STUDIA UBB CHEMIA, LXVIII, 2, 2023 (p. 115-130)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2023.2.08

EVALUATION OF PHOTOPOLYMERIZABLE HEMA-BASED HYDROGELS FOR RELEASE OF ANTI-DIABETIC DRUG METFORMIN HCL

Sebnem SENOL[a]

ABSTRACT. This study targets to prepare a metformin hydrochloride delivery system through the preparation and evaluation of 2-hydroxyl ethyl methacrylate (HEMA) based hydrogels. The current study explores the effect of photoinitiator (Irgacure 184, Irgacure 651), PEG-DA derivatives, 4-Acryloyl morpholine (4-AcM), and gelatine obtained by UV photopolymerization of HEMA hydrogels. Photopolymerization technique which was under UV irradiation was implemented at 365 nm and 300 s. Two different photoinitiators [2,2-Dimethoxy-2-phenyl-acetophenone (Irgacure 651)], [1-Hydroxycyclohexyl phenyl ketone (Irgacure 184)] were used to obtain the impact of photoinitiators on the metformin HCl release behavior of samples. In addition, PEG-DA Mn=258, PEG-DA Mn=700, 4-AcM, and gelatine were used to improve HEMA hydrogels. The prepared hydrogels have been characterized using Fourier transform infrared spectroscopy (FT-IR) and a digital microscope. The behaviors of hydrogels were specified by exploring swelling and release profiles in different medias. *In-vitro* metformin HCl release analyses have been done at pH 1.2, 6.8, and 7.4. UV-Vis spectrophotometer at 244 nm for releasing studies was used. The release results of hydrogels synthesized with Irgacure 651 demonstrated the majority quantity of the drug. Furthermore, the release amounts were higher in pH 1.2 than at mentioned pH medias before.

Keywords: *Photopolymerization, photoinitiators, hydrogels, release system, HEMA, metformin HCl*

[a] *Department of Chemical Engineering, Yildiz Technical University, 34210, İstanbul, Turkey, sebnemsenol_@hotmail.com*

INTRODUCTION

Diabetes is a chronic disease in which the body's ability to regulate excess blood glucose levels is impaired, and is the top ten cause of death worldwide. Diabetes includes a range of life-threatening complications that, if not treated or followed, may lead to problems such as lower extremity amputation, blindness, kidney failure, and premature death, also leading to an increased need for medical care, reduced quality of life, and undue stress on families [1-5]. However, the controlled management of diabetes is a public health priority, with more than 463 million people worldwide living with diabetes. Diabetes death numbers are estimated to have as many as 700 million adults by 2045. Since 2000, the number of men and women diagnosed with diabetes has been increasing rapidly. In European countries, 11.3 million people and 1.7 million people aged 40-59, and 20-39 have diabetes, respectively. 19.3 million people aged 60-79 have diabetes, and this chronic disease is also extensive among older people. The economic dimension of diabetes is extensive. Health payment for diabetes is projected to be around EUR 150 billion in 2019 in the EU, with the average payment per diabetic adult at around EUR 3000 per year [6-7].

Proposed as a first-line treatment for type 2 diabetes by the European Association for the Study of Diabetes and the American Diabetes Association, metformin was validated as an antihyperglycemic agent in different countries [8-10]. Metformin, which is commonly proposed as first-line therapy in type 2 diabetes, has been explained to be safe and effective for as monotherapy and in combination with oral antidiabetic agents and insulins. Metformin reduces cancer factor, which appears to be increased in diabetics, and is an important drug for chemotherapy processes [11].

The drug delivery system (DDS) is a common term that can control the delivery and release of active pharmaceutical ingredients to the site of interest, allowing active substances to maximize therapeutic efficacy [1,2-3,5]. Many delivery methods follow: oral, mucosal, and transdermal administration, lung inhalation, and intravenous injection [12,13,16].

Hydrogels are hydrophilic polymer networks that can absorb large amounts of water, increase their volume, and exhibit many different material behaviors, and were the first biomaterials developed for human use. The widespread use of hydrogels in various industrial and environmental application areas is of primary importance. With more than half a century of industrial use, today it offers a wide range of features for many different purposes [17-22].

Photopolymerization methods, which lead to the occurrence of solid polymer networks starting from monomers, are of great interest in the industry and daily life in a wide variety fields of science. Photoinitiators are a key component

(Irgacure 184, Irgacure 651) that can absorb energy at a specific wavelength of light and create radicals that convert the liquid monomer solution into polymers [23-27].

p(HEMA), which is widely used in various biomedical applications, is stable for many chemical and biochemical reactions [28]. In addition, it is a transparent and biocompatible material with low cellular affinity [29-31]. Polyethylene glycol (PEG)- based hydrogels have been increasingly studied for drug delivery systems due to their adjustable crosslink densities, which can be suitable for photopolymerization with different properties [32-42].

Due to its biodegradability, biocompatibility, and hydrogel-forming ability, gelatine is a hydrophilic protein obtained from denatured collagen obtained from various sources, which has an important role in research on biomedical materials, especially cell culture structures in tissue engineering applications [43-46]. Moreover, gelatine can form covalently cross-linked hydrogels with enhanced biocompatibility and tensile strength [33-34].

4-Acryloyl morpholine (4-AcM) has a high molecular weight, and its derivatives have been widely used in drug delivery release applications for many years [47-50]. In the synthesis of many hydrogels, crosslinkers (EGDMA) are widely used, connecting linear polymeric chains that form a three-dimensional network of chemical bonds between them [51].

In the present study, I paid attention to prepare HEMA-based hydrogels, and analyzing their swelling and releasing behaviors. For this objective, metformin HCl was added into 2-hydroxyl ethyl methacrylate which was unified with PEG-DA, 4- ACM, gelatine with Irg 184, and Irg 651.

RESULTS AND DISCUSSION

FT-IR and SEM analysis of hydrogels

Characterization of chemical structure and determination of functional groups for hydrogels were used by FT-IR spectroscopy (Shimadzu IR Prestige 21). FT-IR images of hydrogels and the determined functional groups are shown in Figure 1.

The FT-IR spectrum of metformin HCl exhibited peaks at 3362 cm^{-1} related to N-H asymmetric stretching, 1478 cm^{-1}, 1450 cm^{-1}, and 1460 cm^{-1} corresponded to C-H asymmetric bending (-CH_3), at 1152 cm^{-1}, 1072 cm^{-1} assigned to C-N stretching, at 945 cm^{-1} and 747 cm^{-1} owing to N-H wagging [52]. The adsorption band observed at 1717 cm^{-1} is characteristic of the carboxylic C=O group in HEMA [53]. The peaks at 1450 cm^{-1} are related to -CH_3 groups. Also, the ring stretching vibration (mainly asymmetric v(C-O-C)) in morpholine is observed at 1152 cm^{-1} [50].

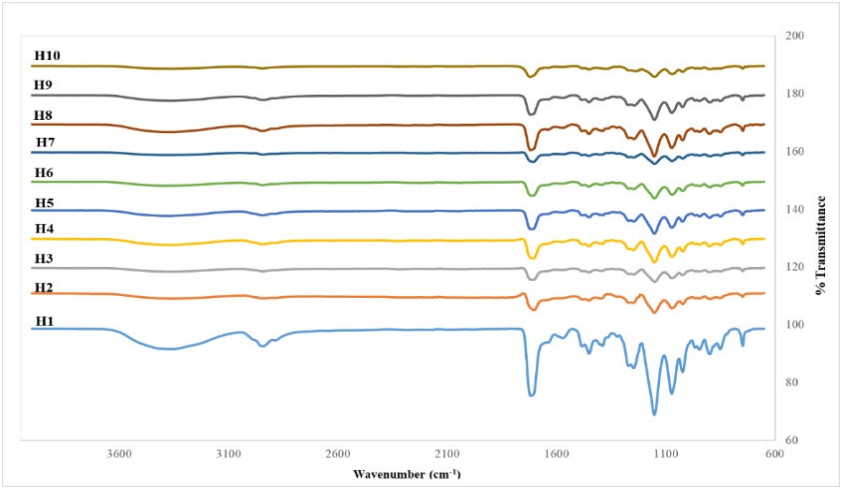

Figure 1. FT-IR analyses of hydrogels

A scanning Electron Microscope (SEM) was applied to explore the samples' morphology. SEM images of hydrogels H2 (a) and H8 (b) hydrogels are shown in Figure 2. H2 and H8 have a porous structure. SEM images presented that the hydrogel surface became nearer with the addition of 4-AcM (H8).

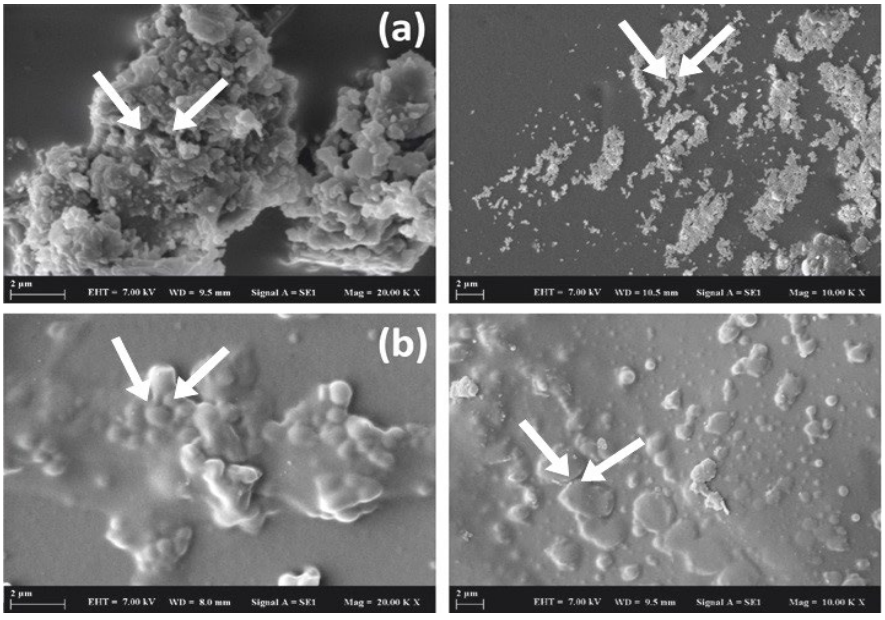

Figure 2. SEM images of H2 hydrogel (a) and H8 hydrogel (b)

Swelling ratios

A gravimetric method measured the swelling ratio at deionized water, pH 1.2, 6.8, and 7.4 at 37°C. The hydrogels were removed and then weighed at a determined time after surface water removal. The swelling ratio was calculated as an equation;

$$\% \text{ Swelling ratio} = \frac{Ws - Wi}{Wi} * 100$$

Wi presents the first weight of the sample and Ws presents the amount of the sample in the swollen state.

The preparation of pH 6.8 and pH 7.4 solutions have been reported in detail [14]. The pH 1.2 media was prepared according to the United States of Pharmacopeia (USP) procedure.

The swelling ratios of hydrogels were obtained by considering time and pH properties. Swelling ratios of hydrogels in deionized water and solutions with mentioned medias at 37 °C are shown in Figure 3-6. Hydrogel samples exhibited same swelling kinetic behavior. The swelling values of the hydrogels increased at the beginning but increased at a slower rate over time until the equilibrium swelling ratio was reached.

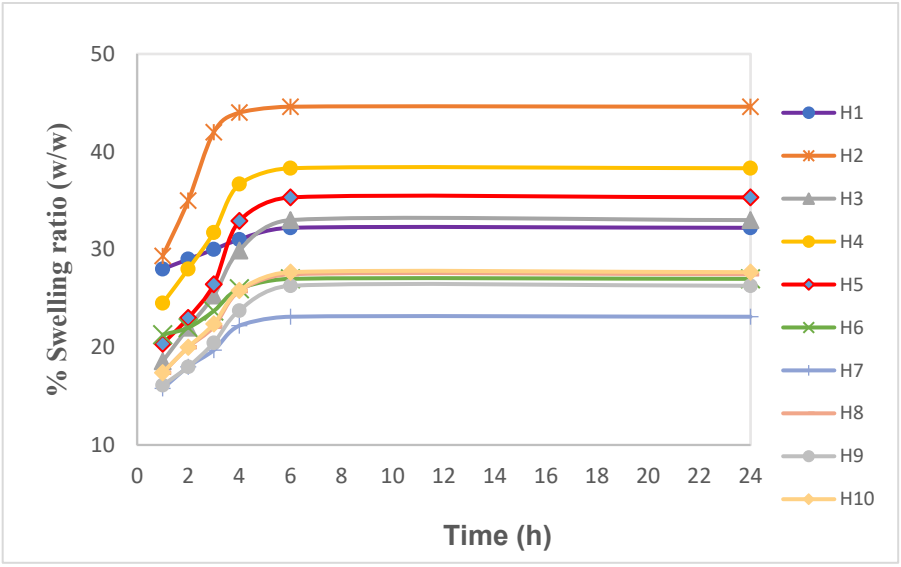

Figure 3. Swelling ratio of hydrogels in deionized water

The presence of PEG-DA increased the swelling rate for all media, and hydrogels generally synthesized with Irg 651 had a higher swelling rate than the others. This is due to the fact that Irg 651 is more hydrophobic and produces more free radicals than Irg 184. The smaller the Mn molar mass of PEG-DA, the more chain ends there are in the networks, and according to Flory's report, it shows that networks with more chain ends have a higher swelling capacity, and therefore hydrogels containing PEG-DA Mn=258 are generally more swollen [16,53,54]. In general, hydrogels exhibited higher swelling properties at deionized water and pH 1.2.

Hydrogels with the highest swelling ratio in a deionized water environment are H2 (44.60 %), H4 (38.12 %), and H5 (35.33 %) respectively. H3 hydrogel has the lowest swelling ratio at 23.25%. It was observed that the presence of PEG-DA positively affects the swelling ratio because of swelling capacity.

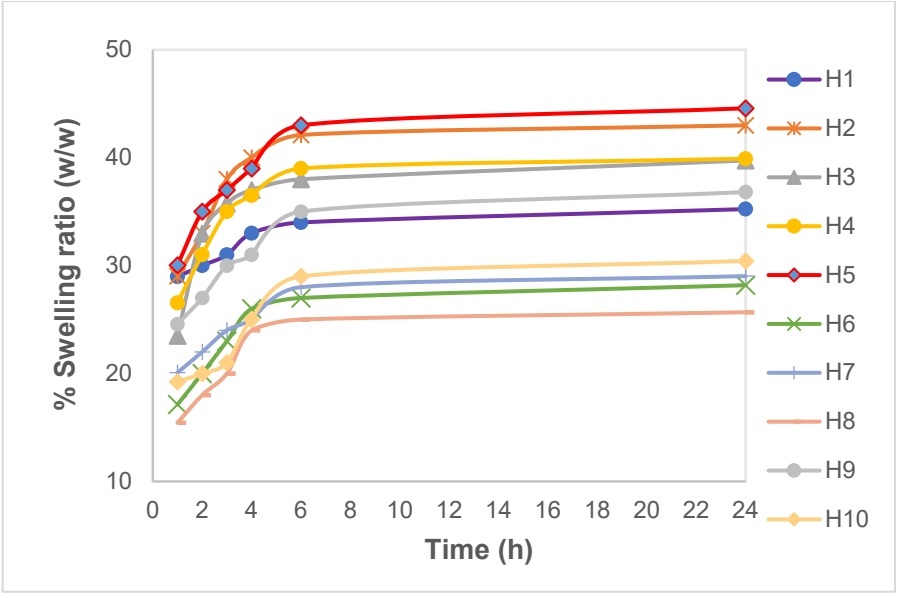

Figure 4. Swelling ratio of hydrogels in pH 1.2

The hydrogels with the highest swelling ratio in pH 1.2 media are H5 (45.38 %), H2 (43.04 %), and H9 ((36.91 %), respectively. Gelatine and PEG-DA increased the swelling ratio in pH 1.2. H8 has the lowest swelling rate with 26.06 %.

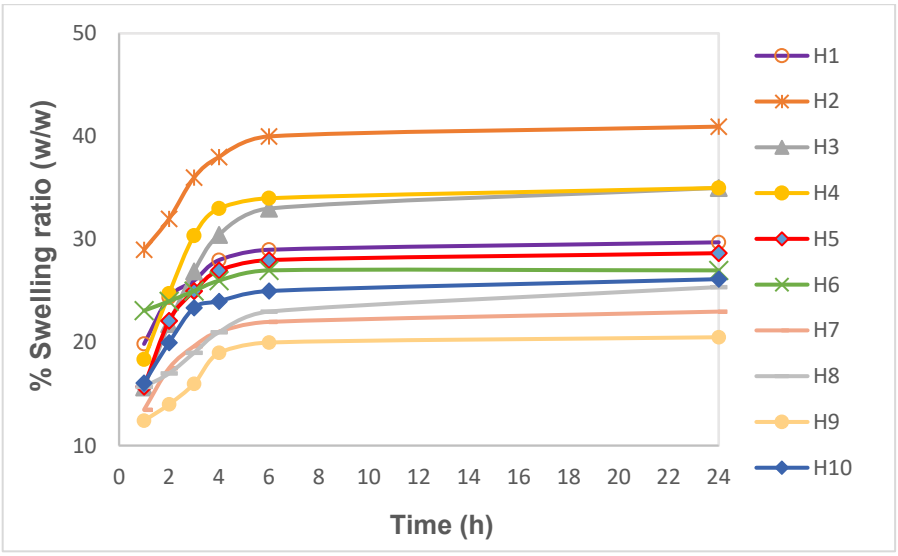

Figure 5. Swelling ratio of hydrogels in pH 6.8

H2 (41.05%), H4 (35.07%), and H3 (35.02%) have the highest swelling ratio in pH 6.8 media, respectively. H1 and H9 have the lowest swelling ratio. PEG-DA has a positive effect on swelling in this media.

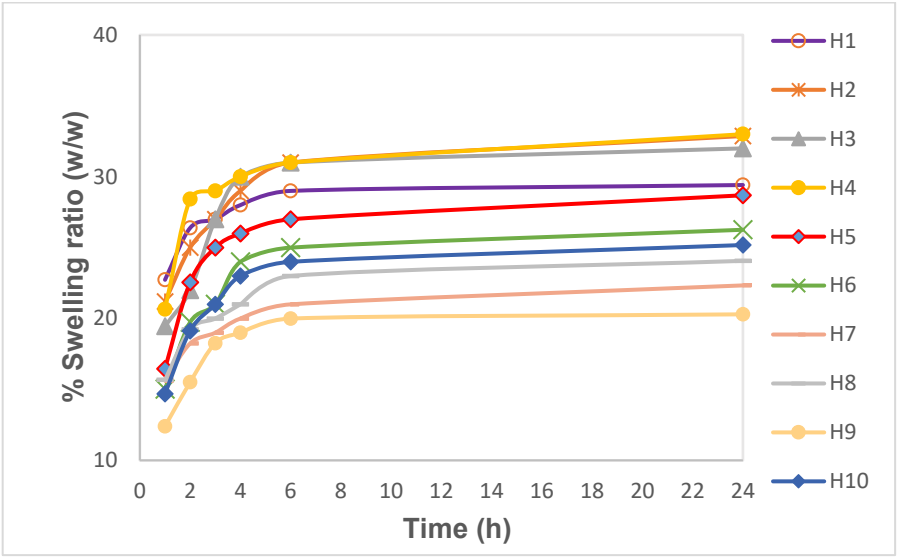

Figure 6. Swelling ratio of hydrogels in pH 7.4

In a pH 7.4 medium, H4 (33.14%) has the highest swelling ratio. This order is followed by H3 and H2. Also, H9 has the lowest swelling ratio at 20.30%. In general, swelling ratios are lower at pH 6.8 and pH 7.4.

Metformin HCl release studies

In this chapter, the interactive relation of the hydrogels with metformin HCl was experienced. Metformin release analyses were done with using a UV-Vis spectrophotometer (Analytik Jena Specord 200/Plus) at 244 nm. The analyses were repeated three times.

Figure 7-9 shows the release ratios of metformin hydrochloride from hydrogels at the described medias at 37 °C.

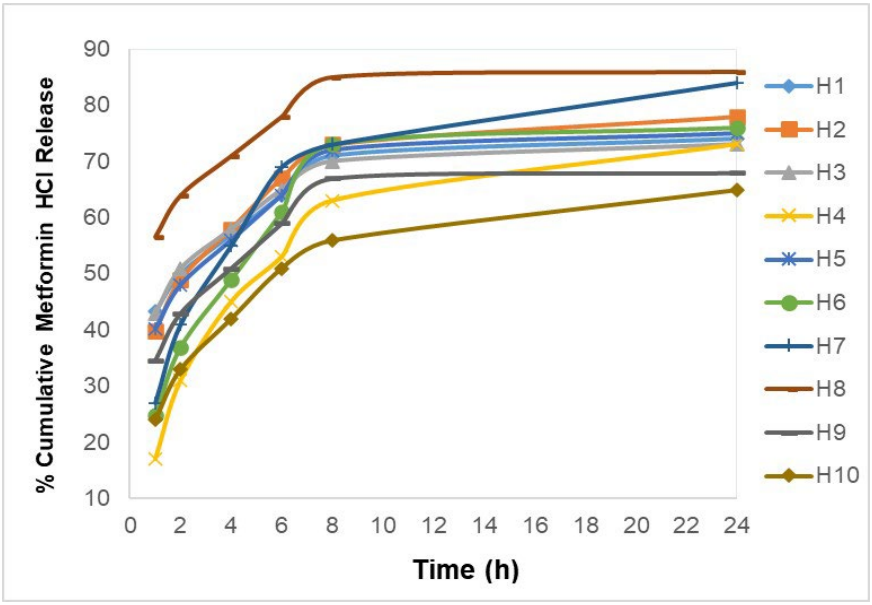

Figure 7. Metformin HCl release ratio in pH 1.2 media

The results showed that hydrogels showed prolonged release profiles. As presented in these figures, at pH 1.2 the highest drug release values have been achieved. H8 and H2 hydrogel exhibited that good performance in pH 1.2, pH 6.8, and pH 7.4. Also, the release ratio from the hydrogel was very responsive to pH. In the meantime, the amount of drug release for most hydrogels was much higher in pH 1.2 than the other explained medias. Furthermore, the release ratio of hydrogels which were synthesized by using Irgacure 184 was slower at pH 1.2 and pH 6.8.

4-AcM increased the release rate of the hydrogel so H8 hydrogel demonstrated the peak level of release ratio (86,16 %) in pH 1.2. H2 exhibited good performance with a 78.03 % release rate. Hydrogels synthesized with Irgacure 651 had a better release in all mediums. Hydrogels synthesized with PEG-DA Mn=258 released better than PEG-DA Mn=700.

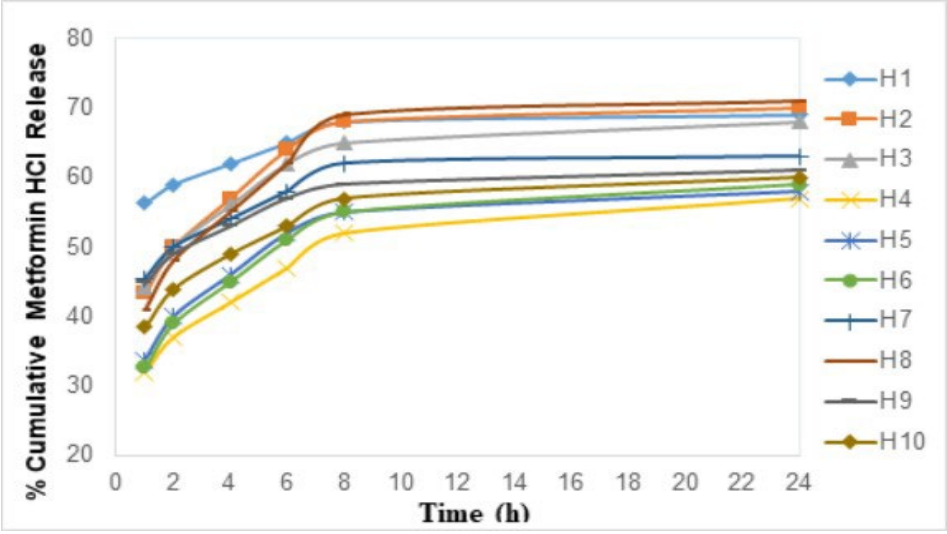

Figure 8. Metformin HCl release ratio in pH 6.8 media

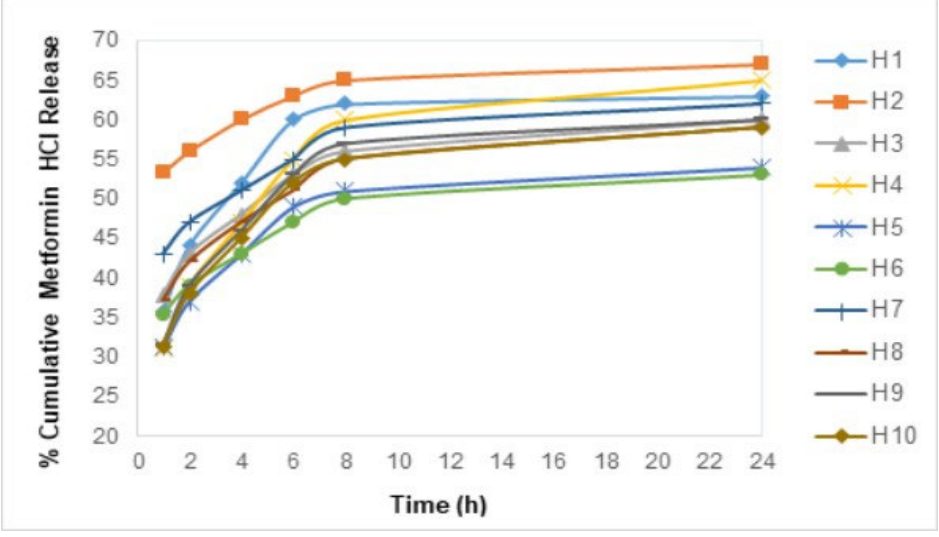

Figure 9. Metformin HCl release ratio in pH 7.4 media

While the maximum release percentage (86.16%) was achieved at pH 1.2, the total release value of metformin HCl in pH 6.8 (simulated intestinal fluids) was around 71.08%. H8 (71.08%) and H2 (70.02%) showed the best release properties, respectively. Also, hydrogels with added gelatin showed the lowest release rate of (60.31 %). In pH 7.4 mediums, H2 has a release rate of 67.19 % in pH 7.4. When the effect of gelatine on hydrogels was compared, the release rates of HEMA/gelatine hydrogels decreased in solutions.

I hypothesize that Irg 651, 4-AcM, and PEG-DA Mn=258 may selectively incorporate into the hydrogels in all solutions to a greater extent than the others. Hydrogels can be applied successfully application for targeted drug delivery used for pH 1.2, pH 6.8, and pH 7.4.

Release kinetics

Zero order, first order, Hixson Crowell, and Korsmeyer-Peppas kinetics models were studied, and analyzed data fitted into Korsmeyer-Peppas kinetic. The model that gives a higher R-squared value is considered as optimal for the release data. The release data were investigated due to the Korsmeyer-Peppas equation, the n values for prepared hydrogels formulations ranged from 0.924 to 0.9878 in all media. According to the Korsmeyer-Peppas model, diffusion is the significant mechanism of metformin hydrochloride release from these hydrogels.

Table 1. Release kinetics of hydrogels

	pH	Zero order		First order		Hixson-Crowell		Korsmeyer-Peppas		Best fit model
		R^2	K_0 [mg/h]	R^2	K_1 [h^{-1}]	R^2	K_s	R^2	n	
H1	1.2	0.9657	$6.7x10^{-3}$	0.9419	0.8527	0.9509	0,0106	**0.9841**	0.2012	Korsmeyer-Peppas
	6.8	0.9621	$2.89x10^{-3}$	0.9596	0.5869	0.9623	0.0042	**0.9674**	0.1868	
	7.4	0.8744	$6.43x10^{-3}$	0.8430	1.0072	0.8545	0.0112	**0,9240**	0.2295	
H2	1.2	0.9330	$1.16x10^{-2}$	0.8966	0.9170	0.9100	0.0145	**0.9598**	0.2486	
	6.8	0.9262	$8.72x10^{-3}$	0.9015	0.8390	0.9104	0.0109	**0.9671**	0.0835	
	7.4	0.9316	$4.2x10^{-3}$	0.9217	0.6375	0.9251	0.0051	**0.9621**	0.1868	
H3	1.2	0.9310	$8.6x10^{-3}$	0.8993	0.8398	0.911	0.0113	**0.9633**	0.1977	
	6.8	0.9149	$6.8x10^{-3}$	0.8930	0.8200	0.9008	0.0091	**0.9575**	0.1617	
	7.4	0.9249	$5.9x10^{-3}$	0.9021	0.9732	0.9102	0.0087	**0.9637**	0.1618	
	1.2	0.8967	$1.73x10^{-2}$	0.8241	1.6577	0.8657	0.0264	**0.9332**	0.5119	

	pH	Zero order		First order		Hixson-Crowell		Korsmeyer-Peppas		Best fit model
		R^2	K_0 [mg/h]	R^2	K_1 [h^{-1}]	R^2	K_s	R^2	n	
H4	6.8	0.9633	$7.6x10^{-3}$	0.9386	1.1535	0.9479	0.0109	**0.9831**	0.1976	
	7.4	0.9299	$1.12x10^{-2}$	0.8995	1.1627	0.9052	0.0151	**0.9556**	0.2707	
H5	1.2	0.9643	$1.05x10^{-2}$	0.9343	1.6577	0.9457	0.0135	**0.9806**	0.2374	
	6.8	0.9124	$6.7x10^{-3}$	0.8835	1.1535	0.8939	0.0105	**0.9519**	0.2011	
	7.4	0.8924	$6.39x10^{-3}$	0.8657	1.1627	0.8754	0.0104	**0.9389**	0.2052	
H6	1.2	0.9594	$1.58x10^{-2}$	0.8980	1.3683	0.9244	0.0231	**0.9563**	0.4316	
	6.8	0.9310	$7.3x10^{-3}$	0.8994	1.1166	0.9109	0.0113	**0.9621**	0.2136	
	7.4	0.9472	$4.8x10^{-3}$	0.9300	1.0516	0.9361	0.0076	**0.9778**	0.1435	
H7	1.2	0.8830	$1.30x10^{-2}$	0.8225	1.2411	0.8459	0.0206	**0.9080**	0.4066	
	6.8	0.9574	$4.8x10^{-3}$	0.9406	0.7960	0.9466	0.0068	**0.9855**	0.1253	
	7.4	0.9636	$4.4x10^{-3}$	0.9485	0.8571	0.9539	0.0069	**0.9878**	0.1289	
H8	1.2	0.9607	$1.01x10^{-2}$	0.9394	0.5805	0.8459	0.0224	**0.9842**	0.1651	
	6.8	0.9625	$1.00x10^{-2}$	0.935	0.9071	0.9453	0.0125	**0.9815**	0.2127	
	7.4	0.9496	$6.3x10^{-2}$	0.9258	0.9953	0.9343	0.0089	**0.9717**	0.1593	
H9	1.2	0.9607	$9.73x10^{-3}$	0.923	1.0679	0.9374	0.0153	**0.9753**	0.2677	
	6.8	0.9119	$4.29x10^{-3}$	0.935	0.8031	0.9016	0.0066	**0.9603**	0.1141	
	7.4	0.9200	$7.67x10^{-3}$	0.9258	1.1415	0.8968	0.0609	**0.9519**	0.2420	
H10	1.2	0.9217	$1.03x10^{-2}$	0.8691	1.3988	0.8891	0.0179	**0.9413**	0.3460	
	6.8	0.9409	$5.8x10^{-3}$	0.9144	0.9534	0.9238	0.0089	**0.9666**	0.1581	
	7.4	0.9075	$7.7x10^{-3}$	0.8761	1.1523	0.8866	0.0126	**0.9433**	0.2371	

CONCLUSIONS

In the completed study, HEMA hydrogels and HEMA with PEG-DA, 4-AcM, and gelatine hydrogels were entirely prepared by the UV photopolymerization method. The swelling studies of hydrogels in different pH mediums indicated that the hydrogels responded to pH and hydrogels were pH sensitive. The incorporation of PEG-DA and 4- AcM into the hydrogels improved the metformin HCl release rate. Also, the swelling and release behavior of hydrogels had been impacted the kind of photo-initiators. In conclusion, *in vitro* release analyses demonstrated that HEMA-based hydrogels can be used as a controlled release of metformin HCl.

EXPERIMENTAL SECTION

Materials

Poly (ethylene glycol) diacrylate (Mn=258, Mn= 700), ethylene glycol dimethacrylate, photoinitiators (2,2-dimethoxy-2-phenyl-acetophenone (Irg 651, 99% purity), 1-hydroxycyclohexyl phenyl ketone (Irg 184, 99% purity)), 4-Acryloyl morpholine, 2-hydroxyl ethyl methacrylate were supplied from Sigma-Aldrich. Hydrochloric acid and sodium chloride were bought by Merck. Sodium hydroxide and monobasic potassium phosphate were provided from J.T Baker. Metformin HCl was gifted by Ali Raif Pharmaceutical Company. All chemicals were not purified.

Preparation and Characterization of Hydrogels

HEMA-based hydrogels were obtained from Irgacure 184, Irgacure 651, 4-AcM, PEG-DA, and ethylene glycol dimethacrylate as seen in Table 1. According to formulations, the reactants were put into glass molds (15 mm (diameter), 1 mm (depth)), and were deaerated by bubbling with nitrogen gas during the photopolymerization. UV irradiation was applied at 365 nm for 300 s.

According to hydrogel properties, 50 % (w/v) HEMA and indicated ratios of Irg 184, Irg 651, PEG-DA, gelatine, and 4-ACM were prepared with using a magnetic stirrer at 50 rpm. 1 % (w/v) Metformin HCl and deionized water, 1 % ethylene glycoldimethacrylate was embedded in, respectively. After the reaction, hydrogels were purified with n-hexane. Then, hydrogels were dried at 25 °C.

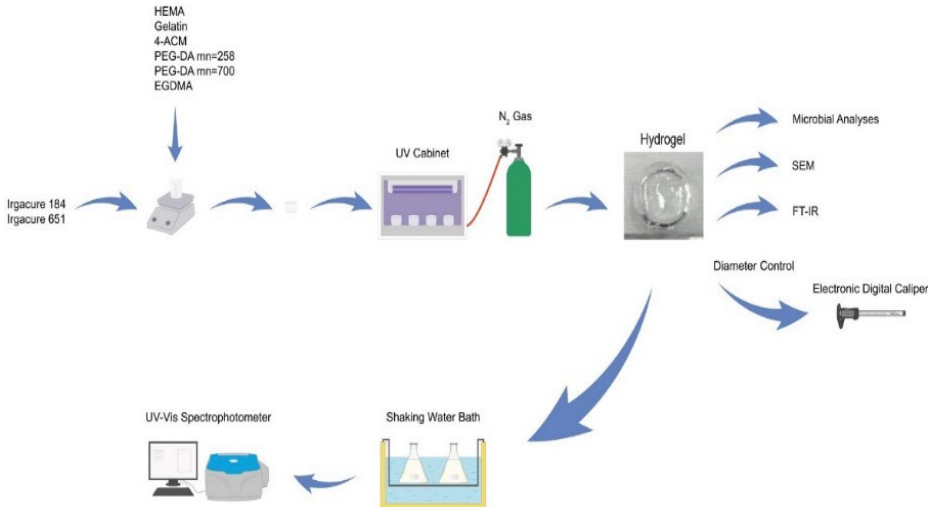

Figure 10. Image of analysis set up

The hydrogels were characterized by a digital microscope. Figure 10. indicates the images of hydrogels which were taken with the digital microscope. The diameter of hydrogels was 0.10 ± 0.02 cm.

Table 2. Types of hydrogels

	HEMA	EGDMA	Irg 184	Irg 651	PEG-DA 700	PEG-DA 258	4- AcM	Gelatine	Metformin HCl
H1	50%	1%	0.75%	-	-	-	-	-	1%
H2	50%	1%	-	0.75%	-	-	-	-	1%
H3	50%	1%	0.75%	-	0.25%	-	-	-	1%
H4	50%	1%	-	0.75%	0.25%	-	-	-	1%
H5	50%	1%	0.75%	-	-	0.25%	-	-	1%
H6	50%	1%	-	0.75%	-	0.25%	-	-	1%
H7	50%	1%	0.75%	-	-	-	0.25%	-	1%
H8	50%	1%	-	0.75%	-	-	0.25%	-	1%
H9	50%	1%	0.75%	-	-	-	-	0.25%	1%
H10	50%	1%	-	0.75%	-	-	-	0.25%	1%

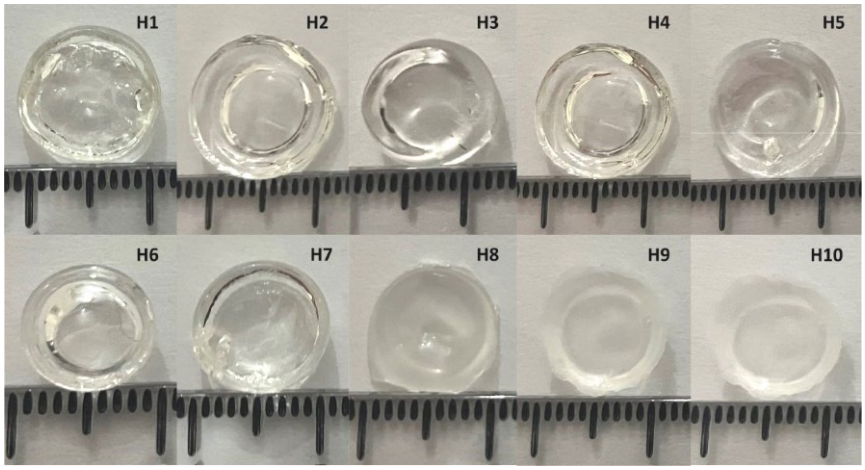

Figure 11. Image of hydrogels

ACKNOWLEDGMENT

The author is also thankful to Murat Şenol, Assoc. Prof. Dr. Emel Akyol, Merve Gülter Ak, and Feryal Ülkücü.

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