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Designing of drug imprinted polymeric microcryogels for controlled release of Darunavir

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Abstract

Darunavir (D.V) is a pivotal antiretroviral medication designed to combat viruses with prolonged treatment requirements, notably gaining recognition as one of the primary choices for treating AIDS, a disease caused by the human immunodeficiency virus (HIV). Bio polymeric materials like microcryogels become the center of attention in most research areas such as controlled release systems. These systems offer the advantage of precise drug administration, ensuring effective therapeutic outcomes through the delivery of specific drug doses. Microcryogels, characterized by their super macroporous, elastic, and spongy morphology, have emerged as a focal point in biomedical applications, particularly when combined with molecularly imprinted polymers (MIPs). In this study, the controlled release and kinetics studies of the D.V were investigated with the D.V imprinted poly (2-hydroxyethyl methacrylate) (pHEMA) based microcryogels. D.V imprinted pHEMA microcryogels with different cross-linker ratios and different loaded drugs were prepared for studies of in vitro release of D.V scanning electron microscopy (SEM), Brunauer–Emmett–Teller (BET) and Fourier transform infrared spectroscopy (FTIR) methods have been considered suitable for the characterization of cryogels that have been designed and whose sensitivity has been enhanced by molecular imprinting. Cytotoxicity of D.V imprinted microcryogels was also inspected using mouse fibroblast cell line L929. The comprehensive analysis results underscore the potential of these meticulously designed microcryogels, showcasing their utility in medical applications. Notably, these microcryogels exhibited controlled drug release, with efficiency levels of up to 85% and a sustained release duration of 40 hours, positioning them as a valuable option for advanced drug delivery systems in the medical field.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) has played a pivotal role in the evolution of antiretroviral drug development. Over the years, spanning from the mid-1990s to the present, the landscape of HIV infection has been transformed into a manageable chronic condition, thanks to early diagnosis, consistent monitoring, and the advent of antiretroviral drug therapies [1]. Over the years, substantial progress has been made in the development of antiretroviral drugs, resulting in the creation of effective, user-friendly, and highly potent protease inhibitors (PI)s. Treatment strategies revolving around HIV-1 protease (PR) inhibitors are geared towards granting patients the opportunity for a prolonged and improved quality of life [2]. The mature human immunodeficiency virus HIV-1 protease (PR) is one of the most important enzymes in D.V drug discovery. D.V, which entered the market in the tenth place and was included in the aspartyl protease group, is one of the most powerful weapons developed against AIDS [3]. On the other hand, D.V is the second generation non-peptidic peptidomimetic PI and antiretroviral drug that requires a high therapeutic dose, to deal with the problems such as severe side effects and drug toxicities [4, 5]. D.V is used orally with ritonavir and other medicated combinations to treat and prevent HIV/AIDS [6]. In recent years, managing the drug dosage is the main focus of treatments for many reasons [7]. During the treatment, many studies are carried out in order to extend the therapeutic period of the patients against the drug and to reduce its side effects [8]. Controlled drug release systems are at the

forefront of these studies and offer an effective treatment method based on delivering drugs at desired dosing intervals [9]. In traditional methods, as the drug is mixed into the blood, the level of active substance in the blood should be kept within a range called the plasma concentration (therapeutic window) of a therapeutic agent effectively. This interval ranges from the minimum effective concentration to the minimum toxic concentration. Drug release systems focus on these two main concentration points, and the drug can be released over a long period in the therapeutic window range [10]. Drug release systems are also suitable for incorporating many innovative methods such as molecular imprinted polymers. Molecular imprinting technique has proven its effectiveness in many medical applications as a highly versatile approach. For example, molecular imprinting has been widely used in the detection of allergens or proteins at low concentrations, to increase the sensitivity in biosensor studies, and in determinations that require high selectivity. The technique is based on preparing a template cavity for the desired molecule to increase molecular affinity [11]. High affinity is achieved thanks to the functional monomers and cross-linker used in the polymerization [12]. In this process, polymer matrices with special recognition and catalytic site properties are also obtained. When the molecule is removed from the polymer, target-specific cavities are occurred [13, 14]. Molecular imprinted polymers exhibit high stability in pH changes, the presence of organic solvents, and high-temperature pressure environments. Therefore, it can easily adapt to the potential changes of the human body, so it can be applied as an effective treatment tool in drug release systems [15–20]. The involvement of cryogel structures in the proven effectiveness of molecular imprinting in drug release systems carries the studies to another point. Hydrogels are considered as a three-dimensional cross-linked natural or synthetic polymer network capable of absorbing large amounts of water or biological fluid, and when this polymer network is formed at low temperatures, the structures formed are called cryogels. These functioning materials are classified as next-generation adsorbents thanks to their unique structural properties and flow dynamics of solutes of all sizes, ranging in size from 10–100 μm [21–25]. These versatile structures, which have been proven in many studies to be useful in biomedical fields, stand out with their ability to resist chemical and mechanical deformation, carriers for antibody, enzyme and cell immobilization, bioaffinity materials and gel bases for swelling kinetics drug release systems [26–30]. The objective of this research is to present an effective and innovative method to the literature by combining molecular imprinting and cryogel approaches. Poly hydroxyethyl methacrylate-N-methacryloyl-(L)-histidine methyl ester (pHEMA) based cryogels were synthesized. D.V is complexed by chelation with copper. N-Methacryloyl (L)-histidine methyl ester (MAH) is included in the structure as a co-functional monomer in imprinting. The release behaviors of D.V were observed in different loading concentrations, temperatures and pH environments. In addition, in order to reach the most stable form of the cryogel structure, different cross-linker ratio cryogels were synthesized. Characterizations of the designed cryogels were made and the results were shared.

2. Experimental

2.1. Materials

D.V was obtained from Hacettepe University Hospital Infectious Diseases and Clinical Microbiology Department. N-Methacryloyl-(L)-histidine methyl ester (MAH) monomer was purchased from NanoReg (Ankara, Turkey). Ammonium persulphate (APS), 2-hydroxyethyl methacrylate (HEMA), N,N,N′,N′-tetramethylene diamine (TEMED), methylene bisacrylamide (MBAAm), and were obtained from Sigma Chemical Co. (St. Louis, MO). Deionized water (DW) was obtained from Thermo Scientific™ Barnstead™ Nanopure water purification device. Remained chemicals were acquired from Merck AG (Darmstadt, Germany).

2.2. Preparation of Pre-complex Used for Molecular Imprinted

The investigation into the impact of chelated Cu (II) levels involved the synthesis of microcryogels containing three distinct Cu (II) concentrations. In the process of creating the MAH-Cu (II) pre-complex, the MAH functional monomer remained constant, while Cu (II) metal ions were incorporated in molar ratios of 1:1, 1:2, and 1:3. These pre-complexes were derived from $Cu(NO3)2{\cdot}5H2O$, resulting in pre-complex solutions of 1:1, 1:2, and 1:3.

Following this, 1.0 mol of D.V was introduced into each of the pre-complex solutions. The ensuing mixture of MAH-Cu(II) and D.V was then dispersed in 1.0 mL of 10 mM phosphate buffer (PBS) with a pH of 7.4. Subsequently, the solution underwent thorough mixing on a rotator at 25°C for a duration of 30 minutes.

2.3. Preparation of D.V Imprinted Microcryogels

The synthesis process unfolded as follows: Initially, 1.3 mL of HEMA monomer was introduced into a beaker. Subsequently, 0.283 g of MBAAm was dissolved in distilled water (DW) and then mixed with the HEMA solution. The total solution volume was adjusted to 12 mL. Next, the MAH-Cu (II)– D.V complex, previously prepared, was incorporated into this mixture and stirred for 10 minutes. To initiate polymerization, TEMED (25 µL) and APS (20 mg) initiator pairs were swiftly added to the mixture.

This resulting blend was carefully introduced into a mold featuring holes with dimensions of 200 µm in diameter and 500 µm in thickness. Polymerization commenced and continued for 24 hours at a temperature of -14°C, positioned between two glass plates. Following this phase, the microstencil mold was subjected to a lyophilization process for 2 hours at -56°C and 0.0010 mbar. Upon completion of this period, the D.V imprinted (MIP) microcryogels formed between the glass plates were extracted from the microstencil mold after reaching room temperature. To eliminate any residual unreacted monomers, the cryogels were subjected to multiple washes with distilled water.

The synthesis steps for microcryogels are visually represented in Fig. 1. The same protocol was applied to create D.V non-imprinted (NIP) microcryogels, with the distinction that D.V drug was omitted from the monomer phase during microcryogel formation.

For all complexes containing different Cu(II) ratios, this methodology was employed. Experimental results demonstrated a more consistent release pattern at a 1:1 ratio; consequently, further studies were conducted at this ratio. In the synthesis of NIP microcryogels, the MAH-Cu (II) pre-complex was utilized in a 1:1 mole ratio. Additionally, in the synthesis of pHEMA microcryogels without employing MAH-Cu (II) and D.V as target molecules, HEMA served as the monomer, and MBAAm functioned as the cross-linker in both MIP and NIP microcryogel production.

Subsequently, the APS initiator was combined with TEMED and introduced into a microstencil mold with identical dimensions. As part of this study, we embarked on the synthesis of MIP microcryogels, which featured varying proportions of MAH-Cu (II) and different ratios of cross-linker. Notably, the quantity of cross-linker played a critical role as one of the parameters influencing the swelling rate, surface area, and release kinetics within the microcryogels.

It's worth noting that MBAAm, serving as the cross-linker, exhibits hydrophobic characteristics. Consequently, an escalation in the MBAAm ratio results in the augmentation of less hydrophilic groups within the polymer structure. Consequently, this augmentation leads to the creation of more robust and enduring polymer networks within the microcryogels.

2.4. Characterization of Microcryogels

2.4.1. FTIR Analysis

The bulk structure of D.V imprinted (MIP), non-imprinted (NIP) and pHEMA microcryogels was examined by FTIR. Before analysis, the microcryogels were dried by lyophilization for 24 hours at -60°C. Then, 2.0 mg of polymer sample was mixed with 98 mg of KBr and pounded in a mortar to obtain FTIR spectra. It was then turned into a fine pellet at a pressure of 600 kg/cm^2 in a hydraulic press. FTIR characterization of the material was performed in the wavelength range of 4600 – 400 cm-1.

2.4.2. Surface Morphology

Surface morphologies of injectable microcryogels were investigated by scanning electron microscopy (SEM, GAIA3, Tescan, Czech Republic). In the first step, the microcryogels were lyophilized at -50°C (0.050 mbar) Then, the samples were coated with gold-palladium (40:60) under vacuum and their surfaces were made conductive, and the surface morphology was placed in the SEM slot, their structures were examined at different magnifications and their images were taken.

2.4.3. Swelling Tests

In order to determine the swelling properties of the cryogels, the dry and swollen weights of the cryogels were taken separately. Dry cryogels, whose weights were measured, were first soaked in water for two hours, and excess water was removed with absorbent paper and the residue was weighed. Eq. 1 is used for determining the swelling rate

$$\text{Swelling \%} = (W_{swelled} - W_{dry}) / W_{dry} \quad \textbf{(1)}$$

2.4.4. Surface Area Measurements

The surface area of the pHEMA-based microcryogel was measured by multi-point analysis using the surface area measuring instrument BET method.

2.5. Investigation of Release behavior of Darunavir from MIP Microcryogels

D.V release studies after drying MIP microcryogels 3 mL PBS buffer, pH 7.4 was studied at 37°C. Cu(II) leakage of injectable microcryogels was investigated with a graphite furnace atomic absorption spectrometer AAS (Analyst 800 / Perkin-Elmer, Shelton, CT). The outcomes are obtained n = 3 considering of environment and drug handling behavior and mean values are denoted. MIP microcryogels were investigated for different loaded D.V concentrations. The concentration of the D.V was determined at 280 nm. Shimadzu, Model 1601, Tokyo, Japan UV/Vis spectrophotometer is used for the entire experiment. Also, to show the effect of pH on the cumulative release of D.V, the effect of pH was inspected at different pH values. In order to see the effect of cross-linker and monomer ratio at different ratios, the cryogels with the mole ratios of HEMA and MBAAm 4, 6, 8 were synthesized. As the cross-linker ratio increased, the release rate decreased. In order to investigate the effect of release at different pHs, drug release from D.V loaded microcryogels at 4 pHs (6.0, 7.0, 7.4 and 8.0) were investigated.

2.6. Cytotoxicity Studies

ISO-10993-5 'Biological Evaluation of Medical Devices' standards was used to investigate the cytotoxic effect and 3-(4,5-dimethyl / thiazol-2-yl) 2,5-diphenyltetra zolium bromide thiazolyl blue (MTT) test was used and fibroblast cell line (L929) was used. The culture medium of the cells was established with 10% fetal bovine serum and 10% L-glutamine in a humidified atmosphere containing 95% air and 5% CO_2 at 37°C containing DMEM and lasted for 3 days. Ultraviolet light was used for the sterilization of cryogels and they were incubated for 72 hours at 37°C. L929 cells have densities of 1×10^3 and these cells were cultured in 96 wells with a volume of 200 µL and incubated overnight in a humidified medium with 5% CO. The cell culture medium was then replaced with the extraction medium and incubated again at 37°C for 24 hours. Cultured cells were treated with 100 µL/well of MTT solution for 4 hours. The plates were then incubated at room temperature for 30 minutes in a dark place. Finally, the wavelength of 540 nm was read by an automated enzyme-linked immunosorbent assay (ELISA).

3. Results

In this study, an antiretroviral drug, D.V imprinted microcryogels and its release kinetics were investigated. D.V-imprinted pHEMA-based microcryogels were synthesized with varying porosity by using polymer precursors with different properties. In addition, the effect of loading amount was observed by adding different amounts of D.V. while preparing the microcryogels. Then, the release kinetics and cytotoxicity of the prepared microcryogels were investigated. Experimental methods were carried out in three basic steps; preparation of D.V imprinted and non-imprinted pHEMA-based microcryogels (MIP and NIP

microcryogels), characterization of D.V imprinted and unimpressed pHEMA-based microcryogels (MIP and NIP microcryogels), investigation of D.V drug release under in vitro conditions

3.1. Characterization of Microcryogels

The amide bands at 1714 cm^{-1}, 1658 cm^{-1} in the structure of MIP microcryogels is observed in FTIR. Also the bands at 1725 cm^{-1}, 1661 cm^{-1} of NIP microcryogels support the characterization of the both structure. (Fig. 2). 3000–2900 cm^{-1} peaks are the markers of aromatic and aliphatic C–H stretch bands. 3400–3200 cm^{-1} broad peaks are identifiers of O–H presence in MIP cryogels. The place of the metal complex in the structure has been proven by amide bands.

Three different cross-linker ratios were synthesized to examine the effects of pore structure and surface area of microcryogels on drug release behavior. The surface morphology of the microcryogels prepared within the scope of this study was examined by scanning electron microscopy and structural morphology by microscope. Optical images and SEM images of microcryogels are given in Figs. 3 and 4. In SEM images, there is a uniform and homogeneous distribution within the microcryogel polymer structure. It is seen that the macropore sizes in the polymeric structure are larger than 20 µm. Due to these macropores, it has been determined that the channels are interconnected in the cryogel structure. Macropores facilitate the diffusion of water from the microcryogel structure into the polymeric material and to the external environment during the swelling and shrinking processes.

This reversible swelling-shrinkage behavior of macroporous microcryogels is considered an important advantage in biomaterials and biotechnology applications. The effect of drug loading rate on swelling behavior and surface area was investigated for microcryogels synthesized at -14°C. The surface area and swelling behavior of microcryogels are compiled in Table 1 at different loading rates.

Table 1
Effect of swelling behavior and surface area of microcryogels loaded with different amounts of D.V.

Microcryogels	Amount of loaded drug (mg D.V/mg microcryogel)	Swelling rate (%), mg H_2O/mg microcryogel	Surface area (m^2/g)
MIP I	0.5	8.17	14.1
MIP II	1	8.65	14.9
MIP III	1.5	9.06	15.3
MIP IV	1.75	9.88	16.1
NIP	-	8.01	12.8
pHEMA	-	7.65	12.1

It is also can be seen that both the swelling ratio and the surface area increase significantly with the increase of D.V loading amount. Increasing the D.V loading amount from 0.5 mg to 1.75 mg increased the

swelling rate from 8.17–9.88%. An almost 1.2-fold increase in swelling rate has been reported to be consistent with an increase in surface area values. The surface area value of 0.5 mg D.V loaded pHEMA microcryogel was 11.7 m^2/g, while this value was reported as 18.5 m^2/g for 1.75 mg D.V loaded. The increase in surface area is an important parameter in D.V release studies, controlling its rate and also in releasing the amount of D.V. The increase in the structural pores of microcryogels causes this behavior. As a result of this increase, the amount of water entering the structure of microcryogels increases. In addition, 1.0 mg of MIP and NIP microcryogels were kept separately at pH 4.5, pH 7.4, and pH 8.5 for 24 hours in order to control the leakage of Cu (II) ions from the structure of MIP and NIP microcryogels, and then the metal ions concentration of the solution taken from the environment was read. Reading of Cu (II) ions was done by AAS. In the reports obtained as a result of the analysis, no Cu (II) ions were detected in each pH solution. From the results, it is possible to say that the pre-complex is stable. Therefore, it has been explained that in drug release applications, Cu (II) ions are not released from the structure of microcryogels and cannot cause any toxic effects.

3.2. Investigation of Darunavir Release Behavior from MIP Microcryogels

3.2.1. Investigation of the Effect of Cu (II) Ion in Different Ratios on the Release Rate

In the preparation of microcryogels, the effect of the increase in Cu (II) on the release rate of D.V was investigated by preparing 3 different mole ratios of MAH-Cu (II) pre-complex separately and selecting 3 different D.V imprinted microcryogels. At this stage, the mole ratio of MAH functional monomer is kept constant and the amount of Cu (II) ions is 1MAH-1Cu (II)- D.V, 1MAH-2Cu (II)-D.V, and 1MAH-3Cu (II)-D.V, 1:1, 1:2, and changed to 1:3. In the examination of the effect of D.V release rate with 3 different microcrystals prepared with different ratios of MAH-Cu (II)- D.V pre-complex, the ratio of monomer and cross-linker was kept constant as 4. In addition, while the amount of D.V loaded was 1.5 mg/mL, the release was at 37°C by keeping the pH of the release at 7.4. The graph of the release rate is given in Fig. 5. Due to the results obtained, the mole ratio of MAH-Cu (II)- D.V to be used throughout the research was chosen as 1:1:1, since the release rate of D.V is more controlled at a mole ratio of 1:1:1 and 62%.

3.2.2. Investigation of the Effect of Cross-linker at Different Rates on D.V Release Rate

To determine the effect of cross-linker and monomer ratio at different ratios, 3 different microcrystals were prepared and the release test was performed with these microcryogels. The effect of the cross-linker is shown in Fig. 6 with a molar ratio of 4, 6, and 8, respectively. Since the cross-linker has a significant role in determining the polymer structure and pore size, its effect on the drug release rate should also be observed. D.V was released from microcryogels at a fixed amount of drug (1.5 mg/mL). In Fig. 6, it’s clear that the controlled release of D.V. is dependent on the MBAAm and cross-linker density and inversely

proportional. A high density of the cross-linker causes the microcryogel to become more rigid due to shrinkage and reduced voids in the microcryogel network. The increased amount of the cross-linker leads to the harder structure of the microcryogels due to the narrowing and reduction of the voids in the polymer network. This may have resulted in increased stiffness of the microcryogel in the unfrozen areas as the number of cross-linker increases. At this stage, the molar ratio of MAH-Cu(II)-D.V was used as 1:1:1. D.V amount was chosen 1.5 mg/mL, release pH: 7.4, release temperature 37°C.

3.3.3. Investigation of the Effect of Drug Loading Amount on Drug Release Rate

D.V imprinted microcryogels were prepared by loading 0.5–1.75 mg/mL D.V drug into MIP microcryogels. D.V release rates from microcryogels loaded with different amounts of drug (0.5, 1.0, 1.5, 1.75 mg D.V/mg microcryogel) are shown in Fig. 7. Figure 7 states that the D.V cumulative release increases with the increase in the amount of drug in D.V imprinted microcryogels. D.V release rate of 25% was obtained when D.V loading amount was 0.5 mg D.V/mg microcryogel. This ratio was 40%, 70%, and 85% for 1.0, 1.5, and 1.75 mg D.V loaded microcryogels, respectively. Almost all of the drugs in the microcryogel structure were released from the structure in microcryogels loaded with 1.75 mg. The concentration difference of the drug releases D.V from the microcryogel structure is the driving force of the mass transfer. Therefore, the driving force is proportional to the concentration of the drug. After an initial rapid release, it reached a plateau and remained constant in all drug release loading experiments almost after the 3rd hour. It appears from the figure that the D.V release time from microcryogels can be extended up to 40 hours.

3.3.4. Effect of pH on Darunavir Release Rate

To examine the effect of pH on release rate, different media pHs of 6.0, 7.0, 7.4 and 8.0 was investigated in Fig. 8. In compliance with the obtained results, the maximum and controlled and steady drug release is obtained at pH 7.4. In cases where Cu (II) ions are used in metal chelate systems, it is expected that D.V release will be lower at acidic pH, since the acidic pH of the environment increases the stability of the metal chelate. In the basic environment, the release occurred faster because the interactions between metal ions and the drug were weaker. Acidic pH values are not preferred considering the possibility of damaging the structure of drugs. Therefore, the physiological pH of 7.4 is preferred for release studies. To understand the effect of the pH on the release rate, different pHs (pH: 6.0–8.0) media are prepared. The release test was carried out with MIP microcrystals (selecting n:4 and the pre-complex ratio 1:1:1 for MAH-Cu (II)-D.V). Release experiments were performed at 37 ^{0}C when the D.V imprinting amount was 1.5 mg/mL.

3.4. Analysis of Release Kinetics

The Korsmeyer-Peppas model was chosen to analyze the in vitro drug release kinetics and mechanism.

$M_t/M_\infty = kt^n$ (2)

In the Korsmeyer-Peppas model, the value of n describes different release mechanisms from cylindrical-shaped matrices and k is the release rate constant. Mt is the D.V release amount at time t and the M ∞ is the release at the equilibrium point. To sum up, Mt/M ∞ is the drug release fraction. In Table 2, R^2 correlation coefficient constants and D.V conduction exponent data are presented.

Table 2
The compilation of release kinetic data according to the Korsmeyer-Peppas Model

Microcryogels	Amount of loaded drug (mg D.V/mg microcryogel)	MAH-Cu(II)-D.V mole ratio	Cross-linker/ monomer ratio (n)	n	k	R^2
MIP I	0.5	1:1:1	4	0.53	0.09	0.98
MIP II	1.0	1:1:1	4	0.41	0.16	0.98
MIP III	1.5	1:1:1	4	0.40	0.25	0.98
MIP IV	1.75	1:1:1	4	0.35	0.39	0.98

According to the calculations, the MIP microcryogels correlation coefficient is the most suitable for D.V release. In terms of n values, Fickian diffusion is preferred if the n value is n = 0.5. If n > 0.5, neither abnormal nor non-Fickian diffusions are working. Finally, if n is equal to 1, non-Fickian or state II kinetics are observed. In this study, non-Fickian diffusion type was presented for all designed microcryogels.

3.5. Cytotoxicity Studies

The mouse fibroblast cell line L929 was also used to measure the cytotoxicity of MIP, NIP and

pHEMA microcryogels. Viability of cell lines was observed for MIP, NIP and pHEMA microcryogels in images 12 and 24 hours after application. The measurements of MIP, NIP, and pHEMA microcryogels loaded with 0.5 mg/mL D.V were 95.02 ± 0.13, 96.11 ± 0.11 and 97.02 ± 0.10, respectively. These results can be considered as evidence that MIP microcryogels are not cytotoxic. The detailed results are shown in Fig. 9.

4. Discussion and Conclusion

This study is a successful combination of molecular imprinting and cryogel applications and is presented as an alternative and effective method for controlled drug release. Controlled release of imprinted D.V in pHEMA microcryogels was investigated at different parameters such as pH, drug concentration and various cross-linker and monomer ratios. The results showed that the controlled release of D.V from microcryogels was promising in terms of and adjustability yet steady kinetics. Biocompatibility of microcryogels has been proven with cytotoxicity tests as well. In conclusion, the versatility of cryogels has been demonstrated and presented as an inspiring approach for use in many medical applications.

The viability of MIP, NIP, and pHEMA microcryogels loaded with 0.5 mg/mL D.V were obtained 95.02 ± 0.13, 96.11 ± 0.11 and 97.02 ± 0.10, respectively. These results can be considered as evidence that MIP microcryogels are not cytotoxic.

Declarations

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

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Figures

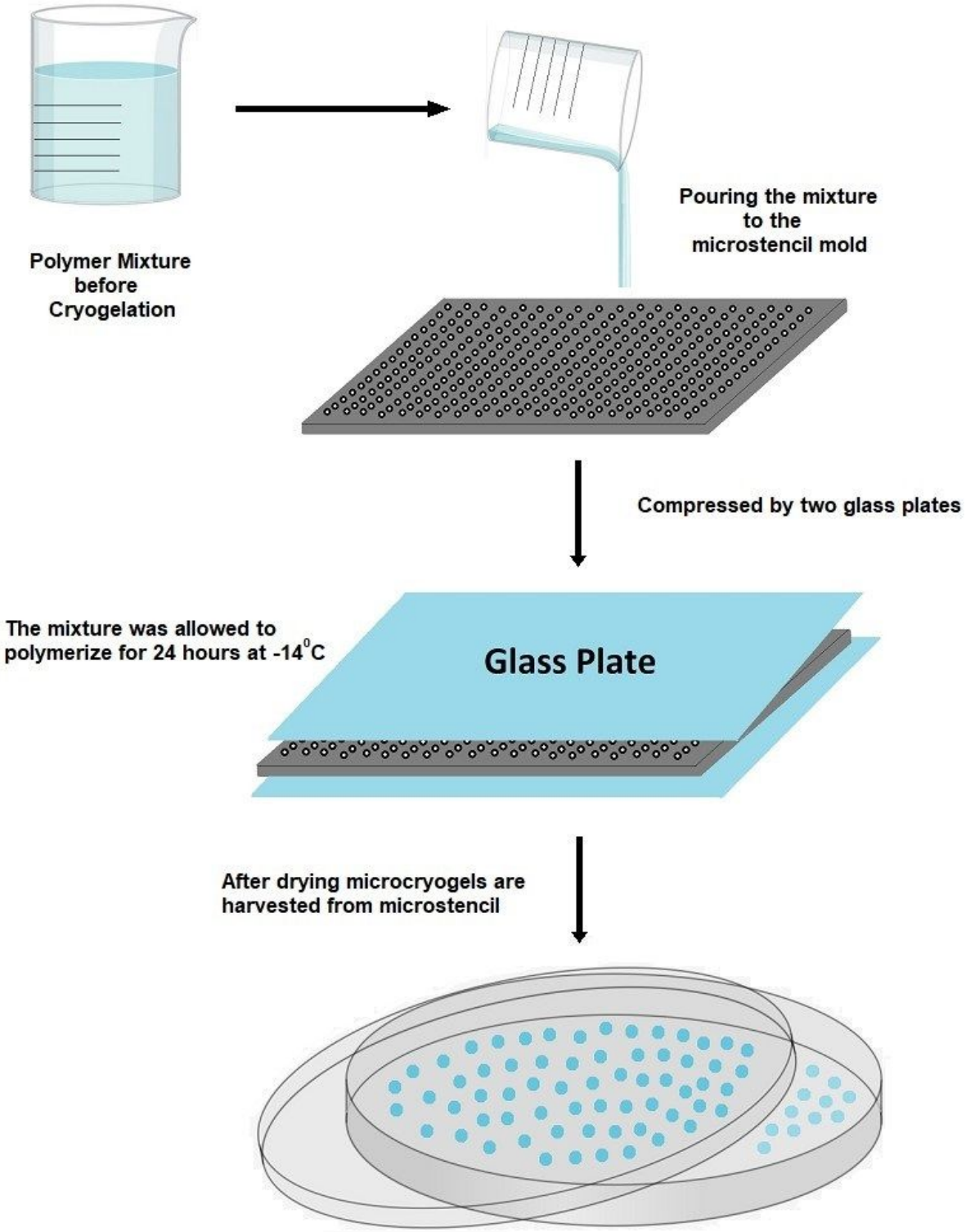

Figure 1

Schematic representation of injectable microcryogels synthesis

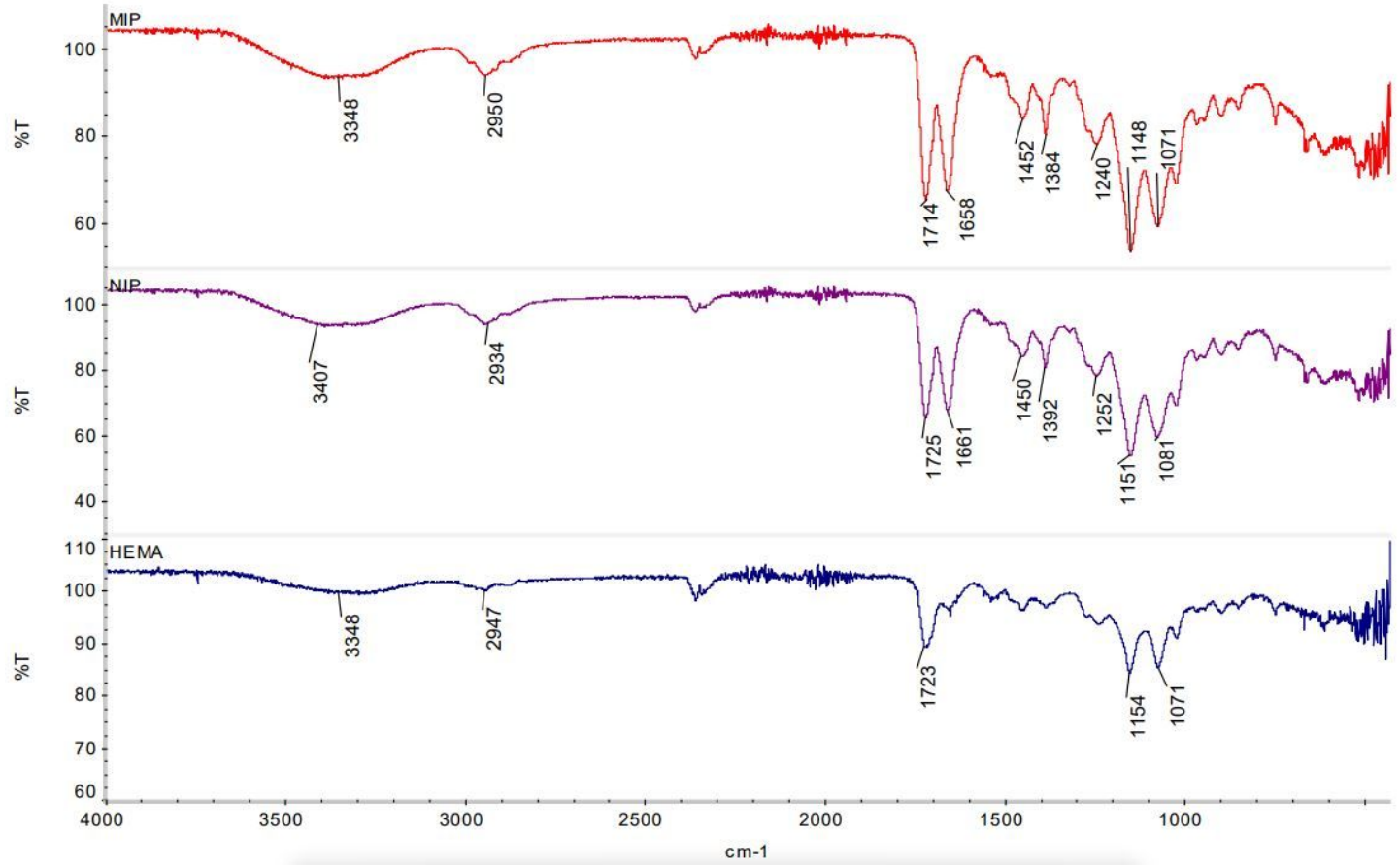

Figure 2

FTIR spectrum of D.V imprinted (MIP), non-imprinted (NIP), and pHEMA microcryogels.

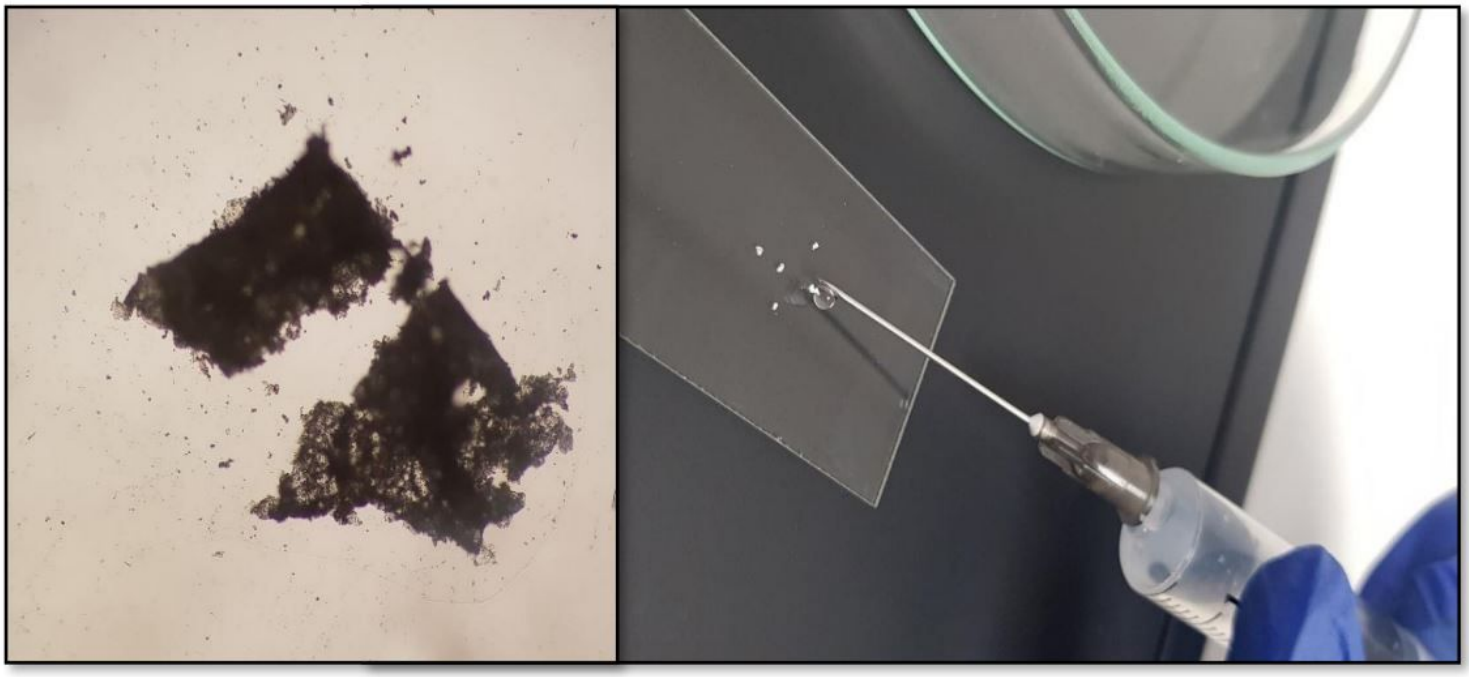

Figure 3

Optical images of microcryogels

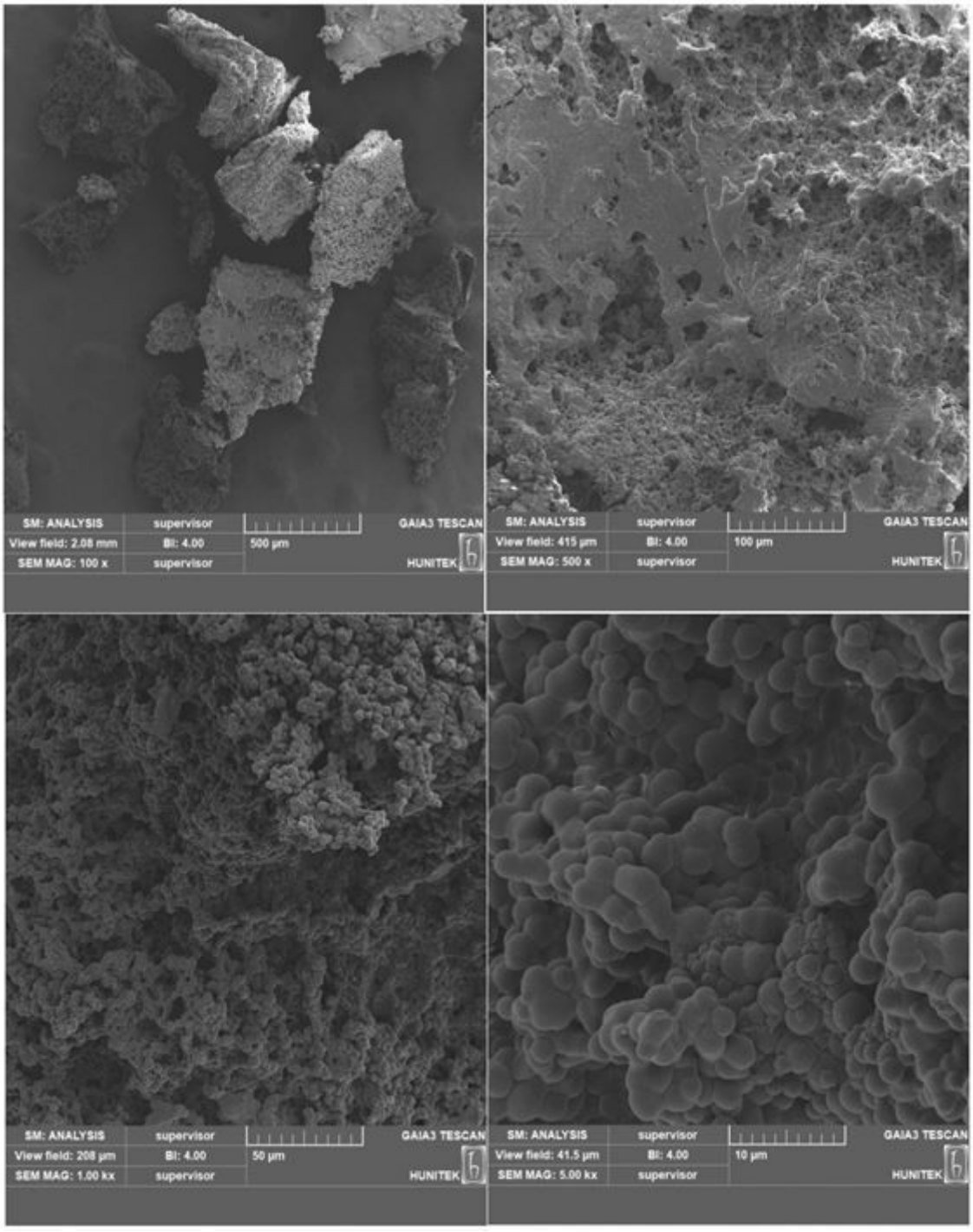

Figure 4

SEM images of microcryogels

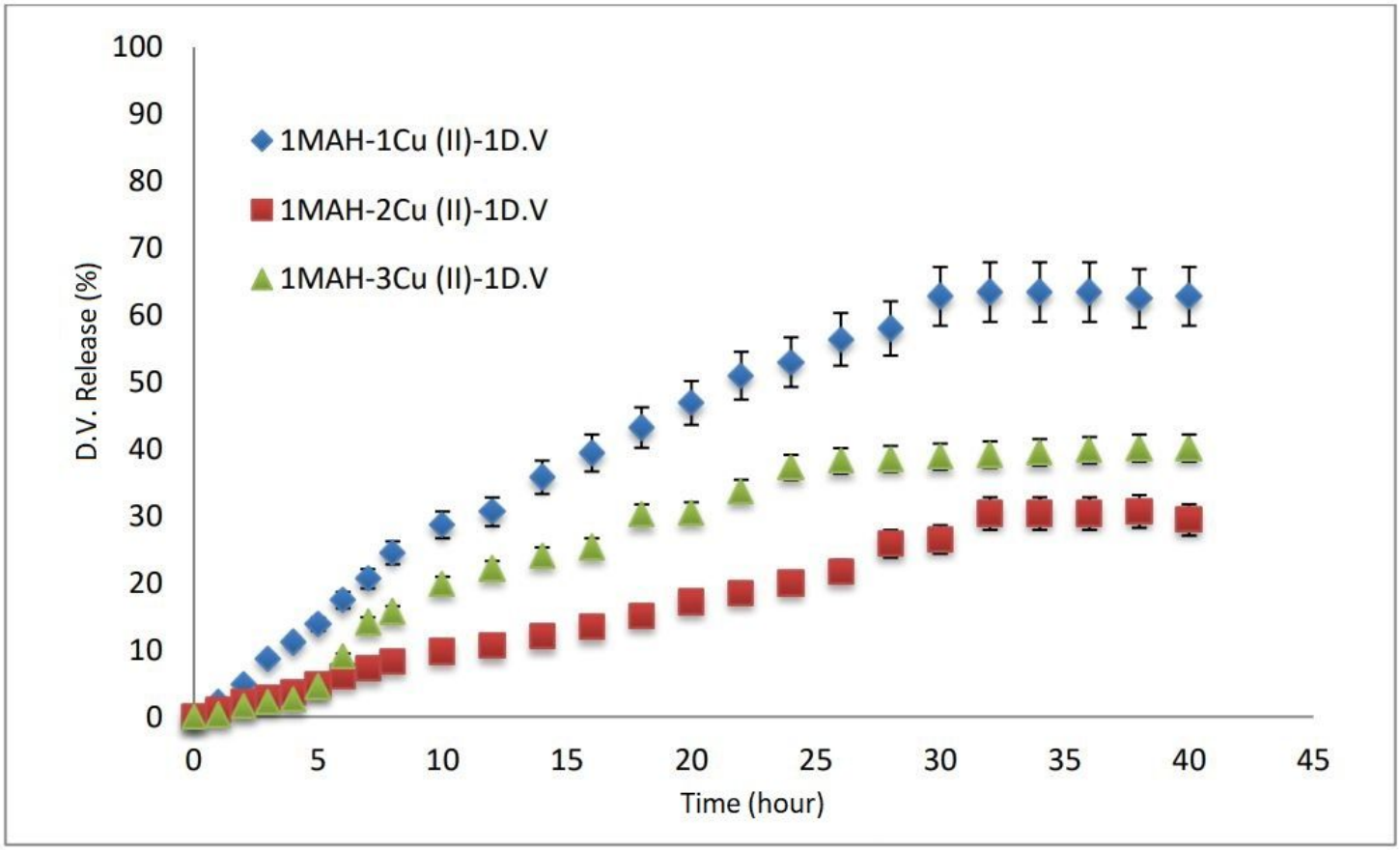

Figure 5

Effect of Cu(II) in at different ratios on the release rate, release medium pH: 7.4; Temperature: 37°C

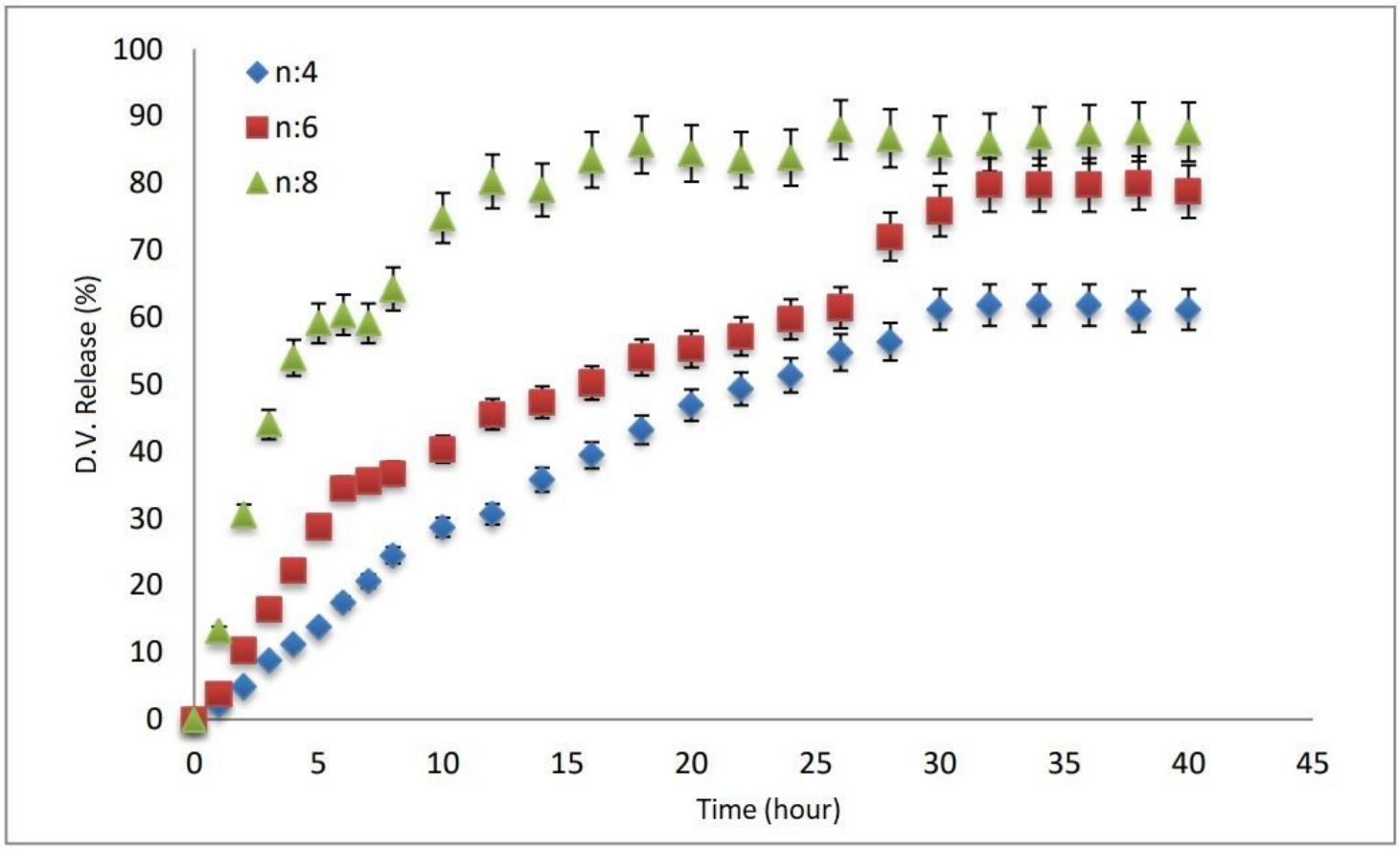

Figure 6

Effect of different cross-linker and monomer ratios on the release rate, release medium pH: 7.4; Temperature: 37°C

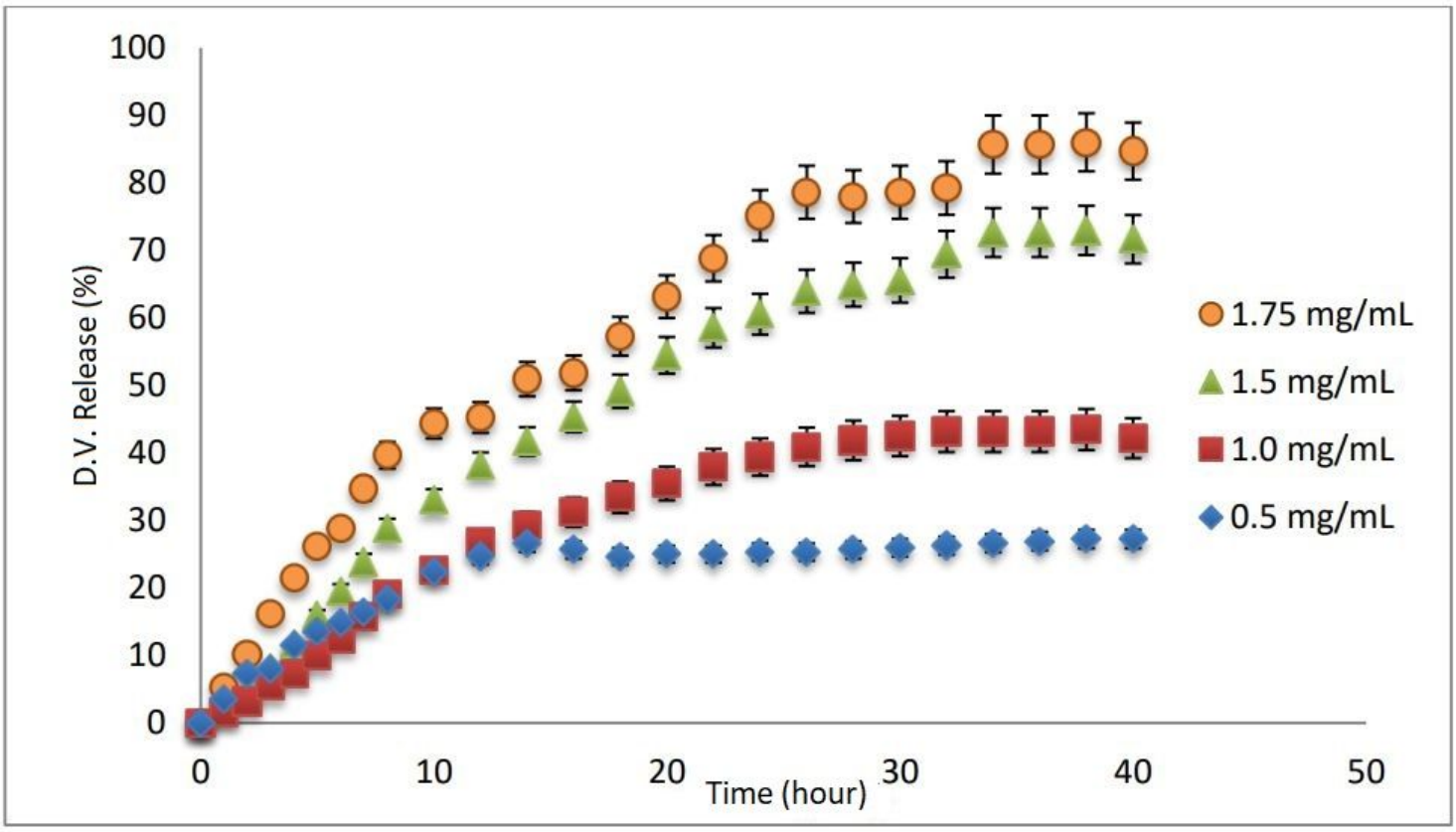

Figure 7

Effect of D.V loading amount on the drug release rate, release medium pH: 7.4; Temperature: 37°C

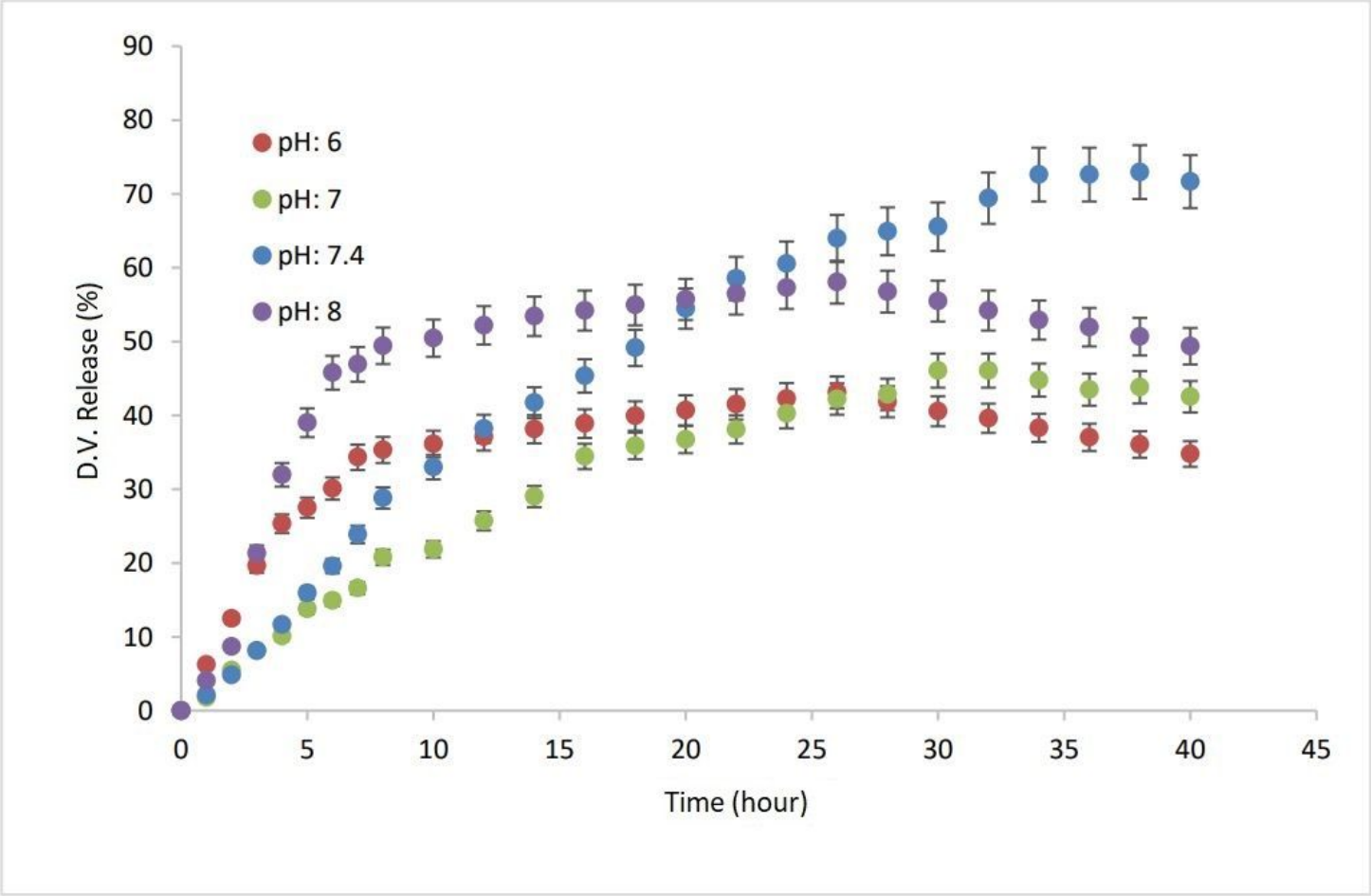

Figure 8

Effect of pH on D.V release rate, Temperature: 37°C

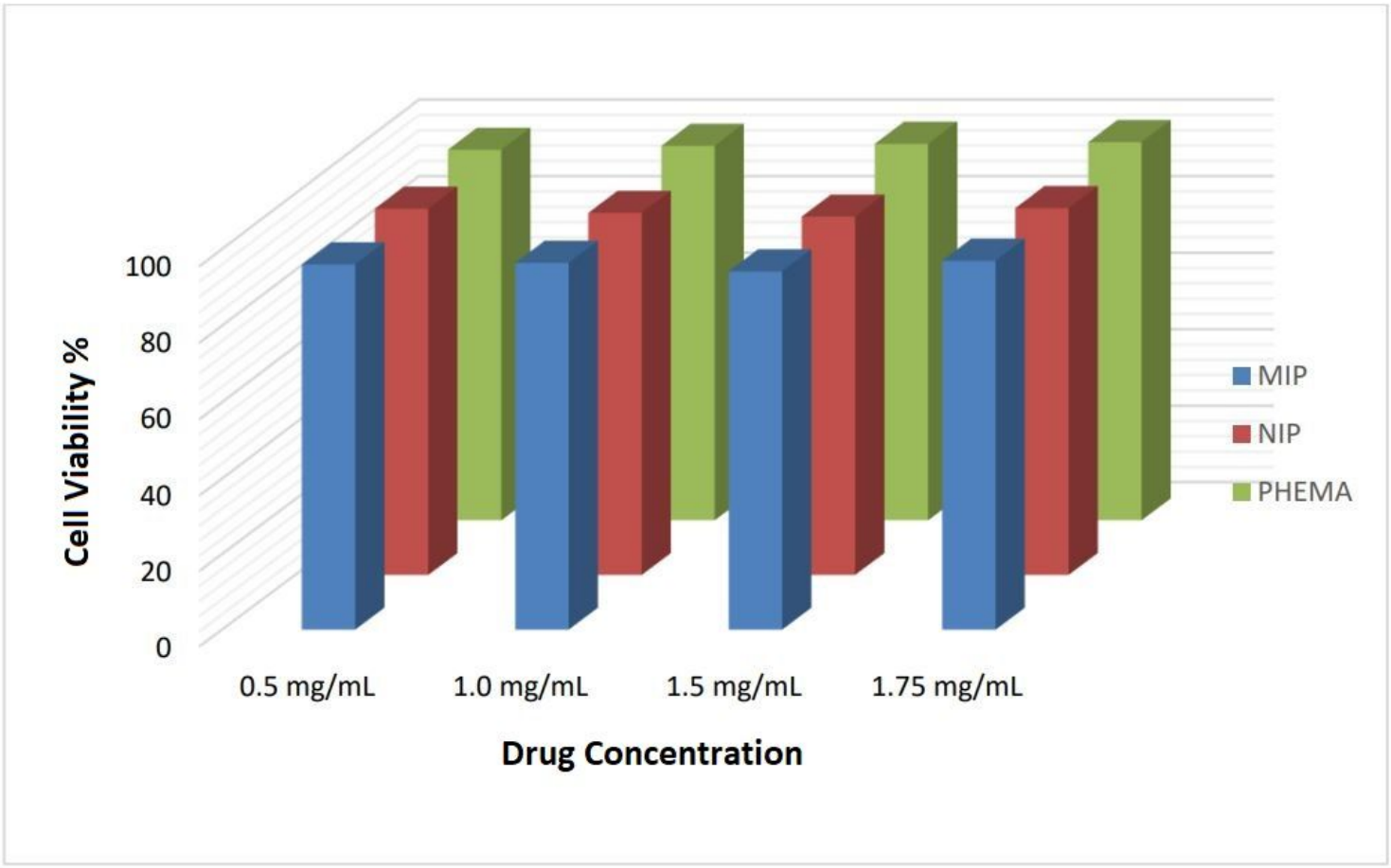

Figure 9

Percentage cell viability values obtained from cytotoxicity studies