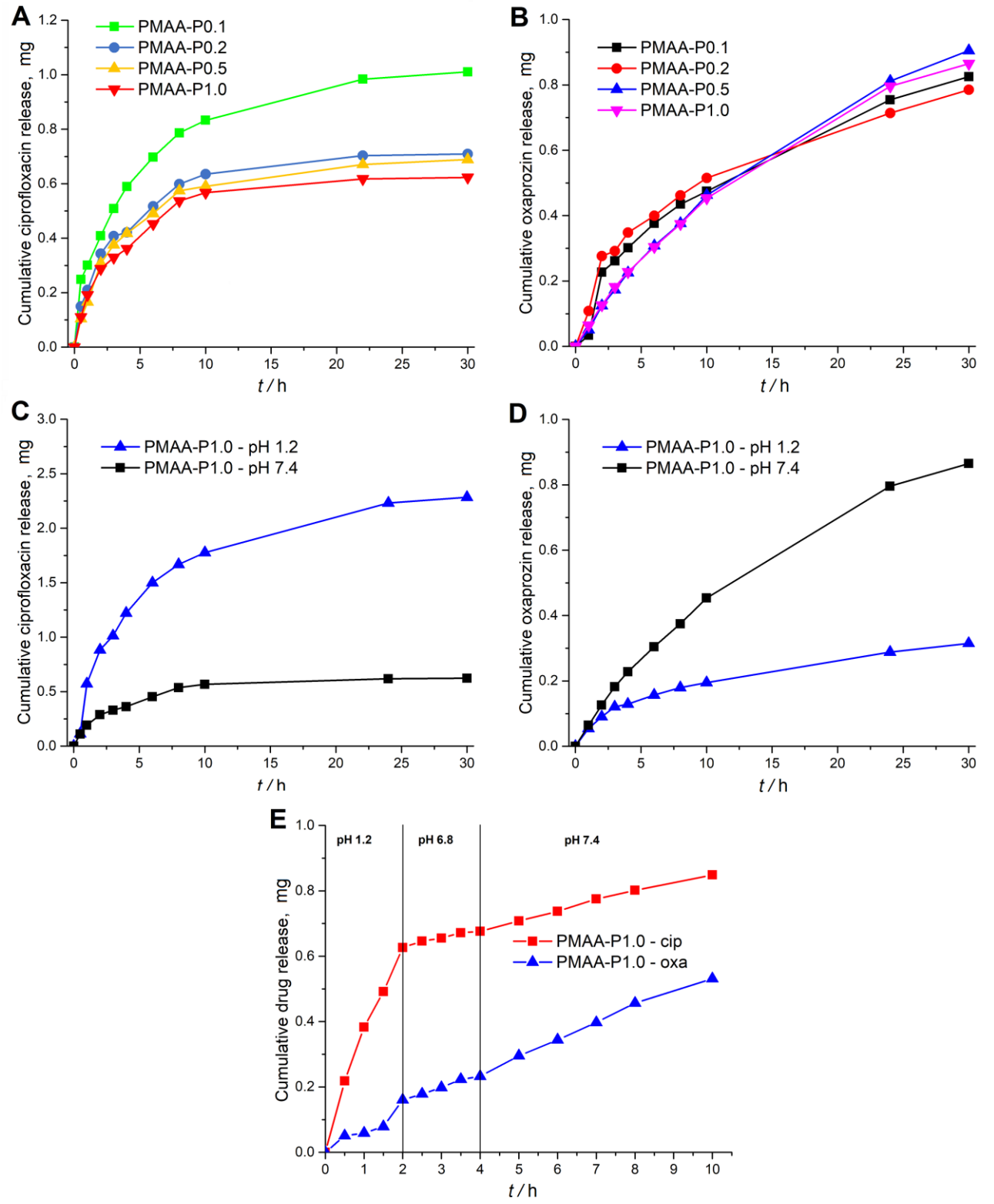

Figure 6. *Cumulative in vitro ciprofloxacin (**A**), and oxaprozin (**B**) release from hydrogels with different contents of PEGDA at* pH *7.4. Cumulative in vitro ciprofloxacin (**C**) and oxaprozin (**D**) release from the PMAA-P1.0 hydrogel at different pH conditions. The in vitro ciprofloxacin and oxaprozin release from the PMAA-P1.0 hydrogel in simulated GIT conditions (**E**)*

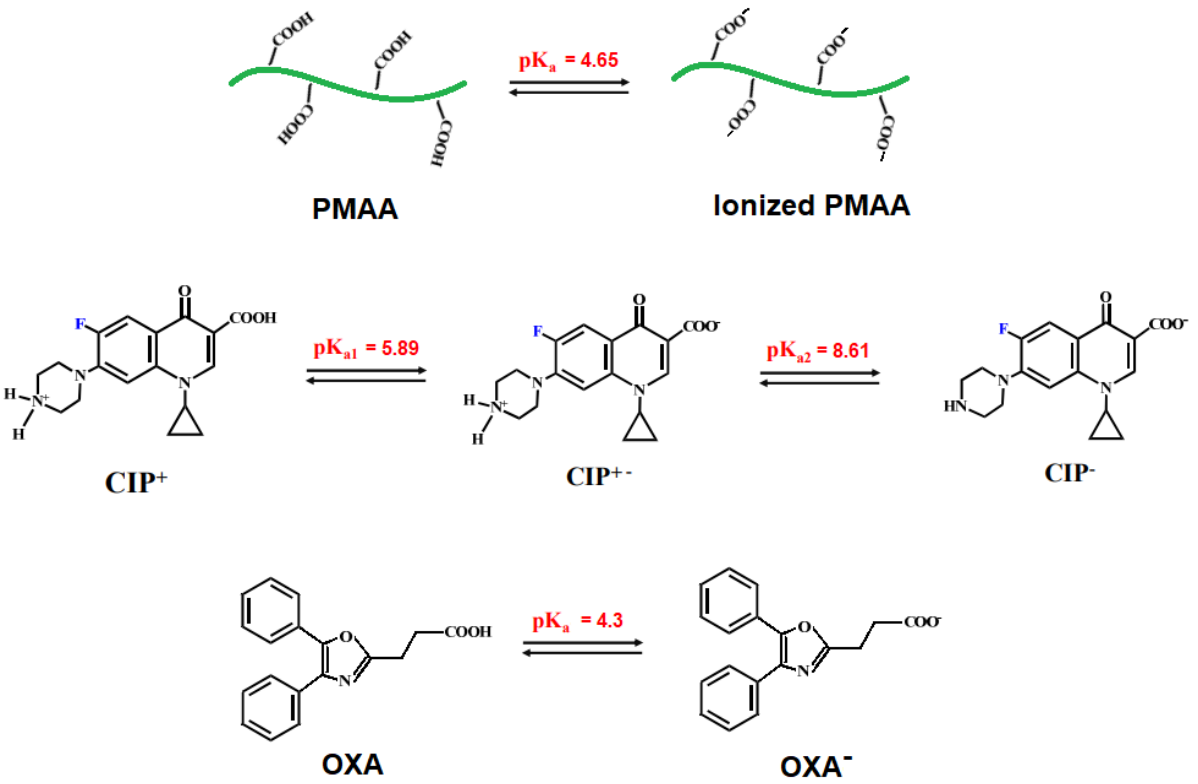

Figure 7. *Structures of the molecules of PMAA, ciprofloxacin (CIP) and oxaprozin (OXA), and their respective ionization states*

However, the situation was reversed in the case of oxaprozin release. The oxaprozin release rate and the total amount of the released drug were significantly higher at pH 7.4 as compared to pH 1.2 (Fig. 6D). In acidic conditions both, the hydrogel and oxaprozin were in the protonated state (the pK_a of oxaprozin is 4.3) and could establish interactions by hydrogen bonds. Moreover, oxaprozin is insoluble in aqueous solutions at pH 1.2, thus its release was additionally hindered. In contrary, when the pH of the medium was 7.4, carboxylic groups in the hydrogel and oxaprozin were in the form of negatively charged carboxylate anions (Fig. 7) that repelled each other, which in combination with the high solubility of oxaprozin in basic conditions led to a significantly higher drug release rate.

Comparison of oxaprozin and ciprofloxacin release curves under different pH conditions demonstrates higher release rates in the case of ciprofloxacin, which indicates higher release tendency of this drug under the applied *in vitro* conditions. This result can be assigned to the ability of this molecule to be in different ionic states.

The *in vitro* oxaprozin and ciprofloxacin release in simulated GIT conditions followed similar trends as in corresponding pH conditions (Fig. 6E). For example, during the first two hours of release in simulated gastric conditions (at pH 1.2), the PMAA-P1.0 hydrogel released more than 0.6 mg of ciprofloxacin, which was more than 70 % of the released amount during 10 h of the experiment at this pH. This means that most of ciprofloxacin would be released in the stomach. On the other hand, during the first 2 h of release in simulated gastric conditions, the same hydrogel released only 30 % of the total amount of oxaprozin released during the experiment at pH 1.2. Most of this drug (≈ 60 %) was released when the hydrogel was transferred to simulated colonic conditions (pH 7.4). The obtained results indicated that PEGDA-crosslinked PMAA hydrogels were more suitable for colon-targeted delivery of oxaprozin in comparison with ciprofloxacin.

4. CONCLUSION

Novel PEGDA-crosslinked PMAA hydrogels were successfully synthesized and investigated for drug delivery applications. Increase in the PEGDA content significantly improved the degree of conversion, compressive mechanical properties and thermal stability of the PMAA hydrogels, while reducing the swelling capacity. The experiments

confirmed an effective crosslinking of PMAA hydrogels by this crosslinker, which is nontoxic and relatively biocompatible. The hydrogels retained the pH-sensitive behavior, which was confirmed by oscillating swelling-deswelling experiments, and swelling studies in simulated GIT conditions. Drug release profiles from the hydrogels demonstrated dependence on the physicochemical properties of the released drug, as a consequence of different physical interactions between the functional groups of the matrix and drug, as well as between the drug and medium. Overall, the presented work demonstrated the possibility to apply PEGDA as a crosslinker for vinyl-based hydrogels, which would be greatly beneficial especially in biomedical applications. Furthermore, such hydrogels can be effectively applied as a vehicle for controlled drug delivery in the gastrointestinal tract.

Acknowledgements: The authors would like to acknowledge the financial support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contracts No. 451-03-68/2022-14/200287, 451-03-68/2022-14/200135, 451-03-9/2021-14/200026 and 451-03-68/2022-14/200178).

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Хидрогелови на бази поли(метакрилне киселине) умрежени коришћењем поли(етилен-гликол) диакрилата, као pH-осетљиви носачи за контролисано отпуштање лекова

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(Научни рад)

Извод

Због своје биокомпатибилне, високо-порозне структуре и могућности да носе и контролисано отпуштају лекове, хидрогелови су нашли широку примену у биомедицини. У овом раду, хидрогелови на бази поли(метакрилне киселине) (ПМК), умрежени помоћу поли(етилен-гликол) диакрилата (ПЕГДА), синтетисани су методом топлотно-индуковане слободно-радикалске полимеризације. Испитан је утицај садржаја ПЕГДА на својства хидрогелова и упоређен са утицајем најчешће коришћеног умреживача – *N,N'* – метиленбисакриламида (МБА). Повећање количине оба умреживача довело је до већег степена умрежења, што је било манифестовано повећањем степена конверзије мономера, смањењем равнотежног степена бубрења и побољшаним топлотним и механичким својствима. Такође, хидрогелови умрежени помоћу ПЕГДА показали су већи степен умрежења у односу на одговарајуће хидрогелове умрежене помоћу МБА. Могућност примене добијених хидрогелова за контролисано отпуштање лекова, испитивано је коришћењем два лека – оксапрозина и ципрофлоксацина. In vitro тестови отпуштања показали су да пресудан утицај на кинетику отпуштања имају међусобне интеракције између лека, хидрогела и медијума, а не степен и брзина бубрења хидрогела. Према томе, отпуштање ципрофлоксацина је било интензивније у киселој средини, а оксапрозина у базној. Отпуштање у симулираним гастроинтестиналним условима показало је да су ПЕГДА-умрежени хидрогелови погодни за контролисано отпуштање оксапрозина у дебело црево..

Кључне речи: Паметни материјали; колон-циљано отпуштање лекова; ципрофлоксацин; оксапрозин