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Preparation and Characterization of Magnetic Gelatin-Polyvinyl Alcohol Hydrogel for using in the Release of Furosemide Drug

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Abstract

The magnetic gelatin-polyvinylalcohol hydrogel was prepared using $K_2S_2O_8$ in aqueous media in the presence of *N*,*N*′-methylenebisacrylamide, and Fe_3O_4 nanoparticles. The magnetic gelatin-polyvinylalcohol (Fe_3O_4/PVA-gelatin) hydrogel was characterized by FT-IR, SEM, and EDX. Also, magnetic characterization of the synthesized Fe_3O_4/PVA-gelatin hydrogel was specified by a vibrating sample magnetometer (VSM). Then, the loading and release of the furosemide drug were investigated using magnetic gelatin-polyvinylalcohol hydrogel. The FT-IR results confirmed the formation of gelatin-polyvinyl alcohol magnetic hydrogel. The effects of temperature and pH on the loading and release of furosemide drug in gelatin-polyvinylalcohol magnetic hydrogel were studied. The gelatin-PVA magnetic hydrogel is sensitive to pH and temperature and provides the controlled release of furosemide. The results showed that the highest loading of the drug was achieved at room temperature after 6 hours. Also, the highest drug release was observed after 4 hours at 40 °C in pH = 7.

Keywords: Gelatin, Polyvinylalcohol, Magnetic hydrogel, Furosemide, Drug release.

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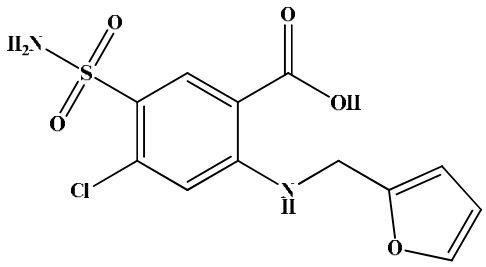
Introduction

Hydrogels are hydrophilic soft polymer networks that may absorb from 10–20% up to thousands of times their dry weight in water. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are called ‘reversible’, or ‘physical’ gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding, or hydrophobic forces [1,2]. The use of PVA-based hydrogels as biomaterials has recently gained great importance given the low toxicity, non-carcinogenic, and high biocompatibility by many of them [3]. Hydrogels have been extensively used in many fields such as drug delivery systems, tissue scaffolds, wound healing dressings, and artificial soft tissues [4-6]. Gelatin is hydrolyzed form of collagen, wherein the hydrolysis reduces protein fibrils into smaller peptides; depending on the physical and chemical methods of denaturation, the molecular weight of the peptides falls within a broad range [7].

Gelatin-based hydrogels have been used as a scaffolding biomaterial for tissue engineering of ‘highly’ vascularized organs and also in cell-based therapies after heart failure [8]. Additionally, due to the presence of bioactive sequences [9], gelatin derivatives have shown promising applications for tissue regeneration [10-16], and cancer studies [12] as well as for vascularization and angiogenic growth factor delivery [17-27]. Hydrogels containing magnetic nanoparticles (MNPs) have been demonstrated to be more suitable for controlling drug delivery and biomedical applications due to their fast response, remote reaction, non-contact action, and super-paramagnetic properties [28–30]. The incorporation of magnetic nanoparticles with biomaterials is an interesting way to create magnetic field-responsive building blocks. For example, alginate magnetic hydrogels have been used to control drug and cell release both in-vitro and in-vivo by causing large deformation and volume changes of over 70% using an external MF [31]. Furthermore, hydrogels with super-paramagnetic iron oxide nanoparticles have been used to increase the temperature of various drug-target systems by magnetic coupling between the magnetic moment of the nanoparticles and the alternating MF, which may be used for cancer hyperthermia treatments [32–34]. When magnetic hydrogels are loaded with specific drugs, they can be used for controlled drug release. Thus, the development of magnetic hydrogels holds high potential applications in tissue engineering and cell/drug delivery. Furosemide, 5-(aminosulfonyl)-4-chloro-2-((2-furanylmethyl)amino) benzoic acid (Scheme 1), is a loop diuretic that has a strong effect with a rapid onset of action and a short duration. The drug in the form of regular pills may cause side effects such as short and severe periods of diuresis, which causes severe discomfort to patients [35]. To overcome the limitations of the conventional release system, it is suggested to develop a new method for existing drugs in order to modify the release mechanism. These changes can increase

drug therapeutic efficacy, reduce toxicity/ side effects, and improve patient compliance, which is attractive to the pharmaceutical industry because the development of new alternatives to expensive drugs requires a great deal of research and time [36, 37].



Scheme 1. The molecular structure of furosemide.

Experimental

General

Polyvinylalcohol (PVA) with a molecular weight of 60000 was purchased from Merck Germany. Gelatin was purchased from Merck. Buffers; pH= 2 (Citric acid/hydrochloric acid/sodium chloride), pH=7 (potassium dihydrogen phosphate/disodium hydrogen phosphate), and pH = 8 (sodium tetraborate/hydrochloric acid) were purchased from Merck, redistilled water was used as a solvent. Potassium peroxodisulphate (KPS) extra pure and *N*,*N*′-methylenebisacrylamide (MBA) was purchased from Merck. Nano Fe_3O_4 particles were purchased from Aldrich.

Preparation of magnetic PVA- gelatin hydrogel

In a three-necked round bottom flask equipped with a condenser and nitrogen gas inlet, a solution of PVA (4.5g), gelatin (1.5g), nano-Fe_3O_4 (0.15g), and *N*,*N*′-methylene bisacrylamide (0.09g) was prepared by using redistilled water (100 mL). The solution was kept under a nitrogen atmosphere at room temperature for 1 h to remove air. Then potassium peroxodisulphate (0.3g) was added carefully and the cross-linking reaction was carried out at 80°C under vigorous mechanical stirring. After 3h the reaction mixture was poured into a Teflon mold and dried overnight at 80°C in a vacuum oven.

Drug loading

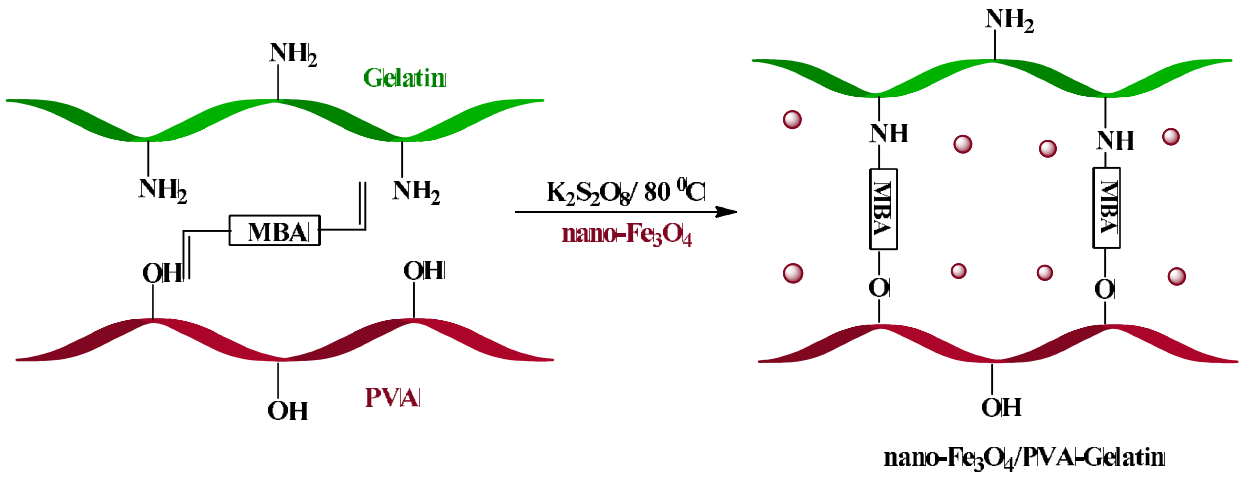
Magnetic PVA-gelatin (0.4 g) was added to 20 ml of drug solution (100 ppm) and put in a dark place for 6h to be completely trapped. To measure drug trapping, the amount of trapped drug at desired times was determined using UV spectroscopy and with the help of a calibration curve depicted a set of drug solutions with known concentrations.

Drug releasing

Drug release of loaded *furosemide* in the PVA-gelatin hydrogel in pH 2, pH 7, and pH 8 solutions buffered were performed at 25°C, 37°C, and 40°C under static conditions. Hydrogel containing a specific amount of *furosemide* drug was added to the release environment (20 ml). In a specific period, 5 ml of filtered samples were trapped and investigated as a function of time to determine the amount of released drug. The amount of released drug was determined using UV-Vis spectrophotometer at λ_{max}= 277 nm and with the help of a calibration curve depicted a set of drug solutions with known concentrations. Also, the cumulative percentage of drug release was calculated using an equation obtained from a standard curve [38].

Results and discussion

In this study, magnetic PVA-gelatin hydrogel is prepared in an aqueous solution at 80°C (Scheme 2). Also, the water absorptivity and the effects of temperature and pH on the loading and release of *furosemide* drug in magnetic PVA-gelatin hydrogel were studied.



Scheme 2. The synthesis of magnetic PVA-gelatin hydrogel.

Characterization of magnetic PVA/gelatin hydrogel

The FT-IR spectrum of magnetic PVA-gelatin hydrogel was assigned (Figure 1). The IR spectra of the hydrogel mark the presence of hydroxyl group of PVA at 3271 cm^{-1} (O-H stretching), vibrations of methylene at 1416 $cm^{-1,}$ and carbonyl stretching of amide linkage of *N,N'*-methylenebisacrylamide at 1644 cm^{-1}. The spectra also contain characteristic bands of C-O-C stretching vibrations due to cross-linking of the chains at 1084 cm^{-1}. This suggests that a polymer comprising two or more networks which at least partially interlaced and cannot be separated unless chemical bonds are broken.

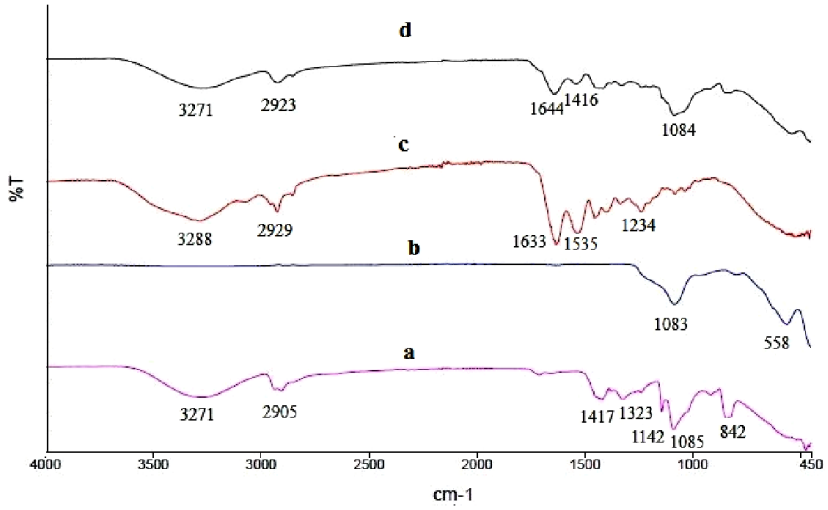


Figure 1. The FT-IR of magnetic PVA-gelatin hydrogel, a- PVA, b- nano-Fe_3O_4, c-Gelatin, d- nano-Fe_3O_4/PVA-gelatin.

SEM Analysis

The surface morphological study of magnetic PVA/gelatin hydrogel explored by scanning electron microscope is presented in Figure 2. The result of SEM analysis revealed that the surface of the magnetic PVA/gelatin hydrogel is porous that facilitates absorption of the drug after swelling. The average diameter of the fine non-agglomerated particles was obtained to be nearly 82 nm for magnetic PVA/gelatin hydrogel. Moreover, the dispersion of nano-Fe_3O_4 in the cross-section of *magnetic PVA/gelatin hydrogel* was examined through the EDX mapping image. The EDX image of *magnetic PVA/gelatin hydrogel* indicated homogenous distribution of nano-Fe_3O_4 in the *PVA/gelatin hydrogel* matrix (Figure 3).

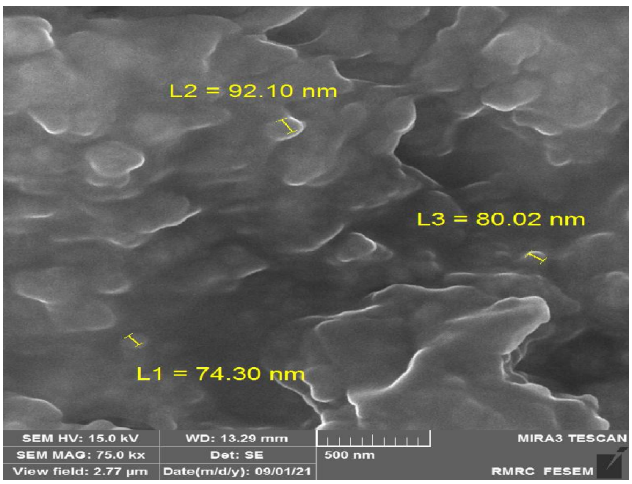


Figure 2. SEM image magnetic PVA-gelatin hydrogel.

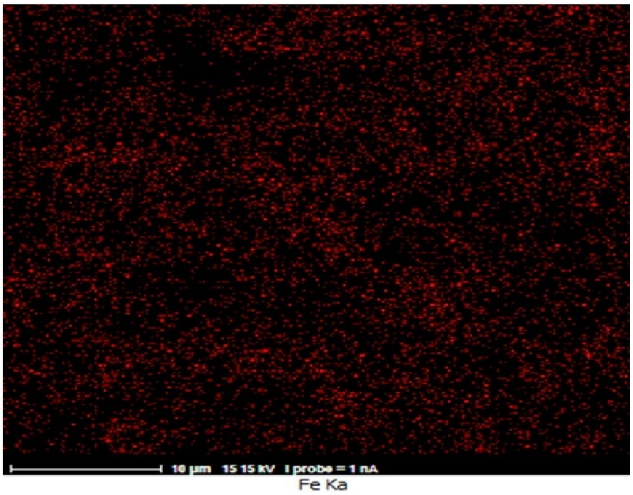


Figure 3. EDX distributions of nano-Fe_3O_4 in the magnetic PVA-gelatin hydrogel.

Also, The EDX spectrum of magnetic PVA/gelatin hydrogel displays the existence of carbon (C), nitrogen (N), oxygen (O), and Iron (Fe) components in the magnetic hydrogel (Figure 4).

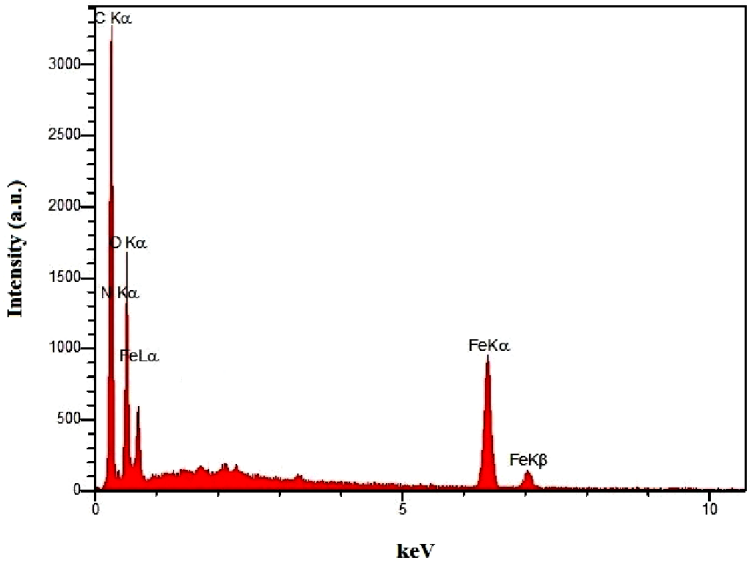


Figure 4. EDX spectra of magnetic PVA-gelatin hydrogel.

VSM analysis

Magnetic characterization of magnetic PVA/gelatin hydrogel was determined by a vibrating sample magnetometer (VSM). Magnetization increased with increases in the magnetic field. The magnetic hysteresis loops of magnetic PVA/gelatin hydrogel are provided in Figure 5. The saturation magnetization about of 2.5 emu/g was determined for magnetic PVA-gelatin hydrogel. Evidently,

the presented result can be attributed to a large amount of diamagnetic PVA and gelatin on the magnetic PVA-gelatin hydrogel.

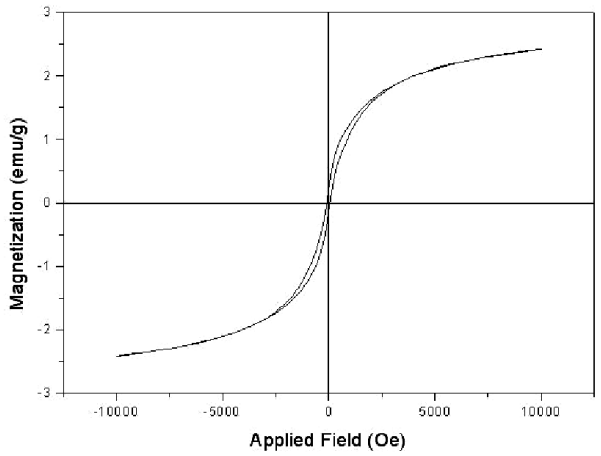


Figure 5. VSM magnetization curves of magnetic PVA-gelatin hydrogel.

Gel permeation chromatography (GPC) was used to determine the molecular weight of synthesized poly(ether sulfones). The molecular weights (M_w) of the poly (ether sulfones) were within the range of 1625–4185 g/mol. The resulting poly(ether sulfones) have moderate molecular weights. The DSC thermogram of synthetic poly (ether sulfones) shows that the glass transition temperatures (T_g) of these polymers ranged from 157-181°C. The endothermic peak of the polymer melting temperature is not seen in these thermograms until degradation; thus, it can be concluded that the resulting polymers are non-crystalline (amorphous) (Figure 3). Due to the presence of bulky groups in the main polymer chains, the polymer chains cannot interact sufficiently with each other. As a result of the weak intermolecular forces in them, their structure becomes amorphous. Of all these polymers, PES-d has the lowest T_g, as it has a more flexible dimethylamine substitution.

Degree of swelling

The degree of swelling could be described as water absorption of the hydrogel (Eq.1). The pre-weighted samples were immersed in redistilled water at 25°C until the gel reached the equilibrium state of swelling. Then, the water on the surface of the swollen gel was removed with tissue paper and immediately weighed. The degree of swelling was defined as follows:

$$\text{Degree of swelling (\%)} = (W_s\text{-}W_d)/W_d \times 100 \quad (1)$$

Where W_s and W_d are the weight of the swollen gel and the weight of the dried gel, respectively. As shown in Figure 6 it is observed that water absorption of magnetic PVA-gelatin hydrogel increase with increasing time to 6h and then is saturated and reaches equilibrium.

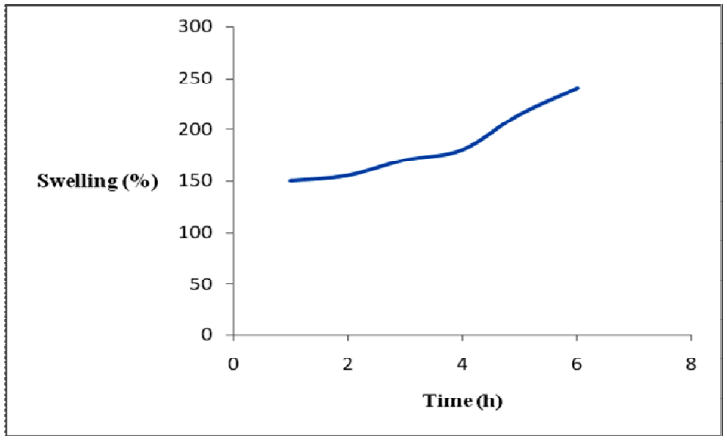


Figure 6. Degree of swelling versus immersion time (h) for magnetic PVA-gelatin hydrogel at 25 °C.

The effect of pH on the release of furosemide

The release of the drug from the hydrogel depends on several factors, including the composition of the hydrogel, its geometric structure, the method of preparation, the type of drug, and the environmental conditions during release, in which pH is the most important factor. Selected pHs were 2, 7, and 8, respectively, which corresponded to the pH range in the acidic medium of the stomach, the neutral medium of the plasma, and the upper limit of the alkaline environment of the intestine [39]. The amount of release of furosemide in acidic, neutral, and alkaline buffers is shown in Figure 7. The highest and lowest releases of the furosemide drug were observed in buffers of pH= 8 and pH= 7 respectively. The result showed that releasing furosemide from PVA-gelatin hydrogel in the alkaline intestine is appropriate at room temperature. This is due to the higher swelling of the magnetic PVA-gelatin hydrogel in pH= 8 at 25°C.

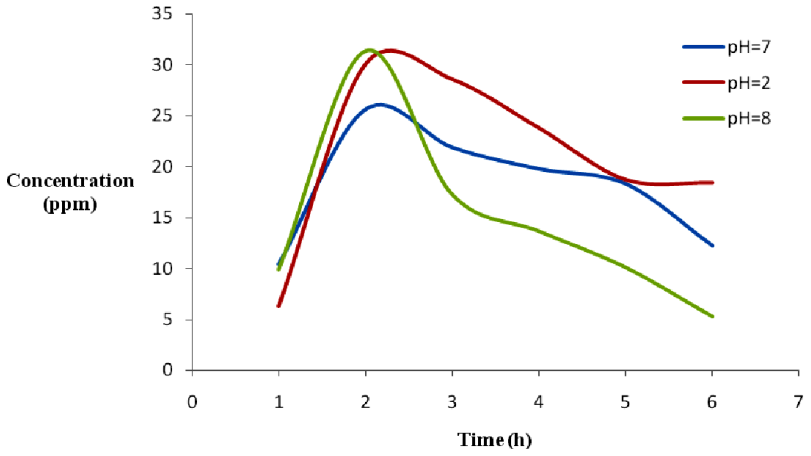


Figure 7. Concentration curve relative to the time in different buffers at 25 °C.

The effect of temperature on the release of furosemide

The use of temperature as a biological stimulus is because the temperature of the human body changes from the normal temperature of 37°C in the presence of pathogenic and febrile agents. These temperature changes provide the appropriate stimulant for drug release in temperature-responsive systems in febrile illnesses. Figure 8 shows the effect of temperature on the amount of release of furosemide drug from synthesized magnetic PVA-gelatin hydrogel. As the temperature increases, the flexibility of the magnetic PVA-gelatin hydrogel is reduced and the amount of water penetration decreases. Consequently, the amount of swelling of the hydrogel decreases, and subsequently, the lower the amount of drug penetrates the hydrogel. The highest drug release was obtained after 4 hours at 37°C in pH = 7 with a value of 41.50 ppm.

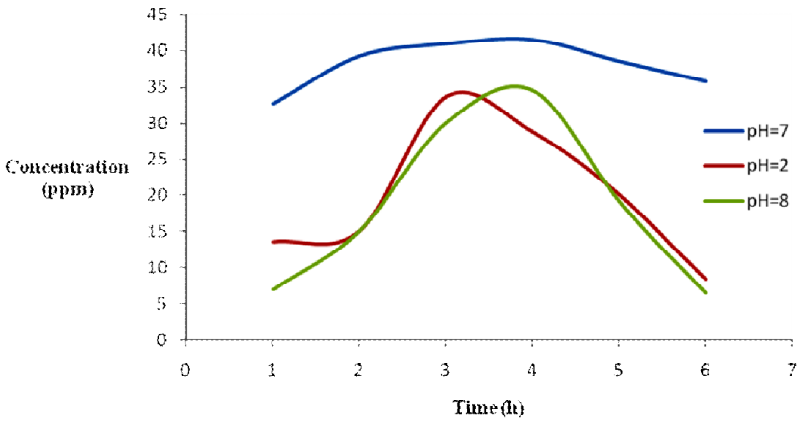


Figure 8. Concentration curve relative to time in different buffers at 37 °C.

At higher temperatures (40°C), the highest drug release was obtained after 4 hours in pH = 7 with a value of 42.19 ppm (Figure 9).

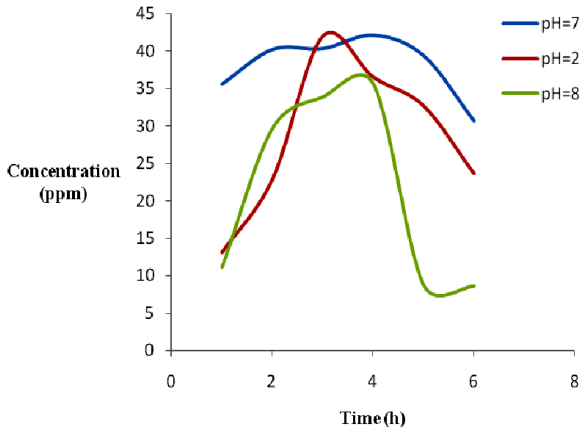


Figure 9. Concentration curve relative to the time in different buffers at 40 °C.

Drug release behavior of the gelatin-PVA hydrogel

We used furosemide as a hydrophilic drug to study the drug release behavior of the gelatin–PVA hydrogel. The release behavior of *furosemide* from the magnetic PVA-gelatin hydrogel was measured over 8 hours in pH 2, pH 7 and pH 8 at 25 °C, 37°C, and 40°C. The drug release shown in Figure 10 represents the total amount of drug released from each hydrogel sample. The magnetic PVA-gelatin hydrogel, which has a high water uptake at 40°C, displayed approximately 42% of the furosemide drug was released within 4 hours because of good water uptake at pH= 7. However, the magnetic PVA-gelatin hydrogel at 37°C and 25°C exhibited lower release profiles about 41% and 25% after 4 and 2 hours respectively. In acidic dissolution media (pH= 2), both drug and hydrogel were fully protonated and abolished the electrostatic association with a resultant high drug release after 3 hours at 40 °C. Conversely, at neutral pH, the ionization states of both the drug and hydrogel favored electrostatic interaction with resultant slower release after 4 hours at 40 °C [40].

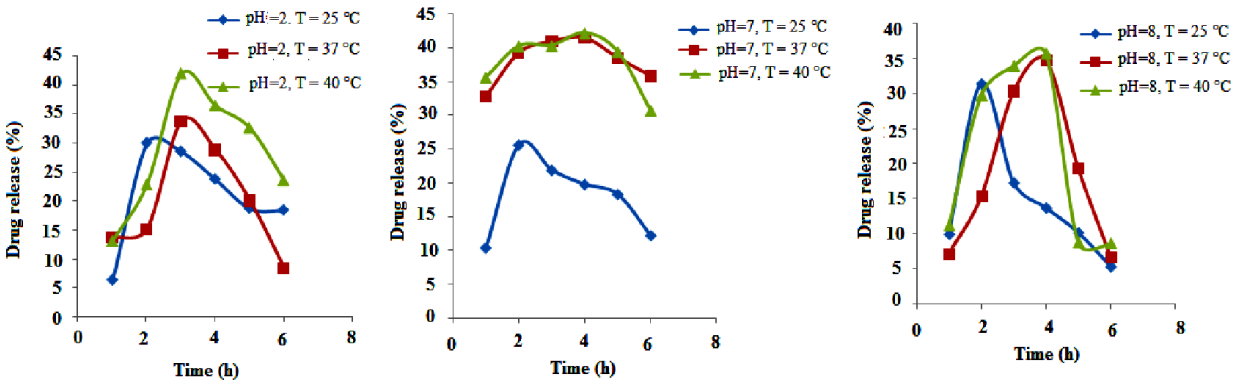


Figure 10. Release profiles of furosemide from magnetic PVA-gelatin hydrogel.

Conclusion

In this work, the magnetic PVA-gelatin hydrogel was prepared from aqueous gelation of polyvinylalcohol and gelatin by potassium peroxydisulphate in the presence of *N*,*N*′-methylene bisacrylamide and Fe_3O_4 nanoparticles. The prepared magnetic hydrogel showed good swelling capacity in water at room temperature. The magnetic hydrogel was investigated for furosemide release to reveal its potential use in drug delivery systems. The PVA-gelatin magnetic hydrogel was shown to have a sustained furosemide release profile at pH 7. These results indicated that PVA-gelatin magnetic hydrogel is useful as a biodegradable matrix for drug release for pharmaceutical applications. In vivo studies are needed to better evaluate the release of furosemide for use in oral drug delivery systems.

Acknowledgments

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