

Nifedipine, Niclosamide and Furosemid Release of A Biocompatible and Protective Hydrogel for Cancer Therapy

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Abstract

In this work, biocompatible and protective pure HEMA hydrogel structures and drug loaded HEMA hydrogel have been successfully synthesized. The hydrogel was prepared using HEMA, MBA, APS and TEMED. The structural and morphological characterization of pure hydrogel and drug-loaded hydrogels were characterized using Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM), respectively. The pH affinity, morphology, structure, drug release, swelling and cytotoxic effect of the resulting materials were studied in detail. Drug releases of drug-loaded hydrogel structures were measured at certain time intervals and recorded as cumulatively (%). In addition, cytotoxicity tests were performed by Alamar blue method on MCF-7, MIA PaCa-2 and HEK 293 cell lines of released drug molecules. This HEMA hydrogel was suggested to be materials of biological attention and of large pharmacological potential as support material in drug release of nifedipine, furosemide, niclosamide and similar drug molecules.

Introduction

In recently different drug delivery application are available in modern medicine for the treatment of diseases [1]. Choosing the appropriate route of application affects how easily a drug reaches the intended target area, drug dosage and dosage regimen [2]. It may thus be highly beneficial to monitor the release behavior of the drug delivery platform [3]. This platform can be useful for continuous treatment or for targeted, site-specific drug release [4]. One potential way to gain control over the release behavior is the application of a smart polymer coating [5]. The smart polymeric coating can serve several purposes at once in such systems. Polymeric encapsulation, which restricts environmental exposure, may achieve stabilization of the solid state of a drug [6]. Besides, these coatings may be helpful for several therapies as they make possible controlled drug release and reduce serious side effects [7]. The main functions of drug delivery systems are based on the mechanism of safely moving the drug to various parts of the body, controlling the amount of medication [8], and duration of treatment [9]. For this reason, there are many structures used for drug transport and protection such as polymeric matrixes, hydrogels, nanospheres, cyclodextrins, crown ethers, and calixarene [10–11].

Characteristics of a carrier material such as biocompatibility, non-toxicity lack of immunogenicity, repeatability, and continuous activation to the target are required in drug release [12]. At stimuli-responsive hydrogels, which demonstrate great potential in various pharmaceutical and biomedical applications, have been one of the more recent developments in carrier material science [13]. Hydrogels have various uses in biomedical applications [14]. At the same time, they are well suited for regenerative medicine and controlled drug delivery [15]. Hydrogels are networks of hydrophilic homopolymers or copolymers that swell water and they are biological fluids that are ab-sorbed [16]. Hydrogels offer important opportunities in drug delivery systems with their characteristic features [17]. In particular, hydrogel structures can cause the slow decomposition and release of a water-insoluble drug. They can also protect the drug from stomach acid and enzyme corrosion and thereby increase the drug's stability [18]. Hydrogels improve the release behavior, degradation, and stability of drugs such as furosemide,

niclosamide and nifedipine by maintaining drug concentration and by overcoming biological barriers for cellular uptake in a therapeutic study [19].

Niclosamide, furosemide and nifedipine drugs are poorly water-soluble anti-inflammatory drugs used to relieve cardiovascular diseases or inflammation. Nifedipine is an effective calcium channel blocker that is commonly used in a variety of cardiovascular diseases for clinical management¹³. In a number of circumstances, furosemide is a loop diuretic used orally to change the amount and/or composition of body fluids, including hypertension heart failure, renal failure, nephritic syndrome and cirrhosis [20]. Niclosamide has been recognized as an anthelmintic drug almost since it became known. And it is a medication active against most tapeworms. Niclosamide is also used in schistosomiasis prevention programs as a molluscicide for the treatment of sewage. But the emergence of the anticancer properties of niclosamide has been in the recent past [21]. Recently, there is remarkable surge in works indicating niclosamide drug's anticancer effect against a lot of cancer disease such as ovarian, prostate, colon and breast. Recently studies show that hydrogel structures and/or nanofiber structures increase the preservation of these drugs by forming host-guest complexes [22]. At the same time, the use of hydrogels as a unifying matrix prevents also the degradation of nifedipine, niclosamide and furosemide [23]. Therefore, the main functions of hydrogels are to prevent drug release behavior and drug degradation in drug delivery applications. In the drug delivery area, there are many studies on the incorporation of polymeric structures such as hydrogels and films. However, there are a limited number of studies on the inclusion of low-resolution active agents such as anticancer drugs, antibacterials, essential oils and aromas into hydrogel [24].

Hydrogels as soft biological tissues may exhibit similar mechanical properties, water content, and transport properties [25]. They can therefore be used as physical models for solid tumors or in drug and biomolecule release studies [26]. HEMA hydrogel was used as a support material for rapid in vitro assessment of three drug release profiles (niclosamide, nifedipine and furosemide). Furosemide, nifedipine and niclosamide are mainly absorbed in the upper small intestine and in the stomach due to their nature [27]. Research has focused on improving continuous release systems of nifedipine, furosemide, and niclosamide to improve the effectiveness of the drug, which exhibits poor water solubility and poor permeability [28]. The fact that only published articles on the synthesis of water-soluble formulations of these drug molecules are the negative side of the problem [29]. Due to the clinical importance of the drug, poor solubility of the drug limits its bioavailability and thus greatly reduces its therapeutic effectiveness [30]. Once made hydrophilic, these anticancer drugs were observed to be ineffectuated to a large extent by the circulatory system in published research. Because of this, the desired clinical results were compromised. Thus, moving a step ahead, hydrogel encapsulated niclosamide, nifedipine and furosemide complexes have also been synthesized recently in order to improve in vivo dissolution profile of drug. The slow and sustained release of drug from such polymeric formulation improves bioavailability of drug and also reduces the fraction of drug being eliminated from the body by circulatory system [31].

In this study, the release of low water solubility drug molecules (furosemide, nifedipine and niclosamide) from hydrogel and their effect on cancer cells were examined. Therefore, synthetic and naturally derived biodegradable HEMA-based hydrogels were created. The preservation of furosemide, nifedipine and niclosamide structures were increased by complexing with the hydrogel structure of HEMA. Then, the release of drug molecules from the hydrogel structure was performed. Cytotoxicity tests were performed of the released drug molecules against MCF-7, HEK 293 and MIA PaCa-2 cell lines. The results showed that HEMA hydrogel skeletons can hold great promise in promoting the preservation and release of drugs into cells.

Experimental Section

Materials

2-Hydroxyethyl methacrylate, $\geq 99\%$, (HEMA), MBA (N-N' methylenebisacrylamide), APS (Ammonium persulfate) and TEMED (N,N,N',N'-Tetramethyl ethylenediamine), drug molecules (nifedipine, niclosamide and furosemide) were purchased from Sigma and Merck. The phosphate buffer solution, the HEMA hydrogel and drug solutions were prepared in our laboratory.

Synthesis Of Hema Hydrogels And Drug Loaded Hema Hydrogels

For the preparation of HEMA-based hydrogel, HEMA (2-hydroxyethylmethacrylate), MBA (N-N' methylenebisacrylamide), APS (Ammonium persulfate) and TEMED (N,N,N',N'-Tetramethyl ethylenediamine) were used as monomer, crosslinker, initiator, and catalyzer, respectively [28]. Drug molecules (nifedipine, niclosamide and furosemide) were added in the poly (HEMA) hydrogels. While the hydrogel was getting in shape, HEMA pores were filled with drug molecules. The inclusion complex that does not contain any covalent bonds was formed [15]. Free and the drug loaded HEMA were filled in freeze-dried during several days for use in 96 well plate to obtain disc shape.

Ft-ir Analysis

The structure of 2-Hydroxyethyl methacrylate (HEMA) hydrogels and drug-loaded HEMA hydrogels were characterized by FT-IR spectra. All the results were recorded with a Bruker Vertex 70 ATR-FTIR instrument in the range $4000-400\text{ cm}^{-1}$.

Morphology

The morphology and structure of the HEMA hydrogels were examined with the scanning electron microscope (SEM). Hydrogel samples were coated with 5 nm Au prior to SEM imaging. The SEM images

of hydrogels were taken at a distance of approximately 1 μm .

Swelling Measurement

Free hydrogels' equilibrium swelling (ES) was determined in distilled water and in different buffer solutions. HEMA hydrogels were immersed in 50 mL of prepared solution at 25°C for 18 h to reach maximum swelling capacity.

$$\text{Equilibrium swelling \% (ES)} = (W_2 - W_1)/W_1 \times 100$$

Where W_1 is the initial dried sample weight, and W_2 is the sample weight for 18 h after swelling. Also the hydrogels were determined the swelling ability in different buffers (pH 2, pH 4, pH 7 and pH 9). They repeated all the experiments three times.

In-vitro Drug Release Of Hema

The amount of drug release of drug-loaded HEMAs in predetermined buffer solution was determined by the UV spectrophotometer (Shimadzu UV 1800) in every hour. All the measurements were completed in 96 h. All results were carried out in triplicate and the results were presented in terms of cumulative release.

$$\text{Cumulative release} = M_t/M_0 \times 100$$

The amount of drug pre-loaded into the hydrogel is M_0 , M_t is the amount of drug released.

Cell Culture And Viability Assay

Cell viability assessment was performed on MCF-7 (Human breast adenocarcinoma), MIA PaCa-2 (Human pancreatic carcinoma) and HEK 293 (Human embryonic kidney 293) cell lines by Alamar blue method. This method is based on color changes during reduction of Alamar blue. It is also a common method used to test cell proliferation, viability and / or cytotoxicity. 96 well-plates containing pure HEMA and drug loaded HEMA were prepared. The cells were seeded into 96 well-plates at a density of 10000 cells/well. Each cell well containing 100 μL DMEM medium was incubated at 37°C with 5% CO_2 for 72 h. Then 10 μL of Alamar reagent (per well) was added to each well and incubated in 37°C, 5% CO_2 for 4 h. After incubation, the absorbance of each well was measured at 560–600 nm with a spectrofluorometric technique [30].

Results And Discussion

HEMA-based hydrogels are structures that usually belong to the group of swelling, drug release, and controlled drug delivery systems. It is simple and easy to prepare HEMA based hydrogels. The preparation

process is presented in the Fig. 1. In this work, free-radical polymerization was used. The reaction between APS and TEMED was the first step of polymerization: Homolytic cleavage of APS fragments accelerated by TEMED created free radicals. We successfully synthesized HEMA based hydrogel. The prepared HEMA hydrogel formed a guest-host complex with drug molecules that have low water solubility (Fig. 1). The binding between the drug and HEMA was provided by non-covalent interactions such as π - π , CH- π , electrostatic interaction, H-binding, and hydrophobic interaction [29, 30]. So, we obtained an useful complex to reduce side effects of drug and also the drug was prevented to degrade. These opportunities provided by the HEMA was most likely due to the incorporation into the non-polar cavities of the HEMA of the non-polar portions of these molecules [31].

The drug molecules have been successfully complexed with HEMA hydrogels to increase its preservation. Placement of these drug molecules into HEMA hydrogel was characterized by FT-IR. FT-IR spectra were recorded to obtain chemical information from samples with various cross-link densities as shown in Fig. 2. At the same time, both pure HEMA structures and interactions between HEMA and drug molecules were shown. The broad peak observed at around 3300 cm^{-1} can be due to O-H stretching vibration in the spectrum of HEMA and drug molecule loaded HEMA (Fig. 2). We observed the peak that located at 1642 cm^{-1} is the most significant difference between the loaded hydrogel samples (Fig. 2). The peak at 1642 cm^{-1} occurred from the C = O bond. Due to C-O-C stretching, the peaks at 1154 and 1070 cm^{-1} also confirmed the existence of the ester group of HEMA. At the same time, the peak observed at 1154 cm^{-1} is explained by the C-N stretching vibrations of the HEMA hydrogel containing the drug molecules of niclosamide and furosemide [32].

The complexation of furosemide, niclosamide and nifedipine with HEMA hydrogel was visualized with SEM after FT-IR characterization. The morphological properties of pure hydrogel and HEMA hydrogels containing drug molecule were evaluated using SEM, as shown in Fig. 3. Hydrogels were clearly observed to have porous internal structures and interconnected pores. The structure of pure hydrogels has been monitored to contain smoother structures compared to drug-loaded hydrogel. The morphologies of hydrogels were observed at in cell-containing mediums and compared the images. Crystal structures were observed on the surface of the hydrogel, as drug releases had occurred from drug-loaded hydrogel in the cells medium. Since these crystal-like structures were not observed in pure hydrogel, these structures can be considered as the drug molecules. This image indicated that the hydrogel structure protects and releases the drug molecules in the presence of cells. As shown in the images, all hydrogel structures after the applied process clearly exhibited the molecules they contained.

The equilibrium swelling behaviors of the HEMA hydrogels in different buffer solutions (pH 2, pH 4, pH 7 and pH 9) and water are shown in Fig. 4. According to swelling of HEMA, buffer solution was absorbed more than water. As the pH was increased, the swelling ability of the hydrogel increased. When the pH exceeds 7, the swelling of HEMA structure was influenced negatively. Rising pH of solutions showed that, the enlarging of hydrogel speed and size also increased. Because, increasing pH cause ionization of the carboxyl groups of MBA. Also, this causes the separation of hydrogen bonds between the carboxylic acid groups of the MBA and the oxygens of the ether groups of the HEMA. Combined with the electrostatic

repulsion force, the dissociation of hydrogen bonds makes the hydrogel network swell rapidly. Therefore, the hydrogels are immersed in more water and a higher swelling ratio is obtained [33].

After characterization of pure hydrogel and drug-loaded hydrogel structures, cytotoxicity tests, and releases of drug were performed. In vitro release profiles are an indication of the effectiveness of the drug release system. Therefore, the in vitro release of the drugs, furosemide, niclosamide, and nifedipine, from the hydrogel of HEMA was examined. The cumulative release profiles of these drug molecules from hydrogel matrices in the phosphate buffer were presented in Fig. 5a. According to the release results of drug-loaded hydrogels, niclosamide, nifedipine and furosemide drug releases were found to be 86, 75, and 80%, respectively (Fig. 5b). Because HEMA is a hydrophilic swellable polymer, when the hydrogels are introduced into aqueous media, the polymeric matrix begins to swell [34]. HEMA hydrogel with swelling ability shows low cytotoxicity without hemolysis. These hydrogels in aqueous solution both provide to reduce side effects of drug and prevent drug degradation [35].

Structural features and monomers types of hydrogels are very significant to decide the application field. For instance, evaluation of the structural properties of hydrogel structures is very important in various biological fields such as drug delivery, biomedical applications, and tissue engineering. As seen in the Table 1, hydrogels containing HEMA structure were used in many areas, especially drug release. In addition, drug release, solubility and vasoactivity studies were carried out from some hydrogel structures of nifedipine, niclosamide and furosemide drug molecules. In this study, HEMA based hydrogel structures containing drug molecules have been designed of cancer therapy. It has been observed that this hydrogel structure swells when contacted with an aqueous solution and / or pH solution. At the same time, this hydrogel structure releases the drug molecules it contains by providing the ability to swell in an aqueous environment. In this section, we shall discuss the swelling of hydrogels for cancer treatment and the release of each drug in the HEMA hydrogel.

Table1 The hydrogel structure can be studied on different application as detailed below:

Hydrogel	Drug	Applications	Reference
Hemicellulose gel	BSA	Drug release	36
HEMA	Acid orange 8 (AO8) and bovine serum albumin (BSA)	Drug release	37
HEMA-TiO ₂	Vitamin B12 (Vit-B12)	Drug release	38
p-HEMA	Contact lenses made of microemulsion-laden gels	A potential ophthalmic drug delivery vehicle	39
HEMA-DEGDMA	Acid orange 8 (AO8)	Controlled release	40
Salecan-g-SS-PIH hydrogel	Doxorubicin	Drug release	15
N-succinyl chitosan/alginate hydrogel	Nifedipine	Controlled delivery	41
Collagen-poly(HEMA)	5-fluorouracil (5-FU), bleomycin A2 (BLM) and mitomycin C (MMC)	Controlled release	42
Hemicellulose-based hydrogels	BSA	Magnetically stimulated release	43
Alginate bead	Chlorpheniramine	Drug release	44
p-HEMA-containing (etafilcon, alphafilcon, polymacon, vifilcon and omafilcon)	Cromolyn sodium, ketotifen fumarate, ketorolac tromethamine and dexamethasone sodium phosphate	Release studies	45
PMAN	Niclosamide	Solubility and vasoactivity	46
HPMC	Furosemide	Forming drug solubilizing micelles	47

The effects of these released drug molecules and hydrogel structure against to healthy and cancer cell lines were investigated. Cell viability measurements were made over time to evaluate the drug release system of the drug-containing hydrogels against MCF-7, MIA PaCa-2 and HEK-293 cells. In vitro drug-release studies were tested with drug-loaded hydrogels to obtain results consistent with cumulative release results. In vitro release studies were done on MCF-7, HEK 293, and MIA PaCa-2 cell lines (Fig. 6b, 6a, 6c). The drugs niclosamide, nifedipine, and furosemide caused 62%, 32%, and 42% deaths in MCF-7 cells, respectively. Also, niclosamide, nifedipine, and furosemide caused 64%, 33%, 50% deaths in MIA PaCa-2, respectively. But, the death rate (around 20%) was less observed in the HEK 293 healthy cell line. Niclosamide has been shown to have more cytotoxic effects on cancer cells than those of furosemide and nifedipine showed.

The cytotoxicity of niclosamide, nifedipine and furosemide against two different cancer cell lines MCF-7, MIA PaCa-2, and an epithelial cell line HEK 293 were evaluated. Obtained cytotoxicity test bar graph were shown in Fig. 6 and estimated IC₅₀ (half maximal inhibitory concentrations) were summarized in Table 2.

Table 2 The IC₅₀ of tested drug molecules in different cell lines (MCF-7, MIA PaCa-2 and HEK 293) after 72-h incubation.

The data is described as a mean \pm SD of three free experimental definitions applied in triplicate.

Compound	Cytotoxicity (IC50, μ M)		
	MCF-7	MIA PaCa-2	HEK 293
Furosemide	132.3 \pm 25.3	114.3 \pm 33.2	N.D.
Nifedipine	184.5 \pm 27.8	151.4 \pm 27.4	N.D.
Niclosamide	93.9 \pm 19.2	87.8 \pm 11,7	N.D.
N.D. not detected			

The Alamar blue experiment was performed using equivalent doses of hydrogels containing the drug molecules. According to in vitro drug release research, the drug release occurred within the first 72 hours. The death of MCF-7, MIA PaCa-2 and HEK 293 cells was at the maximum after 40–44 hours (Fig. 7). However, the lethal effects of drug molecules loaded hydrogels on HEK-293 were much lower than MCF-7 and MIA PaCa-2. Application of hydrogel structures containing the niclosamide to cells for 40 hours showed inhibition rates of 62% at the MCF-7, 64% at the MIA PaCa-2 and 18% at the HEK-293 cell line. As a result of the release of the furosemide from hydrogel to cells, 42%, 50% and 20% deaths were observed in MCF-7, MIA PaCa-2 and HEK 293 cell lines respectively. These results showed similarity with the results from the 72-hour inhibition test. Furthermore, hydrogels containing complex structures of nifedipine and furosemide were applied in equivalent doses. No significant differences were observed in their effect on cell viability. The results of release and incubation of niclosamide showed that it has a higher cell inhibition effect than other drug molecules.

The cytotoxicity tests indicated that all drug molecules had middle and good cytotoxic activity on the cancer cell lines [35]. The hydrogels containing drug molecules were found to cause more death in cell lines compared to control and pure hydrogels. Figure 8 showed that the death rate of cancer cell was higher than healthy cell. As a result, niclosamide is the most cytotoxic drug and furosemide also has moderate cytotoxic. Also, this study suggests that HEMA hydrogels can be used to preserving drug, and developing as support material for drug release.

Conclusion

In this study, HEMA hydrogel was obtained as a biodegradable and / or biocompatible structure. HEMA hydrogel was successfully synthesized using HEMA (monomer), MBA (crosslinker), APS (initiator) and TEMED (catalyzer). Their complex structures were obtained with HEMA hydrogels to reduce side effects of drug and prevent drug degradation. The HEMA hydrogels containing drug molecules were easily prepared in buffer solution with different drug molecules. The characteristic, structure, interaction and morphology of prepared hydrogels were investigated using SEM and FT-IR. The hydrogels containing the drug molecules, they were also used for the release of nifedipine, niclosamide and furosemide. The cytotoxic effects of released drug molecules were detected by Alamar blue method on healthy and cancer

cell lines. The cytotoxicity tests showed that all compounds in tested cancer cell lines exhibited middle and good cytotoxic activity. In addition to reduce side effects of drug molecules, protecting them with hydrogels also allowed for healthier use of drugs. As a result, it can be suggested that HEMA hydrogels prepared within the scope of this study can be drug support materials that can be used in the release studies of nifedipine, furosemide, niclosamide and similar drug molecules.

Declarations

Ethical Approval

Not applicable

Consent to Participate

Not applicable

Consent to Publish

With the submission of this manuscript, I would like to undertake that the above-mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

Authors Contributions

Bahar YILMAZ

Funding

Not applicable

Competing Interests

With this study, the HEMA hydrogel was suggested to be materials of biological attention and of large pharmacological potential as support material in drug release of nifedipine, furosemide, niclosamide and similar drug molecules.

Availability of data and materials

The manuscript was controlled line by line for basic copy editing mistakes, grammar, punctuation, style, spelling checks, data and materials.

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Figures

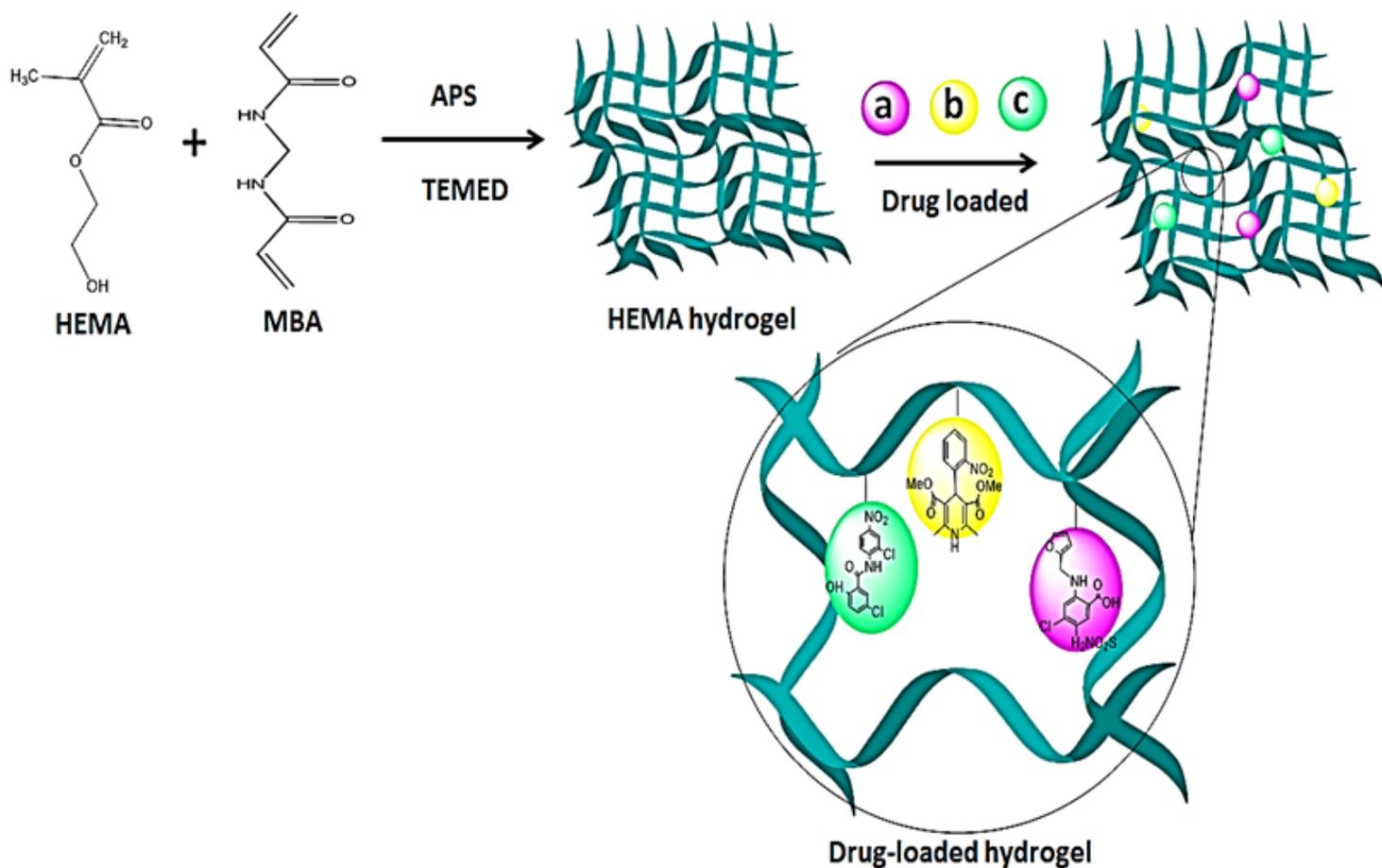


Figure 1

The schematic of the preparation process of HEMA hydrogel and drug-loaded HEMA hydrogel, Furosemide (a), Nifedipine (b), Niclosamide(c)

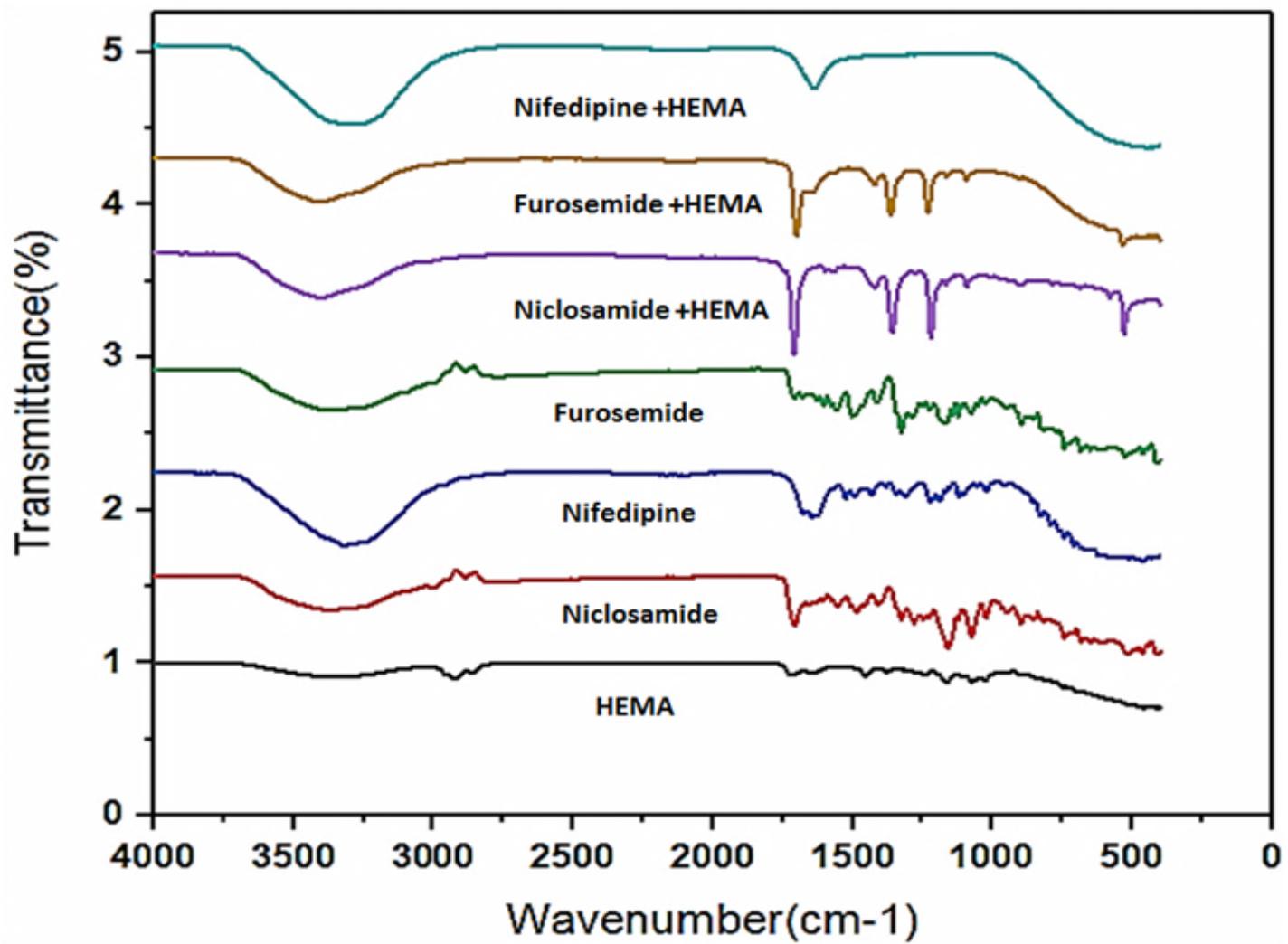


Figure 2

FT-IR spectra of pure HEMA hydrogels and drug loaded hydrogels, in the range 4000–400 cm⁻¹

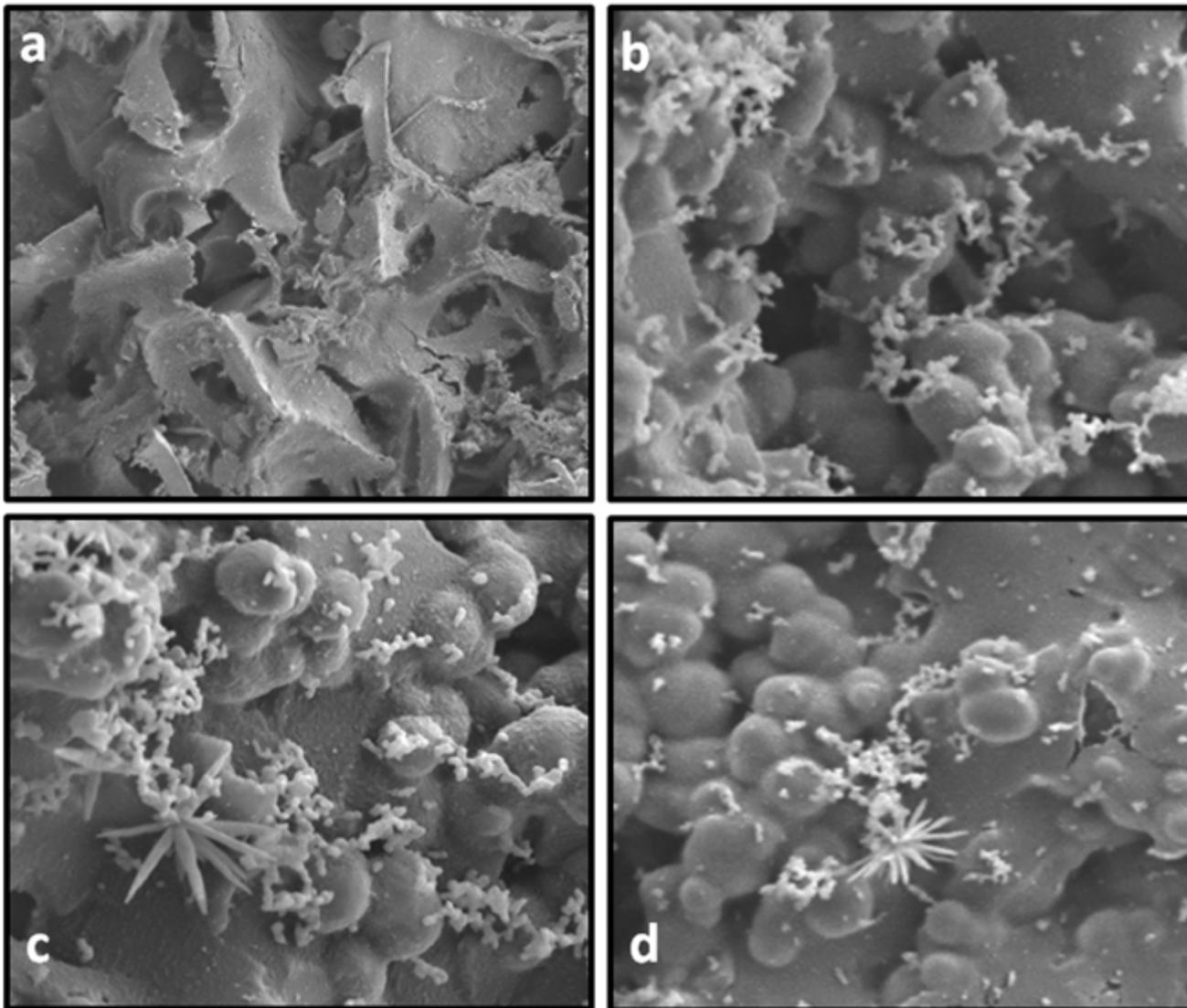


Figure 3

SEM images in cell presence of pure hydrogels and hydrogels containing drug molecule, pure HEMA (a), nifedipine loaded HEMA (b), niclosamide loaded HEMA (c), furosemide loaded HEMA (d).

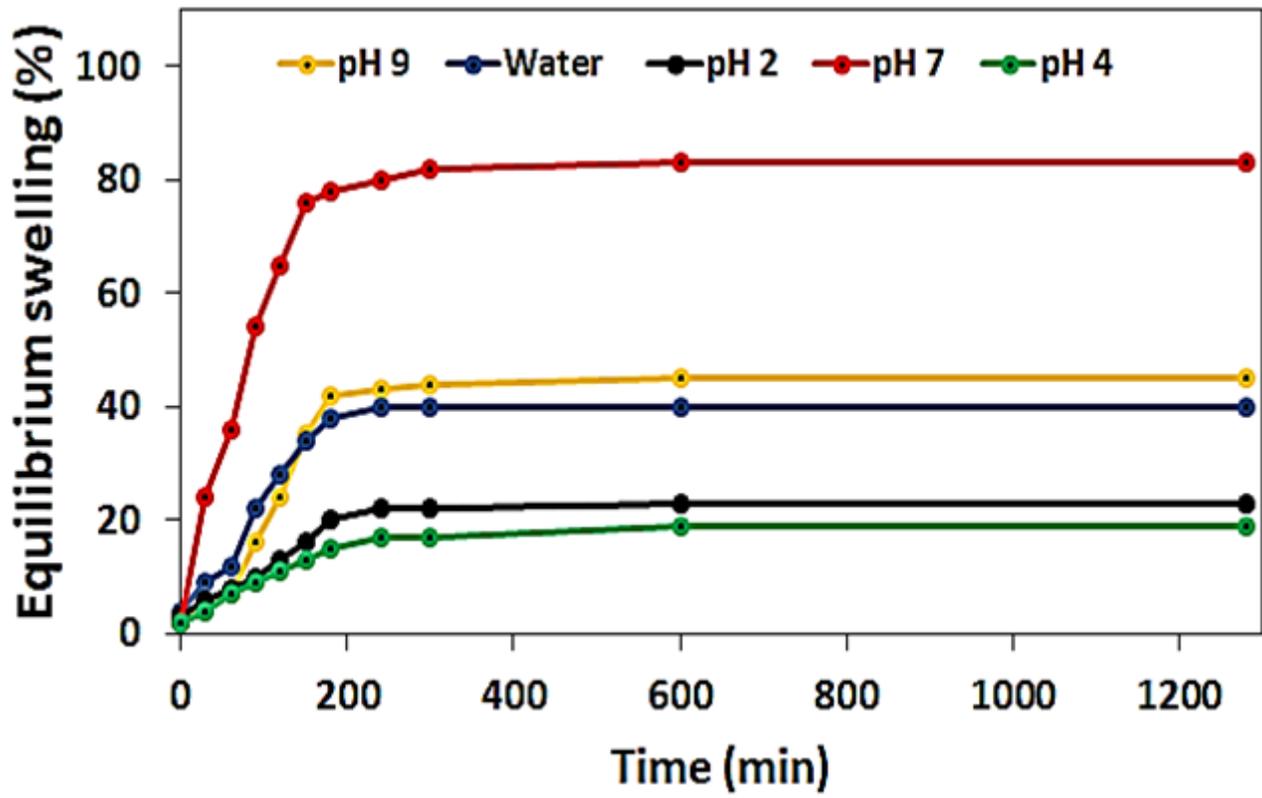


Figure 4

Equilibrium swelling behavior of hydrogels.

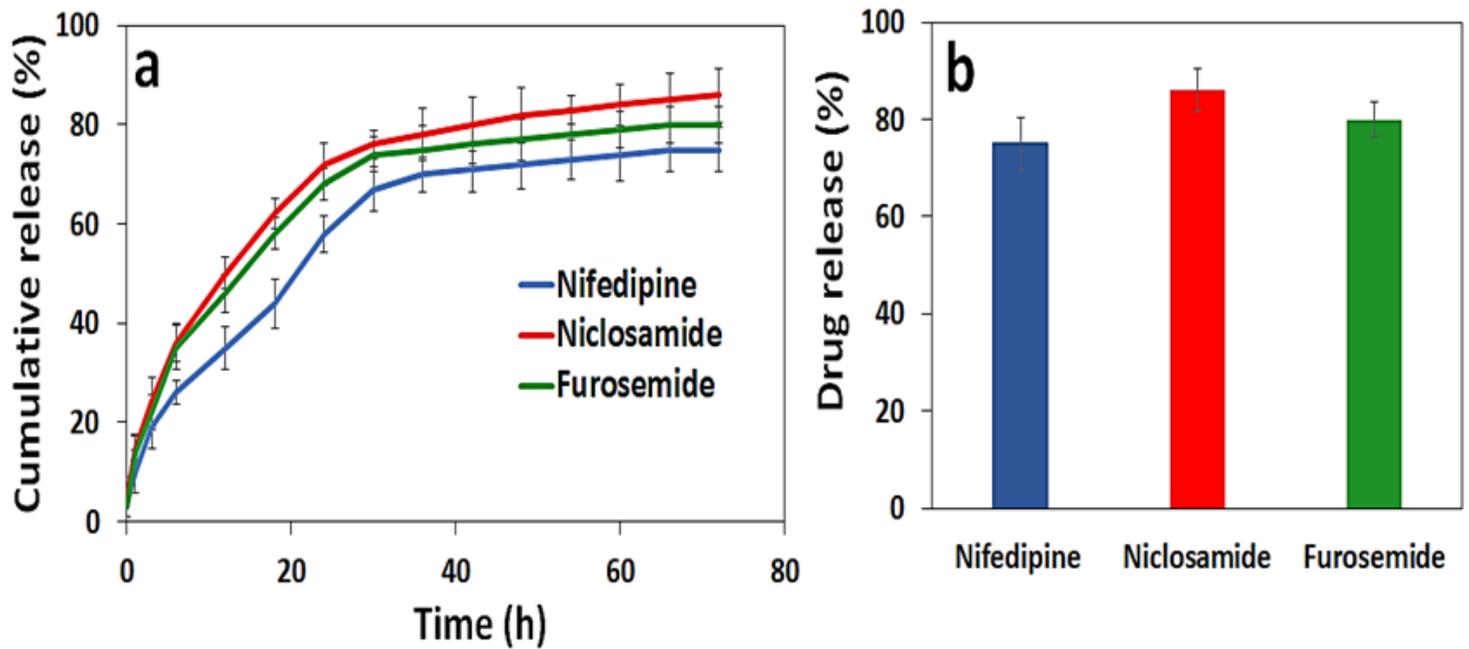


Figure 5

Cumulative release in 72 hours (a), drug release from HEMA hydrogels (b)

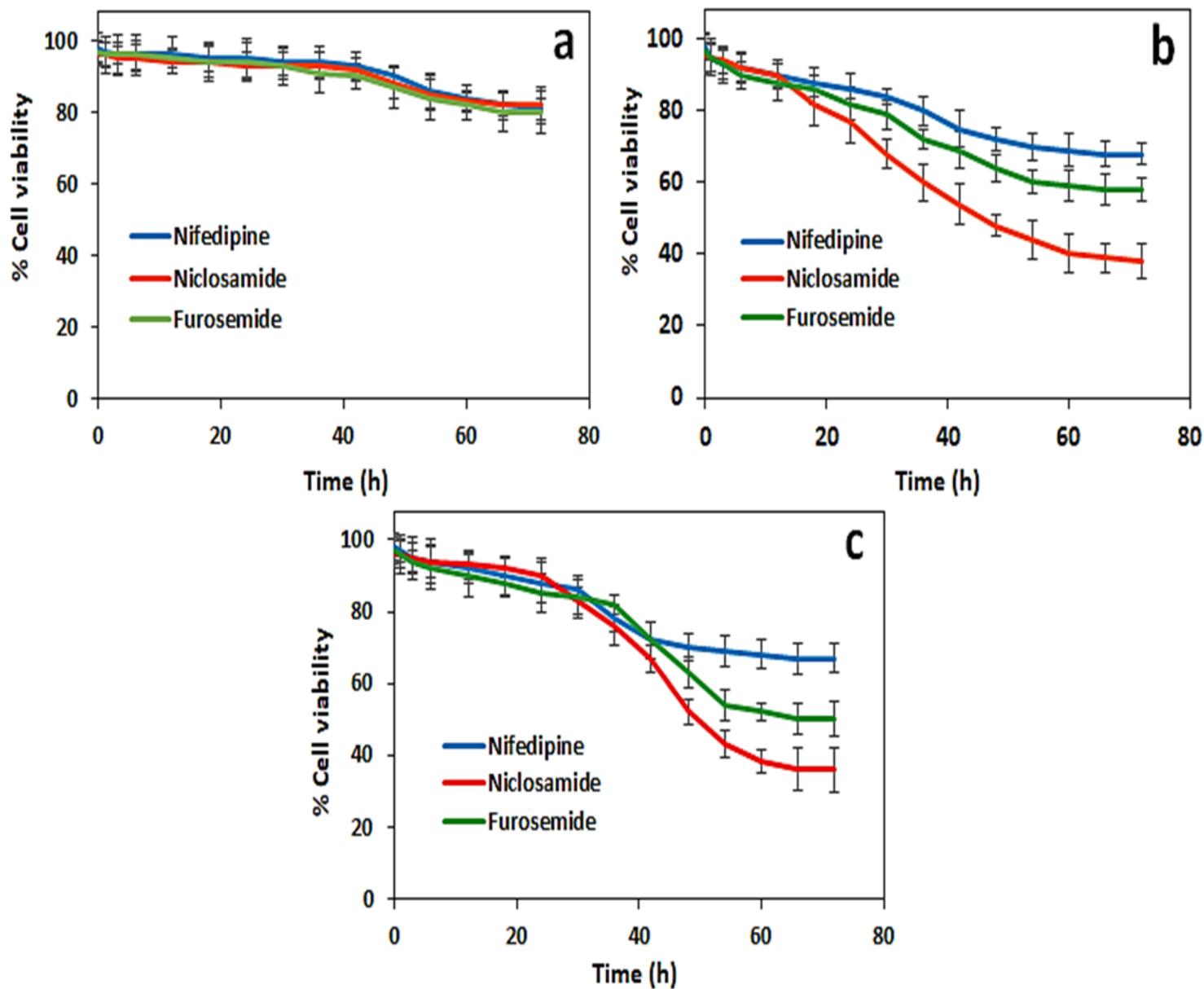


Figure 6

Drug release-induced cell viability by using the Alamar blue assay in; HEK 293 (a) MCF-7 cells(b) and MIA PaCa-2 (c)

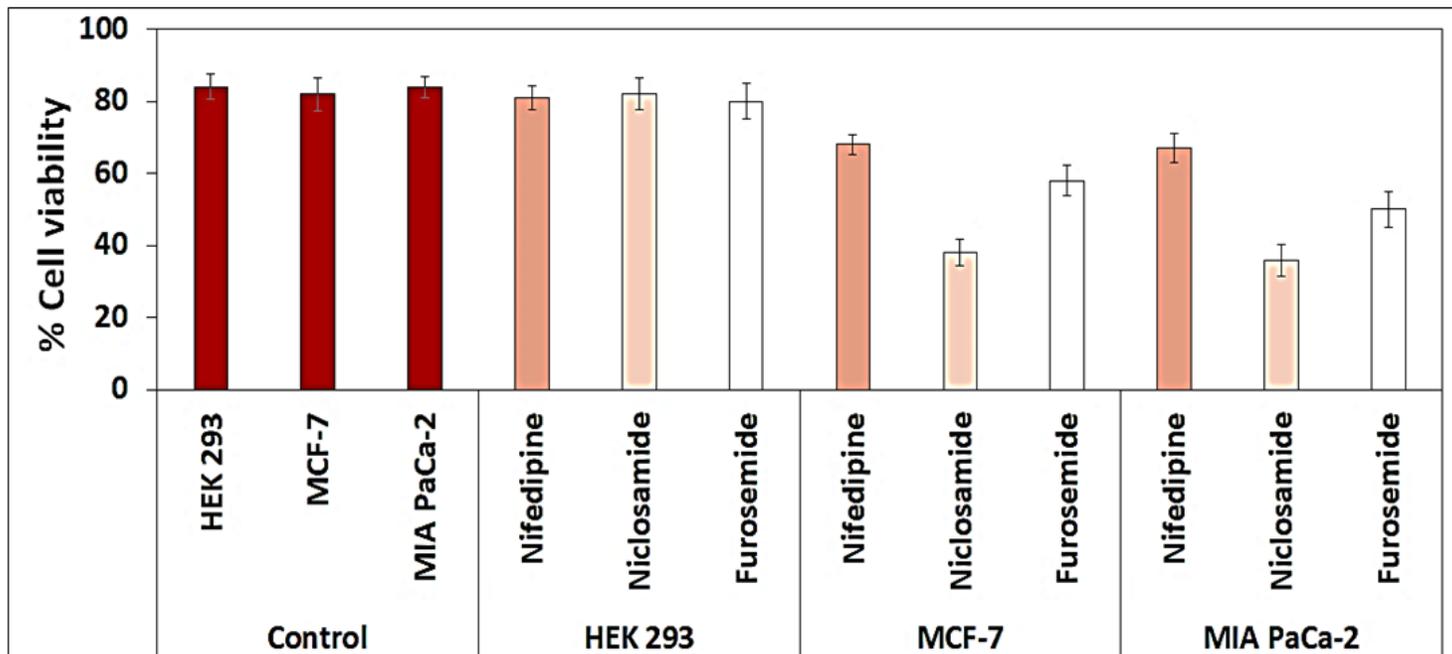


Figure 7

Viability of MCF-7, MIA PaCa-2, and HEK 293 cells exposed to pure HEMA and drug molecules loaded HEMA for 40 h.

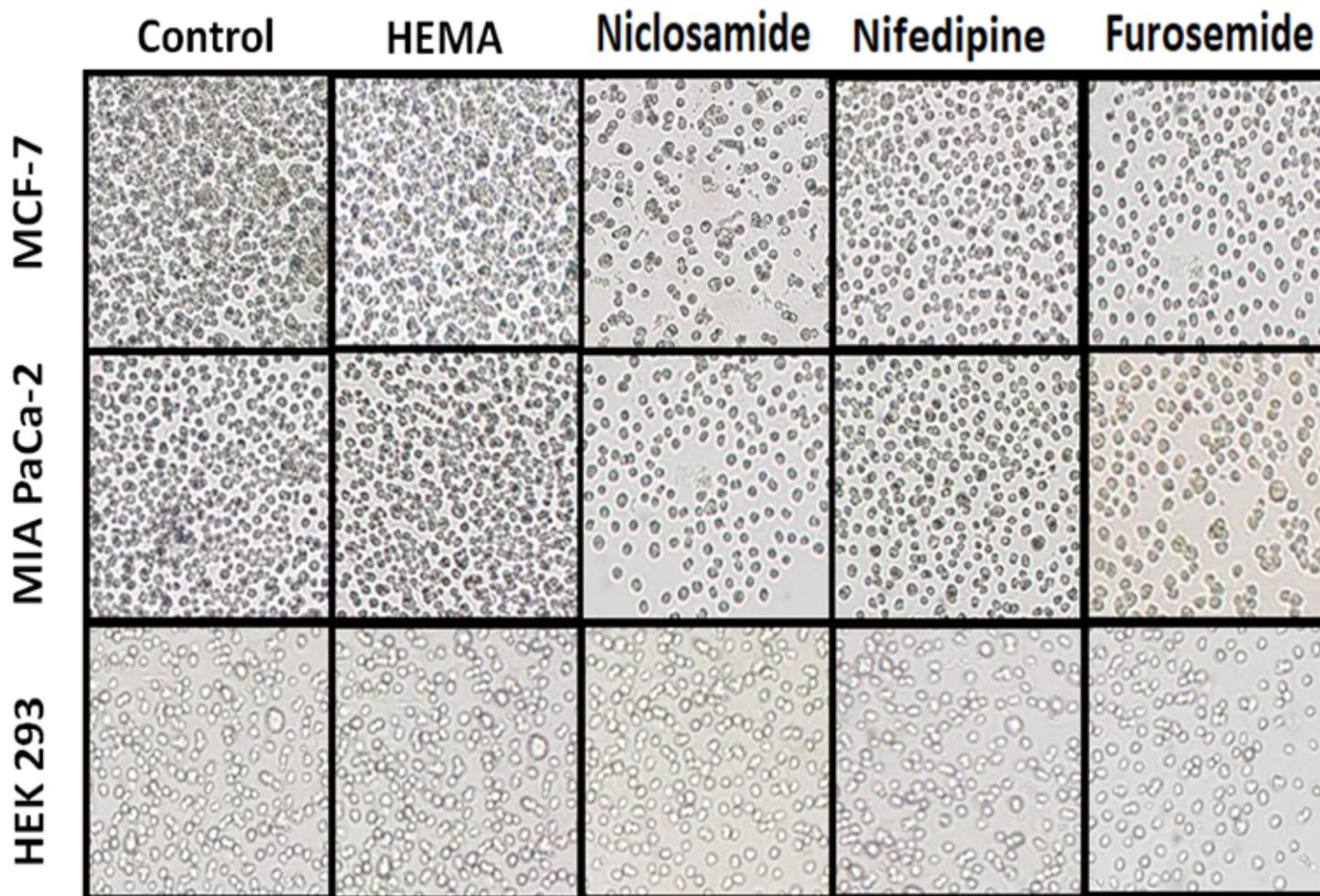


Figure 8

Growth effects of different drug molecules on MCF-7, MIA PaCa-2 and HEK 293 cells after 72-hour incubation.