Studies on the Loading and Release of Metformin HCl Using Hydrogels with EGDMA and MBA as Crosslinkers

Ariyaldi Ariyaldi[1], Noverra Mardhatillah Nizardo[1*], and Maria Lucia Ardhani Dwi Lestari[2,3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok 16424, Indonesia*

[2]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia*

[3]*Pharmaceutical Material Engineering and Processing Research Group, Faculty of Pharmacy, Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia*

* ***Corresponding author:***

email: noverra.mardhatillah@sci.ui.ac.id

Received: April 30, 2024
Accepted: October 16, 2024

DOI: 10.22146/ijc.95826

Abstract: *Hydrogel poly(*N*-vinylcaprolactam-*co*-2-(dimethylamino)ethylmethacrylate) or (P(NVCL-*co*-DMAEMA)) containing the active drug metformin HCl were synthesized using the free radical polymerization method in the presence of DMAEMA and NVCL monomers by using two different crosslinkers: ethylene glycol dimethacrylate (EGDMA) and methylene bisacrylamide (MBA). FTIR spectra confirmed the successful formation of hydrogel and its loading with metformin HCl. SEM analysis revealed the physical shape and surface features. Regarding physical appearance, the texture of the hydrogel is sticky and elastic. When the hydrogel was immersed in a solution, it swelled, and returned to its original shape after drying. Additionally, the chemical degradation temperature is thermally stable at 329.75 °C. The loading test results showed that the active drug in EGDMA was 18.38 (% w/w) and 26.19 (% w/w) in MBA as crosslinkers, loaded within 24 h. The drug-loaded hydrogel containing EGDMA as crosslinkers in pH 7.4 had the highest drug release in 24 h compared to MBA. Then, the drug released from hydrogel with EGDMA in pH 2 had the highest drug release than MBA within 30 min. The P(NVCL-co-DMAEMA) hydrogel has the potential for a drug delivery system. However, additional optimizations are necessary to improve the efficiency of the hydrogels as a carrier.*

Keywords: *hydrogel; ethylene glycol dimethacrylate; methylene bisacrylamide; metformin HCl; controlled release*

■ INTRODUCTION

The purpose of controlling the drug release system is to deliver the active drug to its target within the body, enabling it to function effectively and efficiently. One approach that can be taken is using hydrogel as a carrier of active drugs. Hydrogel has received attention from many researchers for further development as a substance that can deliver active drugs. Hydrogel is a three-dimensional polymer structure that can uptake a massive volume of water or biological fluids, leading to swelling up to thousands of times its original volume when dry [1]. Responsive hydrogel has characteristics that respond to temperature or pH. Therefore, hydrogel has applications in the pharmaceutical field as a controlled-release oral dosage form [2-3]. In this research, poly(*N*-vinylcaprolactam) (PNVCL) was used. It is a thermoresponsive polymer with unique properties such as biocompatibility and solubility. PNVCL has the cloud point transition (T_c) value of 32–34 °C [4]. It enables the utilization of PNVCL in biomedical applications due to its temperature and pH-responsive [5].

Furthermore, pH-responsive hydrogels are synthesized from polymers containing functional groups that can donate or accept protons in response to pH fluctuations [6]. In acid conditions, the tertiary amine group will be protonated in its group and vice

versa [7]. One example of a polymer that is responsive to pH is poly(2-dimethylamino)ethylmethacrylate) (PDMAEMA). Under acidic conditions, PDMAEMA will increase its solubility, which causes the hydrophilic effect on the PDMAEMA structure to be more dominant [8].

During the synthesis of hydrogels, the use of crosslinkers in the hydrogel structure must be considered. The properties of a crosslinker deeply influence the swelling capacity of a hydrogel [9]. As reported by Podaru et al. [10], in the free radical polymerization method for synthesizing hydrogels, methylene bisacrylamide (MBA) has been used as a crosslinker monomer and has shown advantages compared to alternative crosslinkers. Bashir et al. [11] reported using MBA for crosslinking in a hydrogel copolymeric system for controlled drug delivery. The result showed the drug loading and release reached 85% and 77%, respectively, at pH 7.4. The extensive use of MBA as a crosslinker in hydrogel development underscores its critical role in advancing to control the amount of release. Meanwhile, various studies have widely observed that ethylene glycol dimethacrylate (EGDMA) is commonly used as a crosslinker in the preparation of hydrogels. EGDMA has been employed in the synthesis of hydrogels for more applications, such as targeted drug release. Minhas et al. [12] prepared the hydrogels by using EGDMA and they showed a drug release of roughly 80% for 48 h under a pH 7.4 phosphate buffer.

Similarly, the research reported by Bukhari et al. [13], showed that the drug release rate was the highest in a phosphate buffer with a pH of 7.5. Those studies extensively show the versatility and applicability of EGDMA as a crosslinker in the synthesis of hydrogels. Its effectiveness has been observed in drug delivery and biomedical applications because EGDMA, as a crosslinker, is a biocompatible molecule [14]. From the studies mentioned above, EGDMA and MBA are two general crosslinkers used in hydrogel synthesis because they have unique chemical structure characteristics. Yet, no research has compared EGDMA and MBA in terms of their swelling capacity and drug release.

In a previously reported study, the use of MBA and triethyleneglycol dimethacrylate (TEGDA) in PDMAEMA hydrogel was compared for the drug release PDMAEMA hydrogel by comparing the release of drug-loaded by using the crosslinkers between MBA and TEGDA [15]. Taktak et al. [6] observed another swelling behavior and release of the drug using the PDMAEMA hydrogel that they synthesized via an *in-situ* polymerization reaction. However, research has not been conducted on the loading and release of active drugs using EGDMA and MBA as crosslinkers in P(NVCL-*co*-DMAEMA) hydrogels.

Therefore, this study aimed to explore the design and application of P(NVCL-*co*-DMAEMA) hydrogel crosslinked with EGDMA and MBA as carriers to control the release of drugs. Metformin HCl was used as a model active drug due to its good solubility in water but low bioavailability. Its low bioavailability may be attributed to factors such as limited absorption [16-17]. Metformin HCl has a bioavailability of 50–60%; around 30% to 50% of the administered oral dosage is eliminated unchanged through urine within 24 h, while approximately 30% is excreted through feces. The pKa values of metformin HCl are 2.8 and 11.5, with a hydrophilic nature, favoring its existence as a cationic species in physiological pH conditions [18]. This work focused on comparing the use of crosslinkers, EGDMA and MBA, to study which crosslinkers worked better in loading and releasing the metformin HCl. This research also investigated the swelling capacity of hydrogel, focusing on the different crosslinkers that responded to temperature and pH to give further information related to the drug loading and release.

■ EXPERIMENTAL SECTION

Materials

The materials used in this research were metformin HCl (Granules India Limited, India) as a model active ingredient, NVCL (98% purity, Merck, Germany), DMAEMA (98% purity, Sigma Aldrich), azobisisobutyronitrile (AIBN, 98% purity, Sigma-Aldrich), potassium dihydrogen phosphate (KH_2PO_4), 99% purity, Merck), dipotassium hydrogen phosphate (K_2HPO_4, 99% purity, Merck), sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), EGDMA (Merck),

MBA (Merck), distilled water, ethanol, and nitrogen gas. All materials were used as received.

Instrumentation

The instrumentation used to characterize the chemical structure of the hydrogels was attenuated total reflection Fourier transform infrared (ATR-FTIR, Thermo Scientific Nicolet iS50) + NIR spectrometer instrumentation. Differential scanning calorimetry (DSC) was used to investigate the glass transition temperature (T_g) and exotherm of the hydrogel. The loading and release of the active drug were determined by a UV-visible spectrophotometer (Shimadzu UV-1800). The morphology of the hydrogel was performed using scanning electron microscopy (SEM). A water bath shaker (Memmert WNB10) was used for the release study.

Procedure

Synthesis of P(NVCL-co-DMAEMA) hydrogel

Polymerization of hydrogel with the composition P($NVCL_{50}$-*co*-$DMAEMA_{50}$) had been conducted using ethanol via the free radical polymerization technique, as reported by Mishra et al. with some modifications [19]. The synthesis procedure is as follows: NVCL (3.470 g, 25.0 mmol), DMAEMA (3.930 g, 25.0 mmol), and EGDMA as crosslinkers (1% or 0.090 g, 0.5 mmol) were dissolved in ethanol as solvent. A similar procedure was also applied when hydrogel was synthesized using MBA as a crosslinker (1% or 0.077 g, 0.5 mmol). Mixing of the solution was conducted until a homogenous mixture was obtained. The mixture was then purged with nitrogen gas for 20 min before adding the initiator, AIBN (1% or 0.082 g, 0.5 mmol). After that, the mixture was placed in an oil bath which was heated to 65 °C. The reaction was conducted for 5 h, stirring using a magnetic stirrer. Unreacted compounds were removed from the hydrogel using hot ethanol and replaced with new hot ethanol after 12 h of the purification process. The hydrogels obtained were dried in an oven at 40 °C until they reached constant weight. The recipe for the hydrogels synthesis can be seen in Table 1.

Determination of equilibrium swelling ratio

The dry hydrogel was weighed and immersed in the distilled water at room temperature. At specific intervals, the continuous swelling gel was removed and wiped using filter paper, then weighed. The swelling rate of the hydrogel was then calculated using Eq. (1), as reported by Mishra et al. [19].

$$\text{Swelling ratio} = \frac{WS - WD}{WD} \times 100\% \quad (1)$$

In the equation, the ratio of WS is the mass of the hydrogel swelling and the ratio of WD is the mass of the dry gel after drying in an oven for 24 h.

Table 1. Recipe for the synthesis of P(NVCL-*co*-DMAEMA) hydrogels

Code	Formulation	Crosslinker
H-1	NVCL-DMAEMA-EGDMA	1% (0.09 g)
H-2	NVCL-DMAEMA-MBA	1% (0.07 g)

pH Responsive properties of hydrogel

Hydrogels were immersed in a phosphate buffer solution with various pH (2.0, 5.0, and 7.4) using the method by Çakal et al. [20]. The hydrogel was immersed in various pH at room temperature for 24 h. Subsequently, the swollen gel was measured and wiped out the sample by filter paper, and hydrogel was measured again. After swelling, the hydrogel was dried in an oven for 24 h at 40 °C. The equilibrium swelling ratio of the hydrogel was determined using Eq. (1) [19].

Temperature responsive properties of hydrogel

The equilibrium of gel swelling in distilled water was conducted at several temperatures (27, 32, 37, 40, and 45 °C) as reported by Çakal et al. [20]. The samples were immersed in distilled water under varying temperatures for 24 h. Subsequently, the swollen gel was weighed and wiped using filter paper, and hydrogel was measured again. The swollen hydrogel was then dried in an oven for 24 h at 40 °C. The equilibrium swelling of the hydrogel at different temperatures was determined using Eq. (1) as reported by Mishra et al. [19].

Drug loading of metformin HCl in hydrogel

Solution of metformin HCl 20,000 ppm was prepared by weighing 10 g of metformin HCl, transferred it into 500 mL of volumetric flask, then dissolved it with buffer phosphate pH 7.4. The pH 7.4 buffer phosphate media consists of K_2HPO_4 and potassium phosphate, which was then adjusted to pH 7.4 with NaOH.

Following this, 20 mL of metformin HCl 20,000 ppm was transferred into a 50 mL Erlenmeyer and weighed to obtain the weight of the solution. The hydrogel which was already cut in small pieces with an approximate weight of 50 mg was then re-weighed to ensure its exact weight. Each hydrogel was then immersed in Erlenmeyer containing metformin HCl. The immersion process was conducted at room temperature for 24 h. After 24 h of loading, the hydrogel containing the active drug was patted to lose the excess water and then dried in the oven at a temperature of 40 °C for 24 h. The excess of metformin HCl in Erlenmeyer was then weighed and recorded. The amount of metformin HCl loaded was calculated by subtracting the weight of the metformin HCl solution in the Erlenmeyer after the immersion with the initial weight, converted it to the volume by using the density of the metformin HCl 20,000 ppm (1.030 g/mL), and calculated the amount of metformin HCl loaded (in mg). Simply, the calculation of the loading test results was determined using the Eq. (2):

$$\text{Drug loading (\%)} = \frac{\text{amount of loading metformin-HCl (mg)}}{\text{the dry weight of hydrogel (mg)}} \times 100\% \quad (2)$$

Release study of metformin HCl

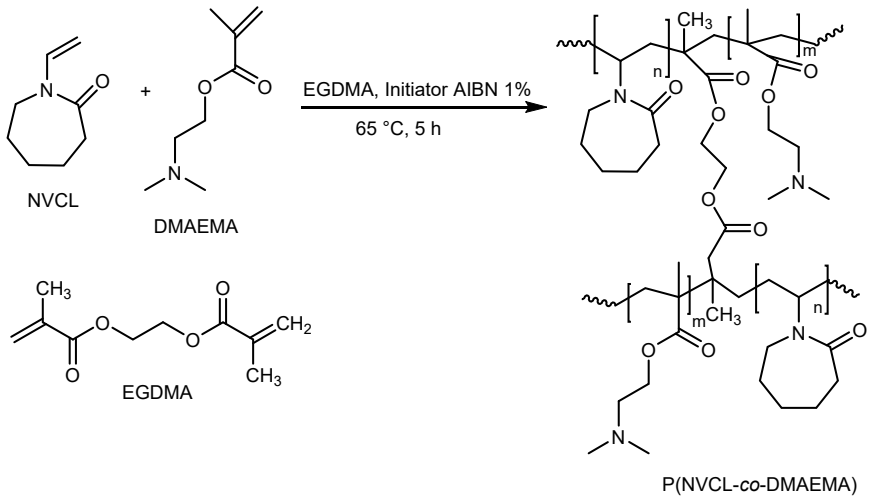
The release of metformin HCl from hydrogel was conducted independently in two different media, buffer pH 2 and 7.4. The buffer pH 2 comprised K_2HPO_4 and potassium phosphate, whilst the buffer pH 7.4 consisted of K_2HPO_4 and potassium phosphate, adjusted with NaOH. Release studies were done in a water bath shaker. Each hydrogel containing metformin HCl was put in a 50 mL release medium in an Erlenmeyer. For the release in pH 2, samples were taken at intervals 30, 60, 90, 120 min. For release in pH 7.4, samples were taken at intervals of 30, 120, 240, 480, and 1440 min. A 5 mL sample was withdrawn at every determined time and replaced with the same volume of release media. Samples were assayed using a UV spectrophotometer at 228 nm. The calculation of the released metformin HCl from hydrogel was determined using the Eq. (3):

$$\text{Drug release (\%)} = \frac{\text{conc. of release results (ppm)}}{\text{conc. of loaded metformin in the hydrogel (ppm)}} \times 100\% \quad (3)$$

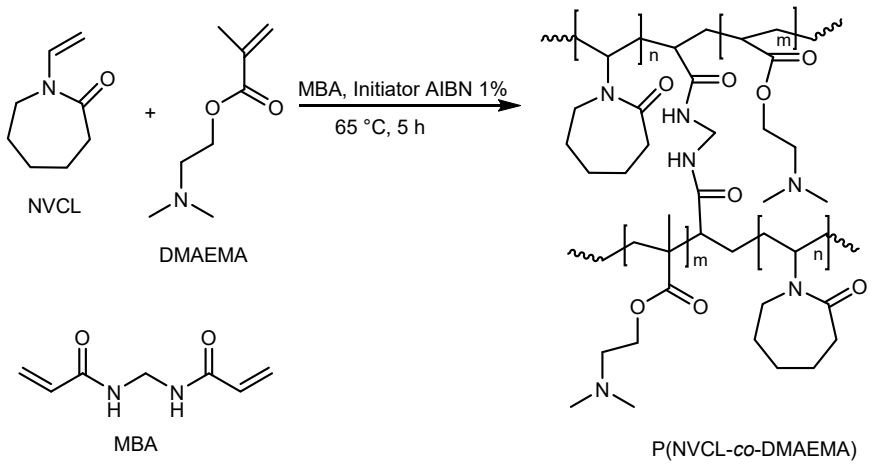
■ RESULTS AND DISCUSSION

Synthesis and Characterization of P(NVCL-*co*-DMAEMA)

The P(NVCL-*co*-DMAEMA) was synthesized by using two crosslinkers separately via free radical polymerization and the hydrogel was characterized by FTIR, DSC, and SEM analysis. The reaction scheme followed is shown in Schemes 1 and 2. The FTIR spectra of the P(NVCL-*co*-DMAEMA) hydrogel using EGDMA and MBA are shown in Fig. 1. By determining the spectra, some differences can be noticed, which indicate that a new compound has been formed. The success of the hydrogel synthesis was indicated by the absence of peak



Scheme 1. Reaction scheme of P(NVCL-*co*-DMAEMA) hydrogel synthesis with EGDMA as a crosslinker



Scheme 2. Reaction scheme of P(NVCL-*co*-DMAEMA) hydrogel synthesis with MBA as a crosslinker

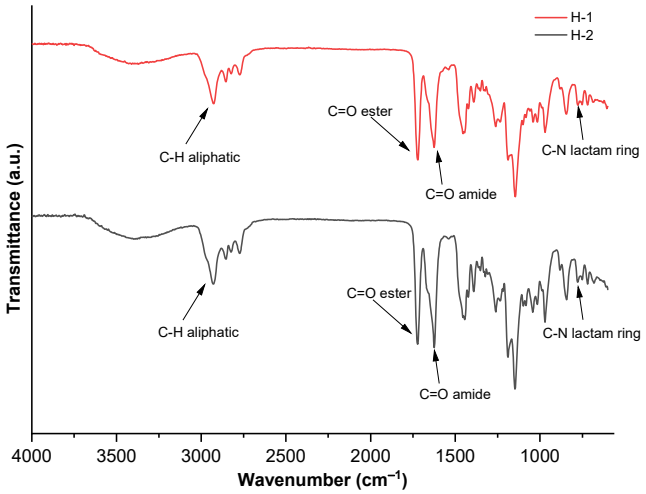


Fig 1. FTIR spectra of P(NVCL-co-DMAEMA) hydrogel using EGDMA and MBA

C=C stretch detected at wavenumbers 3108 and 1658 cm^{-1} [20-21]. Moreover, the disappearance peak of the vinyl group of NVCL and DMAEMA confirmed that there were no monomer impurities remaining in the product and purification was successful. Following this, peak at 1619 and 3270 cm^{-1} the C=O amide peak was detected in the PNVCL structure and the N–H stretch band as reported by Demirel et al. [22]. While at the wavenumber 1718 cm^{-1}, the C=O ester peak was detected in PDMAEMA and it belongs to MBA and EGDMA a similarly as reported by Çakal et al. [20]. As shown in Fig. 2, the spectra of metformin HCl recorded the –NH group from primary amine at the wavenumber of 3366 cm^{-1}, secondary amine at 3179 cm^{-1}, and C–H bending in the range of 1562–1417 cm^{-1}. At 1623 cm^{-1}, there was an absorption from primary –N bending. From the spectra FTIR, it can be observed that the presence of the active drug metformin HCl within the hydrogel showed bands of C–H bending at the wavenumber of 1662 cm^{-1}, the absorption band of primary –N bending at 1564 cm^{-1}, indicating the successful load of the active drugs metformin HCl within the hydrogel. A similar study, at 1472 cm^{-1} spectra from –N primer bending, at 1562 cm^{-1} showed peak –NH bending from imine groups of metformin HCl [23].

In the thermogram of the DMAEMA homopolymer hydrogels, as shown in Fig. 3, the T_g of 72.37 °C was obtained, along with an exothermic peak at 329.89 °C.

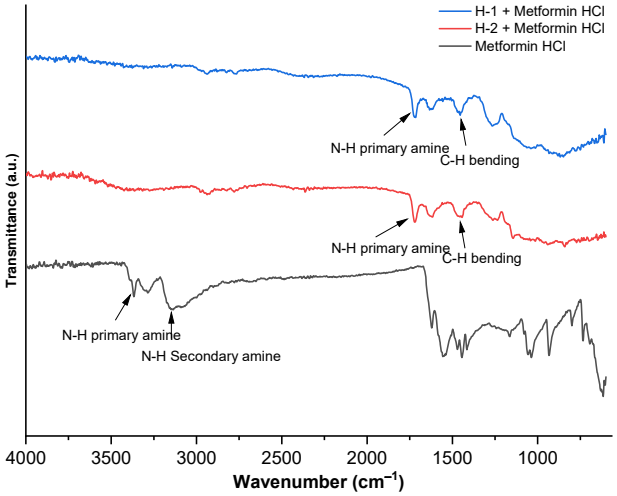


Fig 2. FTIR spectra of metformin HCl, P(NVCL-*co*-DMAEMA) hydrogel using MBA with metformin HCl loaded

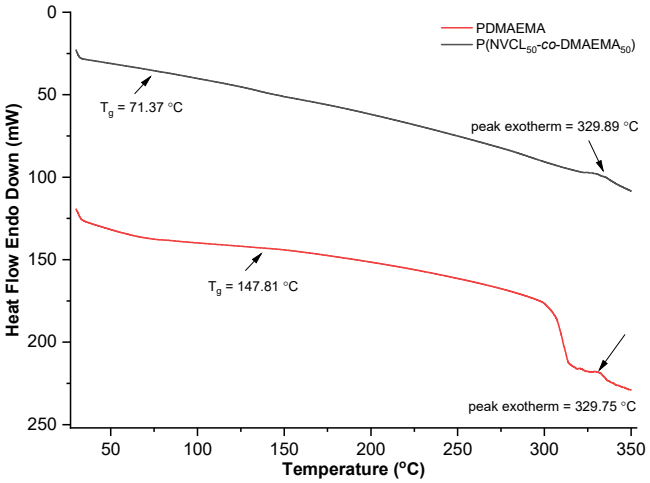


Fig 3. DSC thermogram of PDMAEMA and P(NVCL-*co*-DMAEMA)

T_g is the temperature at which a material, in this case, a hydrogel, transitions from a glass-like state that is rigid and brittle to a softer and more flexible state (like rubber). The T_g value is important for understanding how the hydrogel will behave at different temperatures [24]. In the DSC thermogram of P($NVCL_{50}$-*co*-$DMAEMA_{50}$) hydrogel, it was observed that the T_g value changes to 147.81 °C. Based on the literature, homopolymer PNVCL has a T_g value of 145 °C [5]. The increase of T_g might be attributed to the higher molar masses and the crosslinking point within the P($NVCL_{50}$-*co*-$DMAEMA_{50}$) hydrogel, which restricts chain mobility and increases T_g [8,25]. Following this, as in Fig. 3, the exothermic peak of the homopolymer PDMAEMA is 329.89 °C, while that of its copolymer is 329.75 °C. This phenomenon occurred when PDMAEMA was copolymerized with another monomer, PNVCL. The resulting copolymer may have slightly different thermal properties due to changes in molecular interactions, chain flexibility, and the distribution of functional groups. The small difference in the exothermic peak temperatures between the homopolymer PDMAEMA and its copolymer indicates that copolymerization has a minor effect on the thermal behavior of the material. The shift is likely due to changes in molecular interactions and thermal stability of the copolymer compared to the homopolymer. The previous study also reported the usual results: incorporating itaconic (IA) into polyacrylonitrile (PAN) matrix significantly reduced the intensity of the exothermic peak [26].

Equilibrium Swelling Ratio

Fig. 4 illustrates the equilibrium swelling ratio of the hydrogel with EGDMA and MBA. From the data presented, it is evident that water molecules infiltrate the gel matrix efficiently. The swelling ratio of EGDMA and MBA showed a constant until maximum swelling in 24 h. The swelling ratio for EGDMA reached its maximum in 9 h while for MBA reached its maximum in 12 h. This result is little different from those achieved by Taktak et al. [6], in which hydrogel PDMAEMA using MBA reached its maximum in 6 h [6]. Concerning the potential of swelling of EGDMA and MBA, it was reported that EGDMA is a more appropriate crosslinker for swelling hydrogel than MBA in distilled water at room temperature. The higher swelling capacity of hydrogel with EGDMA was caused by its chemical structure, where hydrogel with EGDMA has more hydrophilic behavior than hydrogel with MBA. Hydrogel with EGDMA likely exhibits more hydrophilic properties due to its chemical structure containing more functional groups capable of strong interactions with water. Hydrogel with EGDMA contains more ester groups and oxygen atoms and has more electronegativity in its chain. These groups tend to exhibit high affinity towards water as they can form hydrogen bonds with water molecules. In contrast, MBAs

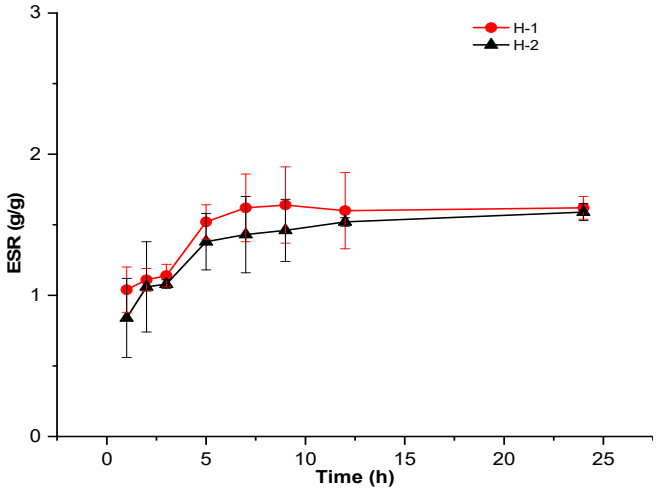


Fig 4. The equilibrium swelling ratio (ESR) of P(NVCL-*co*-DMAEMA) at room temperature

have fewer hydrophilic groups or may contain more hydrophobic groups that are less inclined to interact with water. Although MBA has nitrogen atoms in its structure, it has less electronegativity. Thus, EGDMA displays more hydrophilic behavior and higher swelling capacity than MBA in hydrogel systems. This result is similar to those achieved by Farshforoush et al. [15], who observed the swelling capacity of PDMAEMA hydrogel by comparing MBA and TEGDA.

Temperature-Responsive Behavior of Hydrogel

Hydrogels acquire additional characteristics while maintaining their temperature responsiveness by including hydrophobic or hydrophilic monomers. Fig. 5 shows the impact of temperature on the swelling behavior of hydrogel, comparing the use of EGDMA and MBA as crosslinkers in the hydrogels. The investigation involved swelling samples in various temperatures using distilled water as a solvent. As can be seen, the graph shows that EGDMA gave higher swelling compared to MBA. This happened because EGDMA has more oxygen atoms and more hydrophilic behavior in its structure compared to MBA. Similarly, the research by Park et al. [27] found that the swelling ratio of hydrogels was influenced by cross-linking. Moreover, some studies reported that the crosslinkers within the hydrogel network also affect swelling behavior [28-30]. Furthermore, T_c value of the hydrogel showed that the hydrogels were not influenced by the crosslinkers because there were no changes in the interval of the T_c value. This depicted the phenomenon of hydrogels shrinking at temperatures above 32 °C, using the EGDMA or MBA as crosslinkers. The chemical structureof the hydrogel with EGDMA experiences more interaction with hydrogen than MBA as a crosslinker. Following this, the EGDMA hydrogel showed a sharper decrease when the temperature increased because more deprotonation occurred. This is also supported by the previous report by Taktak et al. [6], who used DMAEMA as a monomer and MBA as a crosslinker. The hydrogel network starts shrinking above the temperature of 32 °C. Similar results were also reported by Demirel et al. [22] in the synthesized P(VCL-*co*-DMAEMA) nanogels by using surfactant-free emulsion polymerization (SFEP). It was observed that above 30 °C, a significant decrease occurred. This phenomenon can be attributed to the stronger hydrophobic interaction among the hydrophobic groups of the hydrogel chains compared to the hydrogen bonding interactions. As a result, the hydrogel experienced a substantial size reduction [22].

pH-Responsive Behavior of Hydrogel

Swelling behavior can be observed in Fig. 6. The data depicts that the swelling rate is substantially more significant at low pH conditions, while the swelling rate considerably decreases as the pH increases. PDMAEMA

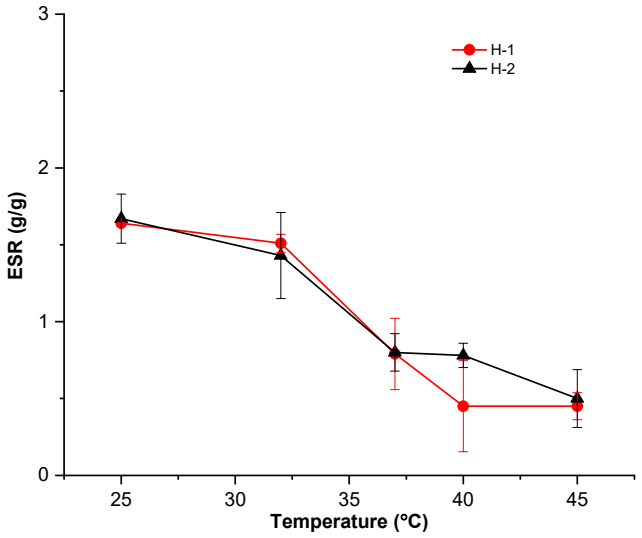


Fig 5. Temperature-responsive behavior of hydrogels

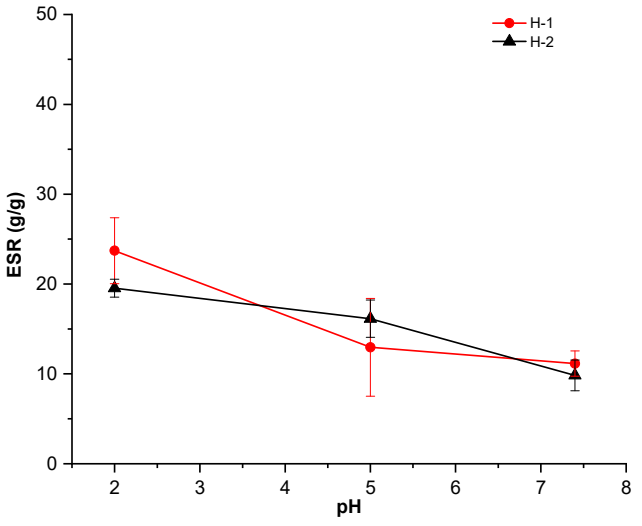


Fig 6. pH-responsive behavior of hydrogels at room temperature

caused this phenomenon. PDMAEMA is a polymer exhibiting pH responsiveness due to the protonation of its base group in an acidic environment [31]. The PDMAEMA polymer chains possess tertiary amine side groups, which attain a temporary cationic charge upon protonation in an acidic condition. This causes the hydrogel chains to separate due to the electrostatic repulsion between chains carrying quaternary amine groups, leading to an increased hydrogel volume and, consequently, a higher swelling rate of the hydrogel [32]. In contrast, at pH 7.4, deprotonation happened through electrostatic repulsion and significantly reduced the swelling ratio of the hydrogel, either using EGDMA or MBA as crosslinkers. These findings align with the results achieved by Taktak et al. [6], deprotonation proceeds entirely at a pH higher than 2, eliminating electrostatic repulsion and substantially decreasing the swelling ratio. Continuously, the swelling of hydrogel using EGDMA is the highest swelling ratio at pH 2 and 7.4 because this hydrogel has more electronegativity, particularly in oxygen atoms in its structure, causing the hydrogel to be more protonated instead of the swelling ratio of the hydrogel using MBA which has less electronegativity in nitrogen atoms in its structure. Thus, because of this phenomenon, hydrogel with EGDMA has the highest swelling ratio.

SEM Analysis of Hydrogel

SEM analysis was conducted to observe the morphology and structure of the hydrogels, hydrogels loaded with metformin HCl, and hydrogels after releasing metformin HCl. All hydrogel samples were prepared by initially freezing the hydrogels for 24 h, followed by the freeze-drying process for 24 h. The freeze-dried hydrogels were then cut in cross-section and analyzed under the SEM at 5.000× magnification. The SEM images in Fig. 7(a) showed that hydrogels with EGDMA were more structured, with some bulges observed on their surface. Fig. 7(b) showed that hydrogel with MBA exhibited a smoother surface compared to hydrogels with EGDMA. Fig. 7(a and b) depicted the disappearance of white crystals on the surface of the hydrogel. Meanwhile, SEM observation of the hydrogels loaded metformin HCl in Fig. 7(c and d) showed the appearance of white crystals that are physically embedded on the surface of the hydrogels. The white crystals that appeared might be attributed to metformin HCl (red circle). Additionally, complementary techniques such as FTIR were conducted to determine whether the white crystal is metformin HCl. The result showed that FTIR can identify functional groups characteristics of metformin HCl, which can help confirm its presence in the sample. Moreover, the result of SEM after releasing the metformin HCl in pH 2 (Fig. 7(e and f)) evidenced that some parts of the embedded metformin HCl had been slightly eroded. However, most of the metformin HCl was still attached to the hydrogels. Physical interactions between the hydrogel and metformin HCl could happen due to the presence of hydrogen bonding, functional group monomers, and crosslinkers that could form hydrogen bonding with metformin HCl.

Loading and *In Vitro* Drug Release

The pH of the media influenced the loading of metformin HCl on hydrogels. In acidic media or pH 2, the hydrogel active sites and molecules of metformin HCl are protonated, causing very weak interaction. This is due to the amine groups in metformin HCl in the solution. In contrast, the hydrogel in a pH 7.4 solution results in complete deprotonation of both amine groups of metformin HCl and the active sites of the hydrogel, such as tertiary amine groups, causing the strength of the drug-hydrogel interaction [33]. Thus, the possible interactions of both drug molecules and hydrogels were hydrophilic interactions. Thereby, the loading process of metformin HCl was conducted at pH 7.4.

The 24 h drug loading results showed that the amount of metformin HCl loaded in hydrogels crosslinked with EGDMA was 18.38 (% w/w), whilst in the hydrogel crosslinked with MBA, it was 26.19 (%w/w). Both crosslinkers have a crucial role in ensuring interactions between the drug and the hydrogel through adsorption, such as hydrogen bond. The hydrogen bond enables interaction between hydrogen atoms bonded by atomic electronegativity, such as the electronegativity of nitrogen atomics from the chemical structure of MBA and

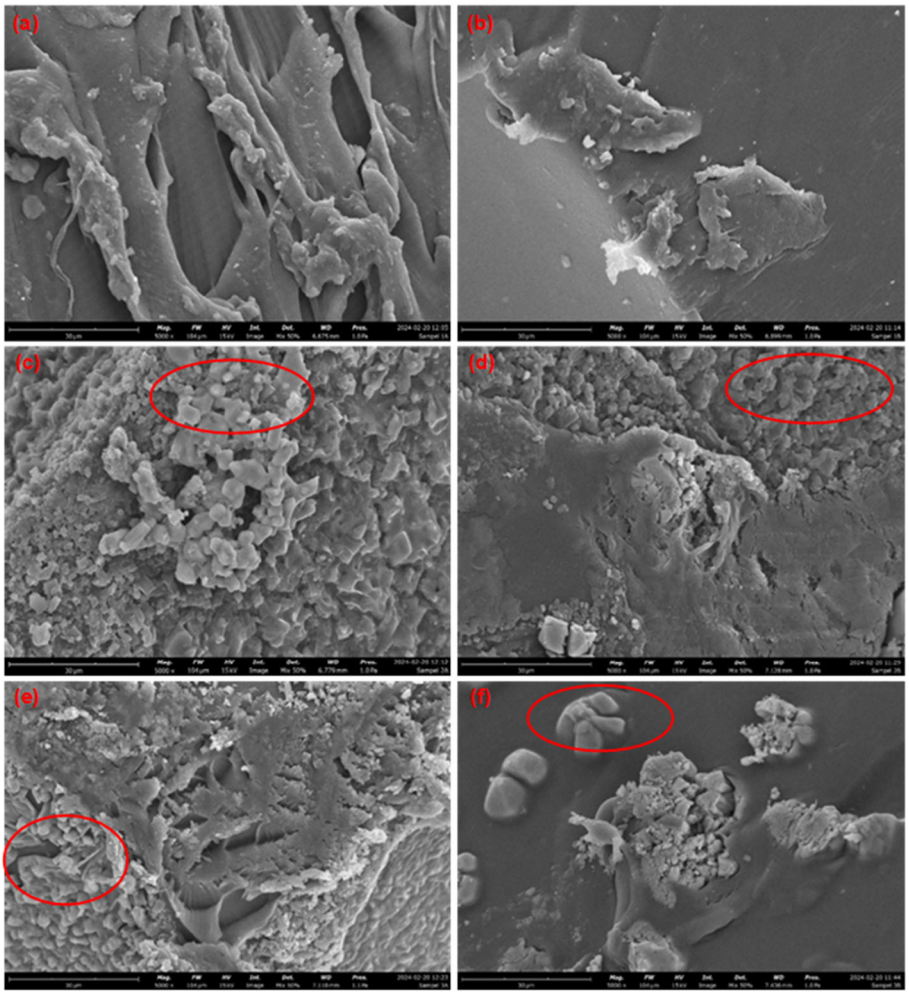


Fig 7. SEM images of hydrogels with magnification 5000×: (a) H-1 unloaded; (b) H-2 unloaded; (c) H-1 drug loaded in pH 7.4; (d) H-2 drug loaded in pH 7.4; (e) H-1 after release in pH 2; and (f) H-2 after release in pH 2. The red circle refers to metformin HCl

the electronegativity of oxygen atomics from the chemical structure of EGDMA. As observed on the SEM images in Fig. 7(c and d), hydrogels appeared to have white crystals of metformin HCl embedded on their surface. In addition, hydrogel with EGDMA as a crosslinker showed the presence of protrusions that might lead to a reduced total surface area available for metformin HCl compared to hydrogel with MBA as a crosslinker.

The release profile of metformin HCl from hydrogel at pH 2 is shown in Fig. 8. The hydrogel crosslinked with EGDMA and MBA released 20.84 and 20.68 (% v/v) metformin HCl within 30 min, respectively. As the release time extended, the amount of metformin HCl also increased. However, after 2 h, the cumulative amount of metformin released from hydrogels with EGDMA was lower than the MBA as the crosslinker. In their study, Mathews et al. [34] concluded that the process of drug release started when the drug diffused into the pores of the hydrogel. Furthermore, the release of active drugs in the hydrogel network is influenced by the diffusion ability and swelling of the active drugs. Thus, the initial rapid release is due to the rapid diffusion process of the active drug [34]. Abedi et al. [35] showed that the hydrogel had a burst release at the beginning. In this study, the SEM results showed that the hydrogels obtained had no pores, and the metformin HCl loaded were embedded on their surfaces. The diffusion process occurs when the concentrated metformin HCl on the surface of

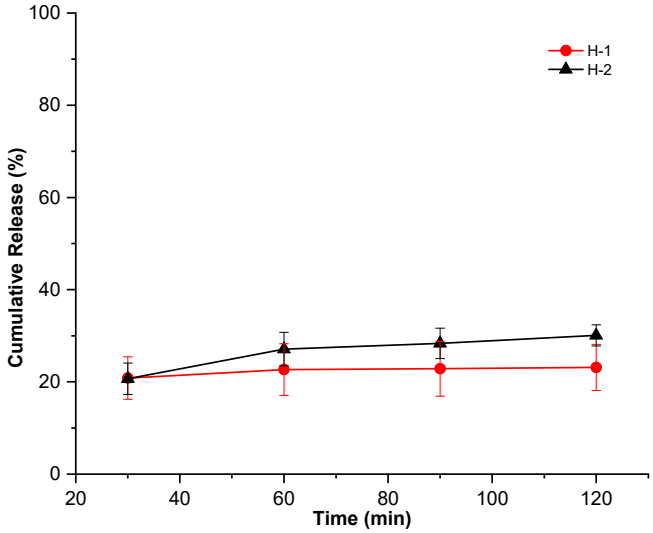


Fig 8. Cumulative release metformin HCl in pH 2 media from hydrogels with different types of crosslinkers

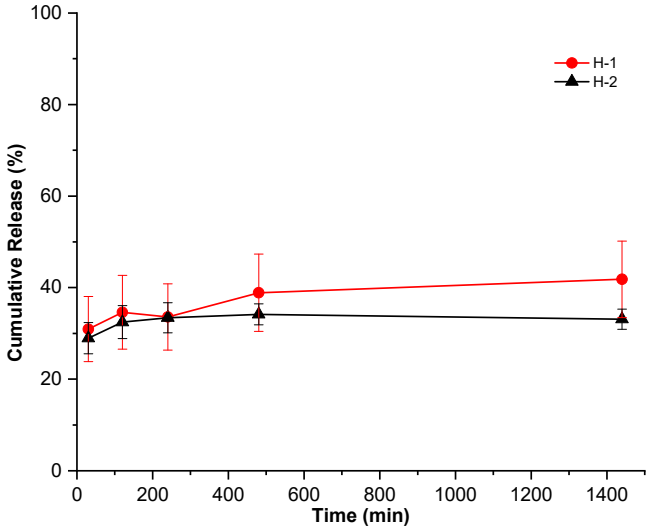


Fig 9. Comparison of cumulative release at pH 7.4 media of different types of crosslinkers

hydrogel starts to move to the lower concentration, the surrounding release medium. The weak interaction between metformin HCl and the surface of hydrogel was most likely responsible for the relatively high percentage of its release. The weak interaction between metformin HCl and the surface of hydrogel was most likely responsible for the relatively high percentage of its release.

Furthermore, swelling from hydrogel in acidic conditions had a high swelling ratio (g/g) compared to alkaline conditions due to DMAEMA monomer, which is a polybasic polymer because DMAEMA has a tertiary amine group that can be protonated in low pH [31]. The presence of metformin HCl in the hydrogel was also strongly affected by the pH value needed to load metformin HCl. The hydrogel active site and metformin HCl are protonated in acidic conditions, weakening the drug-hydrogel interaction. The release of metformin HCl was lower in acidic conditions, and metformin HCl is a highly soluble drug of similar pH to water or alkaline conditions [33].

Subsequently, a release in alkaline conditions or pH 7.4 was conducted, as detailed in Fig. 9. The pH of the medium affects the release, with the profile release at alkaline pH attributed to the weak interactions between the drug molecules and specific carrier surfaces [33]. Besides that, the hydrophilic characteristic of metformin HCl may affect the release process. The performance of metformin HCl, possessing solubility at a neutral pH, involves the deprotonation of its amine groups, resulting in its dissolution in the medium. This aspect might contribute to an increased cumulative release of metformin HCl from the hydrogel in alkaline conditions compared to acidic conditions. Furthermore, other studies state that drug release from hydrogel depends on several factors, including the material used as a conductor, particle size, pH, and temperature [36]. For example, in research, as reported by Cortez-Lemus et al. [37] the release of active drugs increases with increasing pH conditions. The same research was done in the literature by Aderibigbe and Mhlwatika [38], as well as Hu et al [39], which observed that the rapid release of the hydrogel network increased at high pH.

The type of crosslinker also influences the release test results. The percentage of metformin HCl released in pH 7.4 from the hydrogel network using the EGDMA crosslinkers and the MBA as the crosslinkers was 41.82 and 33.11 (% v/v), respectively. The determination of the crosslinkers influences the results. EGDMA might cause the hydrogel to exhibit lower water uptake and reduced swelling capacity than the MBA. Therefore, the drugs slower release from hydrogel might be attributed to this diminished swelling. Additionally, the chemical structure and reactivity of the crosslinkers play an essential role in determining the release kinetics of

metformin HCl from the hydrogel. Hydrogel-EGDMA could result in a more sustained and controlled release of metformin HCl instead of hydrogel-MBA. This result is due to higher reactivity and the ability of EGDMA to form stronger hydrogen bonds. Similarly, a comparison of the release of active drugs using metronidazole was also conducted by Farshforoush et al. [15]. His study found that the TEGDA crosslinker was more efficient in releasing MTZ than the MBA.

Regarding the release of metformin HCl from other materials, Dave et al. conducted the release of metformin HCl with hydrogel (AA-*co*-NIPAM/GO). The results showed that the loading was around 46.53%, and metformin HCl was released at 90.58% in alkaline conditions [16]. Moreover, Barleany et al. [36] used pNIPAAm-chitosan-PVA with metformin HCl as a drug model, resulting in high release in the first hour, which is known as the burst effect or uncontrolled release of metformin HCl. Both studies used NIPAM as the main material to deliver the drug. However, another study reported that PNIPAM has a non-biocompatible property, which restricts its utility in biomedical applications [40]. Therefore, this study provides new insights into how NVCL contributes to modifying DMAEMA using the crosslinkers EGDMA and MBA separately. As the drug model, metformin HCl underwent slow release and minimized the burst effect. In addition, all the materials are biocompatible molecules and safe to be used in medical aspects.

■ CONCLUSION

Loading and releasing metformin HCl in hydrogels was successfully conducted. The SEM results showed that hydrogels containing EGDMA displayed a more organized structure with specific bulges on their surface. In contrast, hydrogel with MBA exhibited a smoother surface than hydrogel with EGDMA. The amount of metformin HCl loaded in the hydrogel with EGDMA was 18.38 and 26.19 (% w/w) in the hydrogel with MBA, indicating a low load of metformin HCl. Surface modification is necessary to enhance the quantity of metformin HCl loaded within the hydrogel. The highest percentage of metformin HCl released from hydrogel with EGDMA and MBA crosslinkers occurred at pH 7.4, approximately 41.82 and 33.11 (% v/v) within 24 h, respectively. Therefore, the P(NVCL-*co*-DMAEMA) hydrogel with EGDMA crosslinker had potential applications as a controlled drug delivery system at pH 7.4. However, further optimizations are required to enhance the effectiveness of the hydrogels as a carrier.

■ ACKNOWLEDGMENTS

The first author would like to thank the *Lembaga Pengelolaan Dana Pendidikan* (LPDP)/Indonesia Endowment Funds for Education within the Ministry of Finance of the Republic of Indonesia for the LPDP scholarship under contract number of LOG-5941/LPDP.3/2024. The corresponding author is funded by the Faculty of Mathematics and Natural Sciences, Universitas Indonesia under Publication Grant scheme 2024. The authors acknowledge the FTIR facilities of the Integrated Laboratory and Research Center (ILRC) Universitas Indonesia.

■ CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

■ AUTHOR CONTRIBUTIONS

Ariyaldi: conducting the experiments, analyzing the data obtained, writing – original draft. Noverra Mardhatillah Nizardo: designing the whole concept and synthesis methodology, writing-review and editing. Maria Lucia Ardhani Dwi Lestari: designing the drug loading and release experiments, writing-review and editing.

■ REFERENCES

[1] Romero, J.F., Díaz-Barrios, A., and González, G., 2021, Biocompatible thermo-responsive *N*-vinylcaprolactam based hydrogels for controlled drug delivery systems, *Bionatura*, 6 (2), 1712–1719.

[2] Sudjaroen, Y., Thongkao, K., and Thongmuang, P., 2023, A physiochemical study on drug delivery of metformin HCl-loaded CS-PLGA nanoparticles, *Int. J. Appl. Pharm.*, 15 (1), 66–71.

[3] Hoffman, A.S., 1995, "Intelligent" Polymers in medicine and biotechnology, *Artif. Organs*, 19 (5), 458–467.

[4] Saikia, A.K., Aggarwal, S., and Mandal, U.K., 2013, Preparation and controlled drug release characteristics of thermoresponsive PEG/poly(NIPAM-*co*-AMPS) hydrogels, *Int. J. Polym. Mater. Polym. Biomater.*, 62 (1), 39–44.

[5] Fallon, M., Halligan., Pezzoli, R., Geever, L., and Higginbotham, C., 2019, Synthesis and characterisation of novel temperature and pH sensitive physically cross-linked poly(*N*-vinylcaprolactam-*co*-itaconic acid) hydrogels for drug delivery, *Gels*, 5 (3), 41.

[6] Taktak, F., 2016, Rapid deswelling of PDMAEMA hydrogel in response to pH and temperature changes and its application in controlled drug delivery, *AKU J. Sci. Eng.*, 16(1), 68–75.

[7] Bustamante-Torres, M., Romero-Fierro, D., Arcentales-Vera, B., Palomino, K., Magaña, H., and Bucio, E., 2021, Hydrogels classification according to the physical or chemical interactions and as stimuli-sensitive materials, *Gels*, 7 (4), 182.

[8] Nizardo, N.M., Fadhilah, R.H., and Anggraningrum, I.T., 2023, Thermo- and pH-responsive behavior of poly(*N*-isopropylacrylamide)-block-poly[(2-dimethylamino)ethyl methacrylate], *Indones. J. Chem.*, 23 (2), 449–460.

[9] Sandjaja, M., and Lestari, M.L.A.D., 2017, Investigation of effect of adding hydrophobically modified water soluble polymers on the structure and viscosity of anionic vesicle dispersion, *Indones. J. Chem.*, 17 (1), 86–94.

[10] Podaru, I.A., Stănescu, P.O., Ginghină, R., Stoleriu, Ş., Trică, B., Şomoghi, R., and Teodorescu, M., 2022, Poly(N-vinylpyrrolidone)–Laponite XLG nanocomposite hydrogels: Characterization, properties and comparison with divinyl monomer-crosslinked hydrogels, *Polymers*, 14 (19), 4216.

[11] Bashir, S., Zafar, N., Lebaz, N., Mahmood, A., and Elaissari, A., 2020, Hydroxypropyl methylcellulose-based hydrogel copolymeric for controlled delivery of galantamine hydrobromide in dementia, *Processes*, 8 (11), 1350.

[12] Minhas, M.U., Ahmad, M., Ali, L., and Sohail, M., 2013, Synthesis of chemically cross-linked polyvinyl alcohol-*co*-poly (methacrylic acid) hydrogels by copolymerization; A potential graft-polymeric carrier for oral delivery of 5-fluorouracil, *Daru, J. Pharm. Sci.*, 21 (1), 44.

[13] Bukhari, S.M.H., Khan, S., Rehanullah, M., and Ranjha, N.M., 2015, Synthesis and characterization of chemically cross-linked acrylic acid/gelatin hydrogels: Effect of pH and composition on swelling and drug release, *Int. J. Polym. Sci.*, 2015 (1), 187961.

[14] Caroline, D.S.M., and Rekha, M.R., 2022, Exploring the efficacy of ethylene glycol dimethacrylate crosslinked cationised pullulan for gene delivery in cancer cells, *J. Drug Delivery Sci. Technol.*, 68, 103067.

[15] Farshforoush, P., Ghanbarzadeh, S., Goganian, A.M., and Hamishehka, H., 2017, Novel metronidazole-loaded hydrogel as a gastroretentive drug delivery system, *Iran. Polym. J.*, 26 (12), 895–901.

[16] Dave, P.N., Macwan, P.M., and Kamaliya, B., 2023, Synthesis and characterization of biodegradable gum ghatti-*cl*-*poly*(AA-*co*-NIPAm)/GO based hydrogel for metformin and sodium diclofenac combined drug delivery system, *Colloids Surf., A*, 673, 131815.

[17] Noreen, S., Hasan, S., Ghumman, S.A., Anwar, S., Gondal, H.Y., Batool, F., and Noureen, S., 2023, Formulation, statistical optimization, and *in vivo* pharmacodynamics of *Cydonia oblonga* mucilage/alginate mucoadhesive microspheres for the delivery of metformin HCl, *ACS Omega*, 8 (6), 5925–5938.

[18] Desai, D., Wong, B., Huang, Y., Ye, Q., Tang, D., Guo, H., Huang, M., and Timmins, P., 2014, Surfactant-mediated dissolution of metformin hydrochloride tablets: Wetting effects versus ion pairs diffusivity, *J. Pharm. Sci.*, 103 (3), 920–926.

[19] Mishra, R.K., and Ray, A.R., 2011, Synthesis and characterization of poly{*N*-[3-(dimethylamino) propyl] methacrylamide-*co*-itaconic acid} hydrogels for drug delivery, *J. Appl. Polym. Sci.*, 119 (6), 3199–3206.

[20] Çakal, E., and Çavuş, S., 2010, Novel poly(*N*-vinylcaprolactam-*co*-2-(diethylamino)ethyl

methacrylate) gels: Characterization and detailed investigation on their stimuli-sensitive behaviors and network structure, *Ind. Eng. Chem. Res.*, 49 (22), 11741–11751.

[21] Durkut, S., 2019, Thermoresponsive poly(*N*-vinylcaprolactam)-*g*-galactosylated chitosan hydrogel: Synthesis, characterization, and controlled release properties, *Int. J. Polym. Mater. Polym. Biomater.*, 68 (17), 1034–1047.

[22] Demirel, G.B., and von Klitzing, R., 2013, A new multiresponsive drug delivery system using smart nanogels, *ChemPhysChem*, 14 (12), 2833–2840.

[23] Ashames, A., Ullah, K., Al-Tabakha, M., Khan, S.A., Hassan, N., Mannan, A., Ikram, M., Buabeid, M., and Murtaza, G., 2022, Development, characterization and *in-vitro* evaluation of guar gum based new polymeric matrices for controlled delivery using metformin HCl as model drug, *PLoS One*, 17 (7), e0271623.

[24] Iwohn, M., Seifermann, M., Reiser, P., Höpfner, J., El Khaled El Faraj, R., Heißler, S., Popova, A., and Levkin, P.A., 2023, OligoHydrogelArray (OHA) for parallelized solid-phase extraction of oligonucleotides, *Adv. Mater. Interfaces*, 10 (21), 2300227.

[25] Giubertoni, G., Sofronov, O.O., and Bakker, H.J., 2020, Effect of intramolecular hydrogen-bond formation on the molecular conformation of amino acids, *Commun. Chem.*, 3 (1), 84.

[26] Zhang, H., Quan, L., Gao, A., Tong, Y., Shi, F., and Xu, L., 2020, Thermal analysis and crystal structure of poly(acrylonitrile-*co*-itaconic acid) copolymers synthesized in water, *Polymers*, 12 (1), 221.

[27] Park, H., Guo, X., Temenoff, J.S., Tabata, Y., Caplan, A.I., Kasper, F.K., and Mikos, A.G., 2009, Effect of swelling ratio of injectable hydrogel composites on chondrogenic differentiation of encapsulated rabbit marrow mesenchymal stem cells *in vitro*, *Biomacromolecules*, 10 (3), 541–546.

[28] Joo, S.B., Gulfram, M., Jo, S.H., Jo, Y.J., Vu, T.T., Park, S.H., Gal, Y.S., and Lim, K.T., 2022, Fast absorbent and highly bioorthogonal hydrogels developed by IEDDA click reaction for drug delivery application, *Materials*, 15 (20), 7128.

[29] Hoti, G., Caldera, F., Cecone, C., Rubin Pedrazzo, A., Anceschi, A., Appleton, S.L., Khazaei Monfared, Y., and Trotta, F., 2021, Effect of the cross-linking density on the swelling and rheological behavior of ester-bridged β-cyclodextrin nanosponges, *Materials*, 14 (3), 478.

[30] Nasution, H., Harahap, H., Dalimunthe, N.F., Ginting, M.H.S., Jaafar, M., Tan, O.O.H., Aruan, H.K., and Herfananda, A.L., 2022, Hydrogel and effects of crosslinking agent on cellulose-based hydrogels: A review, *Gels*, 8 (9), 568.

[31] Kasiński, A., Zielińska-Pisklak, M., Oledzka, E., and Obczak, M., 2020, Smart hydrogels – Synthetic stimuli-responsive antitumor drug release systems, *Int. J. Nanomed.*, 15, 4541–4572.

[32] Reyes-Ortega, F., 2014, "pH-Responsive polymers: Properties, synthesis and applications" in *Smart Polymers and their Applications*, Woodhead Publishing, Cambridge, UK, 45–92.

[33] Putra, I.M.W.A., and Kusumawati, I.G.A.W., 2018, The use of clinoptilolites as carrier of metformin hydrochloride in drug delivery system: *In vitro* drug release study, *Asian J. Pharm. Clin. Res.*, 11 (11), 285–289.

[34] Mathews, A.S., Ha, C.S., Cho, W.J., and Kim, I., 2006, Drug delivery system based on covalently bonded poly[*N*-isopropylacrylamide-*co*-2-hydroxyethylacrylate]-based nanoparticle networks, *Drug Delivery*, 13 (4), 245–251.

[35] Abedi, F., Davaran, S., Hekmati, M., Akbarzadeh, A., Baradaran, B., and Moghaddam, S.V., 2021, An improved method in fabrication of smart dual-responsive nanogels for controlled release of doxorubicin and curcumin in HT-29 colon cancer cells, *J. Nanobiotechnol.*, 19 (1), 18.

[36] Barleany, D.R., Ananta, C.V., Maulina, F., Rochmat, A., Alwan, H., and Erizal, E., 2020, Controlled release of metformin hydrogen chloride from stimuli-responsive hydrogel based on poly(*N*-isopropylacrylamide)/chitosan/polyvinyl alcohol composite, *Int. J. Technol.*, 11 (3), 511–521.

[37] Cortez-Lemus, N.A., and Licea-Claverie, A., 2016, Poly(*N*-vinylcaprolactam), a comprehensive review

on a thermoresponsive polymer becoming popular, *Prog. Polym. Sci.*, 53, 1–51.

[38] Aderibigbe, B.A., and Mhlwatika, Z., 2016, Dual release kinetics of antimalarials from soy protein isolate-carbopol-polyacrylamide based hydrogels, *J. Appl. Polym. Sci.*, 133 (37), 43918.

[39] Hu, Y., Mei, Z., and Hu, X., 2015, pH-Sensitive interpenetrating network hydrogels based on pachyman and its carboxymethylated derivatives for oral drug delivery, *J. Polym. Res.*, 22 (6), 98.

[40] Alexander, A., Ajazuddin, A., Khan, J., Saraf, S., and Saraf, S., 2014, Polyethylene glycol (PEG)-Poly(N-isopropylacrylamide) (PNIPAAm) based thermosensitive injectable hydrogels for biomedical applications, *Eur. J. Pharm. Biopharm.*, 88 (3), 575–585.