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Sustained Release of Drug Facilitated Through Chemically Crosslinked Polyvinyl Alcohol-Gelatin (PVA-GE) Hydrogels. A sustainable biomedical approach

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The present study aimed to prepare hydrogel based on polyvinyl alcohol (PVA) and gelatin (Ge) and characterization of PVA/Ge hydrogel for their potential use as a sustained drug delivery system. Polyvinyl Alcohol (PVA) and-Gelatin (Ge) were cross-linked using glutaraldehyde (GA) as a crosslinking agent and hydrochloric acid (HCl) as a catalyst. Different feed polymer ratio and crosslinking agent concentration were used to prepare a series of PVA/Ge hydrogels. The obtained PVA/Ge hydrogels were investigated for dynamic and equilibrium swelling studies. The effect of polymers ratio, degree of crosslinking and pH of the medium on swelling of PVA/Ge hydrogels was investigated. Furthermore, the values of diffusion coefficient (D), volume fraction, polymer-solvent interaction parameter, molecular weight between crosslink and crosslink density were calculated. For swelling studies, 0.05M USP phosphate buffer solutions of different pH (1.2, 5.5, 6.5 and 7.5) were used. For the drug release study, ciprofloxacin HCl was loaded into selected samples as a model drug. The release of drug from these samples was performed for 12 hours in USP phosphate buffers of pH 1.2, 5.5 and 7.5. The release data from these samples were fitted into various kinetic models like zero order, first order, Higuchi and Peppas models to investigate the release mechanism. It was found that by varying the composition of PVA/Ge hydrogel and GA concentration, a significant difference was observed in drug release kinetics. FTIR spectroscopy and X-ray diffraction were used for the characterization of hydrogels. PVA/Ge hydrogel showed sustained release of the model drug at various pH values suggesting its potential use as a sustained drug delivery system.

Keywords: hydrogel; gelatin; drug release; ciprofloxacin HCl, polyvinyl alcohol.

INTRODUCTION

Researchers from the field of Pharmacy and pharmacology are keen to study biomaterials that have the potential for medical uses including pharmaceutical manufacturing, bioengineering, vaccines, and in preparation of different implantable drug devices. These biomaterials should be biocompatible, non-carcinogenic, non-immunogenic, nontoxic and should not cause any injury to tissue[1–2]. Since biomaterials are foreign in nature, the investigation of host response is important after administration for the estimation of biocompatibility. Among biomaterials studied, hydrogels are found highly biocompatible[3].

Hydrogels may be defined as three-dimensional crosslinked network of copolymers or homo-polymers that when comes in contact with the aqueous environment result in water uptake and swelling. Due to their hydrophilic nature, swelling in water, biocompatibility, and non-toxicity, they have been extensively used in various medical applications[4]. These hydrogels absorb water because of functional groups such as -CONH, -OH, $–SO_3H$ and $-CONH_2$. These hydrogels can absorb huge amount of water (sometimes more than 90%) without being dissolved due to the crosslinks present in hydrogels[5].

Different types of methods have been developed for hydrogel crosslinking. Glutaraldehyde (GA) can be used for crosslinking polymers with –OH functional group (e.g., polyvinyl alcohol)[6]. For hydrogel crosslinking, extreme conditions need to be applied i.e., acidic pH, increased temperature, use of methanol as a suppressor. On the other side, amine containing polymers can be crosslinked with the aldehyde (glutaraldehyde) under mild conditions. This has been investigated for the preparation of crosslinked proteins e.g., albumin, gelatin and amine containing polysaccharides[7].

Different types of hydrophilic polymers have been used in hydrogel formulation and Gelatin (Ge) is one of them. Ge is widely used in hydrogels as a natural polymer due to its low price, biodegradation, compatibility, and natural origin[8]. Ge is obtained by hydrolysis of collagen, which is found in nature and obtained from bones, animal skins and tissue. Ge is composed of different amino acids. Ge characteristic features include high amino acids content such as proline, glycine and hydroxyproline[9]. Polyvinyl alcohol (PVA) is a water-soluble polymer obtained by hydrolysis of polyvinyl acetate[10]. As PVA has no carcinogenic or toxic effects, it is widely used in different fields of research since 1924. PVA is

currently extensively used for a variety of applications like artificial manufacturing of vessels, intestine, kidney, and lenses. PVA made hydrogel are currently under research for drug delivery system[11]. On the other hand, gelatin has a unique property of swelling in acidic and alkaline medium. It contains ionizable groups that help in swelling at a wide range of pH. These ionizable groups include $–NH_3$ and –COOH. This property of gelatin can be very helpful in sustained release of drug. PVA and Ge are both hydrophilic polymers. The properties of both polymers can be combined to design hydrogel for sustained drug delivery.

PVA/Ge blends[12], hydrogel films[13], spongy cryogels[14], nanofibers[15] and wound dressing membranes[16] have been prepared so far by physical crosslinking using freeze--thaw method and esterification method. The present work aimed to prepare disc of PVA/Ge hydrogel using glutaraldehyde as a crosslinker for sustained release of drug. Ciprofloxacin HCl was used as a model drug. Hydrogels were characterized by performing dynamic and equilibrium swelling studies, studying the effect of polymer ratio and crosslinked density on swelling, porosity, sol-gel fraction, analysis of drug release pattern, FTIR spectroscopy and X-ray diffraction.

Materials and Methods

Materials

To prepare chemically crosslinked polyvinyl alcohol/gelatin hydrogel, polyvinyl alcohol (PVA) and gelatin (Ge) (Merck, Germany) were used as polymers. Glutaraldehyde (GTA) (Merck, Germany) was used as a crosslinking agent. Acetic acid glacial 100% (Merck, Germany) and distilled water were used as solvents. HCl (Fluka, Switzerland) was used as a catalyst. Potassium bromide (KBr) (Fisher Scientific UK) was used in FTIR. Analytical-grade chemicals were used in the study.

Preparation of PVA/Ge hydrogels

Chemically crosslinked PVA/Ge hydrogels with different ratios of polymers and crosslinking agent were prepared as given in Table 5. PVA solution was prepared by dissolving weighed amount of PVA in distilled water at temperature of 60 °C using reflux condenser. The solution was left to cool down at room temperature. Ge solution was prepared by dissolving weighed amount of Ge in 3% acetic acid solution at 37 °C using a reflux condenser until completely dissolved. Ge solution was left to cool down at room temperature and then added to PVA solution and mixed. Varying amounts of GA and HCl were added gradually to the stirred mixture. Distilled water was used for volume makeup. Then after thorough stirring, the mixture was introduced into several glass tubes. The tubes were then kept at 45 °C for 1 h, 50 °C for 2 h, 55 °C for 3 h, 60 °C for 4 h and 65 °C for 12 h in water bath for crosslinking. After cooling the tubes at room temperature, the hydrogels obtained were sliced into disc of 7 mm and immersed in distilled water for total removal of water-soluble moieties and then dried in vacuum to constant weight[15]. Figure 1 shows the presumptive structure of PVA/Ge hydrogel.

Table 1. Sample designation and polymer ratio in the preparation of hydrogels

Sample code	Polymeric composition Ge/PVA	GA /100g solution
S1	11/89	0.608
S2	20/80	0.608
S3	27.2/72.8	0.608
S4	30/70	0.608
S5	28.5/71.5	0.608
S6	27.2/72.8	0.608
S7	27.2/72.8	0.576
S8	27.2/72.8	0.640
S9	27.2/72.8	0.704

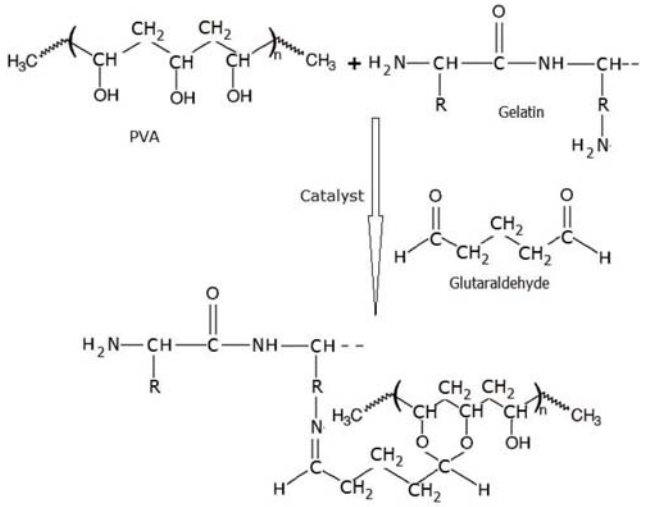

Figure 1. Presumptive structure of PVA/Ge hydrogel

Swelling study of the Prepared Hydrogels

Dynamic and equilibrium swelling studies

Swelling study was conducted in 250 ml of 0.05M USP phosphate buffer solution of pH 1.2, pH 5.5, pH 6.5 and pH 7.5 to investigate the dynamic and equilibrium swelling ratio of the prepared gels. Washed, dried, and weighed hydrogel was left to swell at desired pH at a temperature of 37 °C. At regular intervals, hydrogels were withdrawn from the buffer solution, the first filter paper was dried and then its weight was taken and again kept in the same buffer[16]. Swellings of the gels were taken at time t. The formula used to estimate the dynamic swelling ratio of each hydrogel is as follows:

$$q = W_h/W_d \quad (1)$$

W_h and W_d represent the swollen weight of gel and the initial weight of the gel at time t. The process remains continuous till the equilibrium weight was reached. The following formula was used to determine equilibrium swelling.

$$S\ (Eq) = W_h/W_d \quad (2)$$

W_h and W_d represent the weight of gel at equilibrium swelling and the initial weight of dry gel respectively[17].

Water diffusion coefficient

Diffusion coefficient (D) of the swelled gels was determined by slowly drying swelled gels at room temperature and weight after 15 minutes until constant weight was obtained. The following equation was used to calculate D values of hydrogels.

$$D = \pi\left(\frac{h.\theta}{4.Q_{eq}}\right)^2 \quad (3)$$

D represents the diffusion coefficient of the gels, Q_{eq} is the swelled hydrogel at equilibrium, θ represents the slop of the swelling curves and h is the original width of the dry hydrogel disc prior to swelling[18].

Sol-gel analysis

PVA/Ge hydrogel discs of 3–4 mm size were dried for 7 days at room temperature and then at 45 °C in a vacuum oven to attain constant weight and subjected to Soxhelt extraction with deionized water as a solvent at boiling temperature for 4 hrs. Uncross linked polymer was removed from the hydrogel with this extraction process. The resultant hydrogels were oven dried at 45 °C till constant weight. Sol fraction and gel fraction were then calculated by using the following equations[19].

$$\text{Sol fraction (\%)} = \left[\frac{W_o - W_i}{W_o}\right] \text{X100} \quad (4)$$

$$\text{Gel fraction (\%)} = 100 - \text{Sol fraction} \quad (5)$$

W_0 denotes the dry weight of the hydrogel before the extraction process and W_1 represents the weight of the hydrogel which is dried after the extraction process.

Porosity measurement

For the porosity study, hydrogels dried and weighed were placed in absolute ethanol for one night and then re-weighed after surplus ethanol on the surface was removed with filter paper. The equation used for estimation of percent porosity is given below[20].

$$\text{Porosity} = \left[\frac{M_2 - M_1}{pV}\right] \text{X100} \quad (6)$$

M_1 denotes mass of gel before dipping and M_2 denotes mass of gel following dipping in pure ethanol. P represents the density of absolute ethanol and V represents the volume of hydrogel disc.

Analyzing network parameters of PVA/GE Gels

Molecular weight between crosslinks (Mc)

The theory of Flory-Rehner was applied to determine Mc value of PVA/Ge hydrogel. According to this theory, Mc value tends to increase with the increase in the swelling ratio of gels. The following equation is used to calculate Mc value[21–22].

$$Mc = -\frac{d_p v_s\left(v_{2,s}^{1/3} - v_{2,s}/2\right)}{\ln(1 - v_{2,s}) + v_{2,s} + x v_{2,s}^2} \quad (7)$$

Volume fraction of the polymer V_2, s was calculated by the following equation:

$$V_{2,s} = \left[1 + \frac{d_p}{d_s}\left(\frac{M_a}{M_b} - 1\right)\right]^{-1} \quad (8)$$

d_p and d_s are the densities (g/ml) of the hydrogel and solvent respectively. Ma and Mb are the masses (g) of the swollen and dry hydrogels respectively. V_2, s represents volume fraction of the swollen hydrogel in the equilibrium state and χ is the Flory-Huggins polymer solvent interaction parameters.

Solvent interaction parameters (χ)

Solvent interaction parameters were measured to investigate the compatibility of polymer with the molecules of surrounding fluid. Polymer volume fraction in the swollen state is the amount of fluid imbibed and retained by the hydrogel. The values of (χ) are calculated by Flory-Huggin's theory. The following equation was used to calculate χ values[23].

$$\chi = \frac{\ln(1 - v_{2,s}) + v_{2,s}}{v_{2,s}^2} \quad (9)$$

V2,s represents volume fraction of the swelled gel at equilibrium and χ is the Flory-Huggins polymer solvent interaction parameters.

Density of crosslinks (q)

Crosslinking density is used for characterization of crosslinked hydrogels. The following equation was applied for determination of density of crosslinks[21, 24].

$$q = \frac{M_C}{M_r} \quad (10)$$

Where M_r is molar mass of the repeating unit and is calculated as:

$$M_r = \frac{mGeMGe + m_{PVA}M_{PVA} + m_{GA}M_{GA}}{mGe + m_{PVA} + m_{GA}} \quad (11)$$

m_{Ge}, m_{PVA} and m_{GA} represent masses of Ge, PVA and GA respectively used in hydrogel preparation. While M_{Ge}, M_{PVA} and M_{GA} represent the molar masses of Ge, PVA and GA respectively.

Ciprofloxacin HCl loading and release of PVA/Ge hydrogel

For the calculation of the percent drug loading of hydrogels, three different methods were used. The following equations were used to calculate % drug loading by the first method.

$$\text{Total drug} = W_D - W_d \quad (12)$$

$$\text{Percent drug loaded} = [(W_D - W_d)/W_d] \times 100 \quad (13)$$

W_d and W_D are masses of dried gels before and after placing in drug solution. In another method, drug loaded in hydrogels was determined by repeatedly extracting the drug from gels in distilled water. Each time 25 ml fresh deionize water was used until the whole drug was extracted from the gel. Drug concentration was measured using spectrophotometer. The sum of drug from all the extracts was considered the actual amount of loaded drug.

In the last method to calculate the drug loading in hydrogel, weighed gel disc was dipped in drug solution till equilibrium swelling. The swollen gel was weighed after removing the excess solution from the surface with filter paper. The difference in weight before and after swelling is the weight of the drug solution. The volume of drug solution absorbed by the gel disc can be calculated by knowing the density and weight of the drug solution. After calculating the volume of the drug solution, amount of drug absorbed by gel disc was calculated.

Drug release was studied by measuring the amount of drug released in dissolution apparatus (Pharmatest,

PT-Dt 7, Germany) with the help of UV-visible spectrophotometer. The pre-weighed hydrogel disc was placed in 500 ml buffer at a temperature of 37 °C and the buffer was stirred at a rate of 100 rpm. 0.05 M USP phosphate buffer solutions of pH 1.2, 5.5 and 7.5 were used as dissolution medium. Ciprofloxacin HCl release study was conducted at λmax 278 nm up to 12 hours after regular intervals. Each time 5 ml sample was taken for UV analysis and replaced by fresh buffer solution[25].

Release pattern of ciprofloxacin HCl

For the analysis of release of ciprofloxacin HCl, zero--order[26], first-order[27], higuchi[28], and korsmeyer-peppas models[29] were applied. To understand drug release mechanism, the release behavior was analyzed using semi empirical power equation proposed by peppas. The following models are used for release calculations.

Zero-order kinetics: $Ft = K_o t$ (14)

Where F represents the fraction of drug release in time 't' and Ko is the zero-order release constant.

First-order kinetics: $\ln (1\text{-}F) = -K_1 t$ (15)

Where F represents the fraction of drug release in time 't' and K1 is the first order release constant.

Higuchi model: $F = K_2 t^{1/2}$ (16)

Where F represents the fraction of drug release in time 't' and K_2 is the Higuchi constant.

Korsmeyer-Peppas model: $M_t/M\infty = K_3 t^n$ (17)

M_t is the mass of water absorbed at any time t, $M\infty$ is the amount of water at equilibrium and K_3 is the kinetic constant and n is the exponent describing the swelling mechanism. When n equal to 0.45 means Fickian diffusion, but when the value of n is greater than 0.45 and less than 1 means non-Fickian diffusion[30].

FTIR spectroscopic analysis

For FTIR spectroscopic analysis, hydrogel samples (drug-loaded and unloaded) were crushed to powder with pestle in an agate mortar. Hydrogel powder was mixed with potassium bromide in 1:100 ratios and dried at 40 °C. The mixture was compressed to a 12 mm semi--transparent disk by applying a pressure of 55 kN for 2 min. The FTIR spectrums over the wavelength range 4,500 - 400 cm^{-1} were recorded using FTIR spectrometer.

X-ray diffraction (XRD) study

X-ray diffraction (XRD) for pure drug, drug-loaded and unloaded hydrogel was performed using Bruker D8 Discover (Germany) apparatus. Measurement conditions included target (CuKα), voltage (35 KV), and current (35 mA). A system of diverging, receiving, and anti-scattering slits of 1°, 0.2° and 2° respectively, was used. Eva software was used for the data processing (Evaluation Package Bruker, Germany). Patterns were obtained using scan speed of 4 degree/minute with 2θ between 5° and 80°.

RESULTS AND DISCUSSION

Effect of pH on swelling and on drug release of PVA/Ge hydrogels

The effect of pH on swelling was investigated in buffer solutions of pH 1.2, 5.5, 6.5 and 7.5. The dynamic and equilibrium swelling ratios were found high in buffer solution of pH 1.2, 5.5, 6.5 and 7.5 as shown in Table 2. In PVA/Ge hydrogel, the swelling at different pH values is mainly controlled by Gelatin as PVA has no ionizable groups in its structure. Similar results were found by Sundaram Gunasekaran et al.[31], who observed that in chitosan-PVA hydrogel, PVA has no effect on the time needed to reach swelling equilibrium. Gelatin contains ionizable groups such as – NH_3+ and -COOH. It was found that at low pH, gelatin acts as base and takes up H+ ions from the medium forming – NH_3+ and -COOH and gelatin become positively charged. In alkaline medium, gelatin acts as an acid gives H+, forming – COO- and – NH_2 groups and gelatin become negatively charged. In an acidic environment, the swelling is controlled mainly by the – NH_3+ and in basic medium by COO-.

Table 2 shows that in basic medium, the swelling is higher. This behavior is due to the presence of the hydrophobic functional groups (mainly – COO-) in the gelatin structure. These results correlate to the finding of Deyi Zhu et al.[32], who prepared gelatin-based hydrogel crosslinked with microbial transglutaminase. They found that gelatin-based hydrogel swelling is pH dependent and shows high swelling ratio at pH <2 and pH >7. The effect of pH on drug release was investigated in buffer solutions of pH 1.2, 5.5 and 7.5. For drug release study, ciprofloxacin HCl was used as a model drug due to its hydrophilic nature. Effect of pH on ciprofloxacin HCl release was studied by immersing the ciprofloxacin HCl loaded samples in buffer solutions of different pH (1.2, 5.5 and 7.5). Figure 2 shows the effect of pH on drug release from PVA/Ge hydrogel. It was observed that drug release was high in the medium of pH 1.2 and pH 7.5 as compared to pH 5.5.

Table 2. Effect of polymers and crosslinker concentration on dynamic and equilibrium swelling ratio of PVA/Ge hydrogels

Dynamic and equilibrium swelling ratio in solution of different pH								
Sample No	pH 1.2		pH 5.5		pH 6.5		pH 7.5	
	q	Eq	q	Eq	Q	Eq	q	Eq
S1	3.6	7.6	3.32	5.88	3.64	7.7	3.78	7.8
S2	3.15	6.25	2.83	5.3	3.22	6.29	3.48	6.3
S3	2.62	5.46	2.56	4.94	2.7	5.4	2.89	5.67
S4	2.59	5.5	2.31	5.03	2.75	5.5	2.86	5.75
S5	2.8	6.24	2.69	5.35	3.0	6.3	3.05	6.66
S6	2.76	6.37	2.68	5.39	3.0	6.5	3.1	6.77
S7	2.82	6.0	2.63	5.37	2.76	6.09	2.91	6.1
S8	2.76	5.5	2.46	5.1	2.71	5.6	2.7	5.8
S9	2.53	5.2	2.35	5.0	2.41	5.4	2.65	5.5

q: Dynamic swelling ratio and Eq: Equilibrium swelling ratio

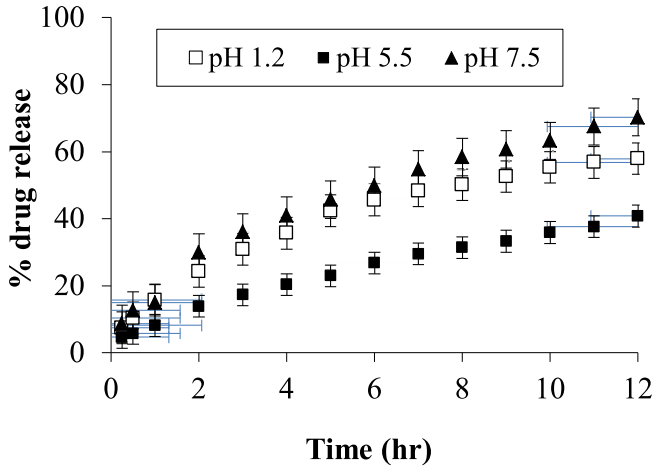

Figure 2. Effect of pH on drug release from PVA/Ge hydrogel (70:30) using GA as a crosslinking agent (0.608%) in 0.05 M USP phosphate buffer solutions

Effect of PVA contents on swelling and on drug release of PVA/Ge hydrogels

The concentration of polyvinyl alcohol (PVA) used in PVA/Ge hydrogel was varied from 7 g to 7.5 g and 8 g per 100 g of solution using glutaraldehyde as crosslinking agent (0.608 wt%) to investigate the effect of PVA contents on the swelling and drug release. It was observed from Figure 3 and Figure 4, that drug release and swelling of hydrogel increases with an increase in PVA concentration due to the availability of more free hydroxyl groups of PVA[33]. Increasing PVA contents results in greater hydration of its chains because of the hydrophilic nature of the PVA. Drug release studies were carried out for 12 hrs in 0.05 M USP phosphate buffer solutions of different pH. As shown in Figure 4, drug release was observed 70.26%, 83.3% and 84.34% at pH 7.5, 40.8, 52.3 and 54.67 at pH 5.5, and 57.9%, 70.5% and 72.2% at pH 1.2 with respect to composition of 30/70, 28.5/71.5 and 27.2/72.8 respectively.

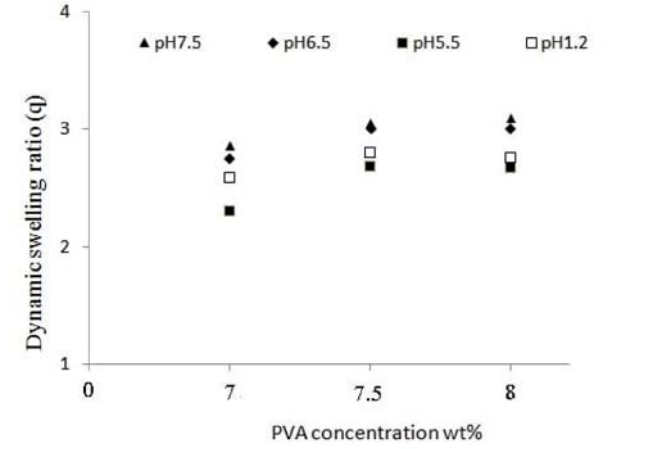

Figure 3. Dynamic swelling ratio (q) of PVA/Ge hydrogels with different concentrations of PVA (7, 7.5 and 8 g) using glutaraldehyde as crosslinking agent (0.608 wt%) in solutions of different pH in 0.05 M USP phosphate buffer

Effect of Gelatin concentration on swelling of PVA/Ge hydrogels

To study the effect of gelatin (Ge) concentration on swelling, three formulations of PVA/Ge hydrogels with

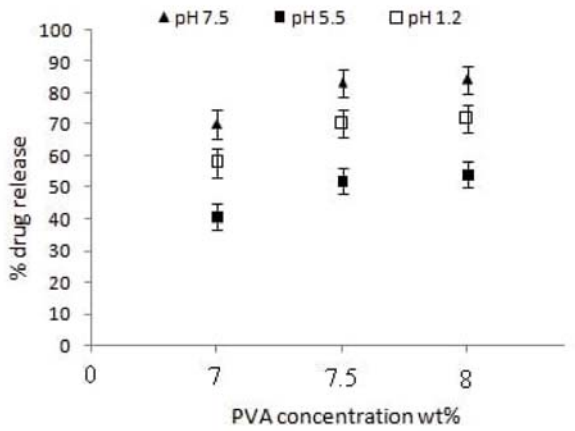

Figure 4. Release of ciprofloxacin hydrochloride from PVA/Ge hydrogels using different concentrations of PVA (7, 7.5, 8 g) and GA as crosslinking agent (0.608 wt%) at various pH values in 0.05 M USP phosphate buffer solutions

different concentrations of Ge varied from 1 g to 2 g and 3 g keeping PVA and GA concentration constant were synthesized and subjected to swelling studies in solutions of different pH values. It was observed that at pH 1.2, 5.5, 6.5 and 7.5, swelling ratio with increased Ge concentration was not significant as compared to swelling ratio with decreased Ge concentration as shown in Figure 5. The swelling ratio was observed to decrease with increase in Ge concentration. This is because of increase in density of crosslinks with increase in Ge concentration. The higher the Ge concentration, higher will be the density of crosslinks. Ge network is a triple-helix which acts as a crosslink and exhibit higher swelling at low concentration because of loose structure of network while swelling decreases as the concentration of Ge increases. These results are consistent with those reported by Bajpai et al.[34], Congde Qiao et al[35] and Xiaohong Hu et al[36]. They all suggested a decrease in swelling ratio by increasing gelatin concentration.

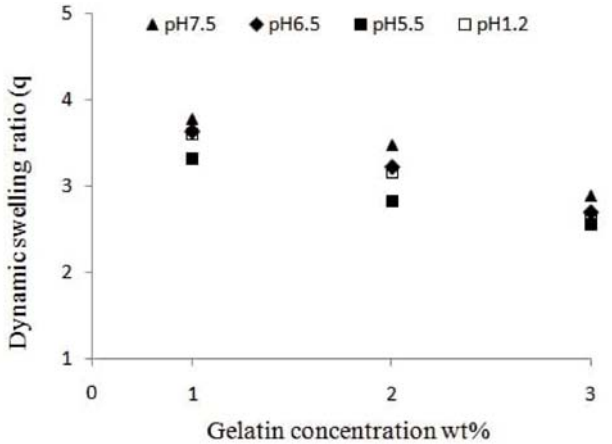

Figure 5. Dynamic swelling ratios (q) of PVA/Ge hydrogels with different concentrations of Ge (1, 2 and 3 g) using glutaraldehyde as crosslinking agent (0.608 wt%) in solutions of different pH in 0.05 M USP phosphate buffer

Effect of Glutaraldehyde on swelling and on drug release of PVA/Ge hydrogels

A series of three PVA/Ge hydrogels with different concentrations of crosslinking agent (0.57%, 0.64%, and

0.704%) were prepared to investigate the effect of glutaraldehyde (GA) on swelling and release behavior of drug from hydrogels. It was observed that swelling of hydrogel decreases with increase in GA concentration as shown in Figure 6. This may be due to the increased crosslinked density and as the crosslinking of PVA increases, the number of free hydroxyl groups decreases, as a result water uptake decreases with increasing crosslinking density. A similar decrease in swelling ratio was reported by Parka et al[37], who prepared PVA/methylcellulose (MC) blend hydrogel and suggested that by increasing GA concentration swelling ratio decreases significantly. Figure 6 and Figure 7 show that increase in GA concentration from 0.57% to 0.64% and 0.704% results in decrease in swelling ratio and decrease in percent drug release. As shown in Figure 7, drug release was observed 85.6%, 76.61% and 62.98% at pH 7.5, 58.2%, 48.8% and 40.3% at pH 5.5 and 71.9%, 69.4% and 54.9% at pH 1.2 with respect to feed crosslinker concentration of 0.57, 0.64 and 0.704 g respectively.

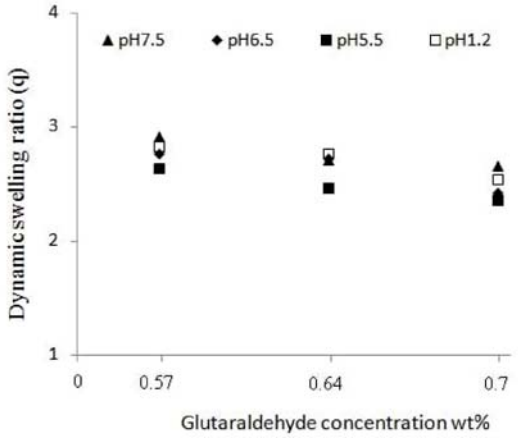

Figure 6. Dynamic swelling ratios (q) of PVA/Ge hydrogels with different concentrations of GA (0.57, 0.64 and 0.7 wt%) in solutions of different pH in 0.05 M USP phosphate buffer

Diffusion coefficient of polymers (D)

To measure solute diffusion into hydrogel, diffusion coefficient (D) is applied indirectly. Fick's law of diffusion was used during membrane permeation method or sorption and desorption phenomenon. It was found that diffusion coefficient decreased with the increasing PVA concentration because swelling of polymer increases as the concentration of PVA increases. Diffusion coefficient increased with increasing gelatin and crosslinking agent concentration. Table 3 shows the increase and decrease in diffusion coefficient[38–39].

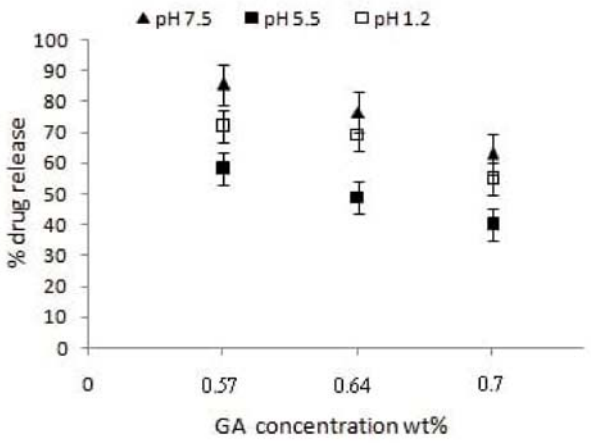

Figure 7. Release of ciprofloxacin hydrochloride from PVA/Ge hydrogels using different concentrations of GA as crosslinking agent (0.57, 0.64, 0.7 g) at various pH values in 0.05 M USP phosphate buffer solutions

Molecular weight between crosslinks (M_c) and solvent interaction parameters (χ)

An increase in values of molecular weight between crosslinks (M_c) was observed by increasing the concentration of polyvinyl alcohol (PVA). Higher swelling of polymer was reported due to PVA hydroxyl group into polymer chain. Crosslinked density (q) is also related to the values of PVA and average molecular weight between crosslinks as shown in Table 3. Solvent interaction parameters (χ) were studied to check the effect of solvent interaction between polymer and solvent. It was reported that greater the values χ weaker the values of interaction between polymer and solvent[24, 39].

Gel fraction analysis

It was observed that gel-fraction of hydrogels increased with increased concentration of polyvinyl alcohol (PVA) and crosslinker glutaraldehyde (GA). Sol- fraction of hydrogels was observed to decrease with the increased concentrations of PVA and GA. By increasing gelatin concentration, gel-fraction decreased as shown in Table 4. Figure 8 shows the effect of polymers concentration and crosslinker concentration on gel-fraction of hydrogel.

Porosity measurement

Table 3 shows that the porosity of PVA/Ge hydrogel increases by increasing the concentration of polyvinyl alcohol due to increasing viscosity of the hydrogel solution. Viscous solution efficiently prevents escaping of the bubbles from hydrogel solution that results in increased porosity due to formation of interconnected channels.

Table 3. Flory-Huggins network parameters of PVA/Ge hydrogels

Sample No	$V_{2,s}$	χ	M_c	M_r	q	D 10^{-7} (cm²/sec)
S_1	0.07742	-0.52740	1940.28	1302.34	1.4898	1.30517
S_2	0.08540	-0.53042	1876.14	1194.69	1.5703	1.20461
S_3	0.10192	-0.53680	1271.65	1104.67	1.6511	2.52398
S_4	0.10503	-0.53802	1296.42	1057.30	1.2261	2.03673
S_5	0.09013	-0.53223	1335.64	1082.00	1.2344	2.02954
S_6	0.07591	-0.52684	2419.43	1104.67	2.1901	0.58373
S_7	0.08114	-0.52880	1988.45	1110.96	1.7898	0.74316
S_8	0.09410	-0.53376	1783.30	1099.44	1.6220	0.90632
S_9	0.11313	-0.54122	347.254	407.997	0.8510	3.35658

V_{2s}: Volume fraction of the polymer at swelling equilibrium in phosphate buffer solution. M_c: average molecular weight between crosslinks. M_r: molar mass of the repeating unit. χ: solvent interaction parameter. q: crosslinking density. D: is diffusion coefficient

Table 4. Gel fraction and porosity of different formulations of PVA/Ge hydrogels

Sample No	Ge/PVA ratio	GA (wt %)	Gel fraction (%)	Porosity (%)
S1	11/89	0.608	94.55	17.11
S2	20/80	0.608	91.43	14.02
S3	27.2/72.8	0.608	89.66	12.98
S4	30/70	0.608	88.62	10.27
S5	28.5/71.5	0.608	90.20	13.50
S6	27.2/72.8	0.608	92.86	16.34
S7	27.2/72.8	0.576	90.54	17.66
S8	27.2/72.8	0.640	91.55	14.10
S9	27.2/72.8	0.704	94.95	13.54

By increasing gelatin and glutaraldehyde concentration, porosity decreases as shown in Figure 8. Increase in glutaraldehyde concentration results in increased in entanglement between polymers which result in decreased porosity.

Drug release mechanism

The drug release constant (k) and (r) values were obtained for zero order, first order, Higuchi model and Peppas. Table 4 shows values of (r) for zero order and first order obtained from drug loaded PVA/Ge hydrogels using different concentrations of PVA and crosslinking agent. It was found that the values of (r) obtained for first-order release constants were higher than (r) values of zero order. From the results, it is clear.

That most samples showed drug release from PVA/Ge hydrogel following first-order release. The values of (r) from Higuchi model showed that the drug release mechanism is diffusion controlled. As the plot of drug released versus the square root of time is linear, which indicates diffusion-controlled system[40]. The effects of PVA and GA on release exponent "n" values are given in Table 5. The value of 'n' for the release of ciprofloxacin HCl at different pH (1.2, 5.5 and 7.5) has been evaluated from the slope and intercept of the plot ln Mt/M∞ versus ln t and the results showed that the values of 'n' are between 0.45 and 1.0 which indicates a non-Fickian or anomalous diffusion mechanism, and the swelling and relaxation of polymer are involved in drug release mechanism[41–46].

Fourier Transform infrared spectroscopy (FTIR)

PVA/Ge hydrogels were analyzed by FTIR for confirmation. Figure 9 shows spectra of pure PVA, Ge, PVA/

Table 5. Effect of different concentrations of PVA and GA on drug release kinetics and release exponent of PVA/Ge hydrogel in a buffer of different pH

Sample	PVA content	pH	Zero order		First order		Higuchi	
			r	K0 (h-1)	R	K1 (h-1)	r	K2 (h-1/2)
S4	7	1.2	0.959	4.227	0.982	0.067	0.994	0.178
		5.5	0.988	2.993	0.995	0.039	0.997	0.132
		7.5	0.971	5.042	0.994	0.092	0.996	0.210
S5	7.5	1.2	0.973	5.188	0.995	0.095	0.997	0.216
		5.5	0.991	4.089	0.998	0.058	0.996	0.167
		7.5	0.950	5.677	0.994	0.134	0.992	0.241
S6	8	1.2	0.994	5.477	0.996	0.099	0.992	0.222
		5.5	0.996	4.367	0.998	0.063	0.990	0.176
		7.5	0.987	6.226	0.987	0.135	0.997	0.256
	GA content							
S7		1.2	0.994	5.507	0.996	0.100	0.991	0.223
	0.57	5.5	0.997	4.075	0.996	0.063	0.986	0.164
		7.5	0.994	6.376	0.979	0.144	0.990	0.258
S8		1.2	0.997	5.158	0.992	0.089	0.986	0.207
	0.64	5.5	0.988	3.495	0.995	0.049	0.995	0.143
		7.5	0.984	5.747	0.998	0.115	0.997	0.237
S9		1.2	0.989	3.836	0.996	0.058	0.995	0.151
	0.70	5.5	0.990	2.794	0.993	0.036	0.995	0.106
		7.5	0.987	4.412	0.995	0.073	0.994	0.175
Sample	PVA content	pH	Release exponent (n)		r		Order of release	
S4	7	1.2	0.548		0.9969		Non-fickian	
		5.5	0.584		0.9969		Non-fickian	
		7.5	0.554		0.9944		Non-fickian	
S5	7.5	1.2	0.570		0.9984		Non-fickian	
		5.5	0.730		0.9974		Non-fickian	
		7.5	0.511		0.9939		Non-fickian	
S6	8	1.2	0.597		0.9959		Non-fickian	
		5.5	0.708		0.9964		Non-fickian	
		7.5	0.634		0.9979		Non-fickian	
	GA content							
S7		1.2	0.598		0.9954		Non-fickian	
	0.57	5.5	0.481		0.9864		Non-fickian	
		7.5	0.602		0.9964		Non-fickian	
S8		1.2	0.574		0.9934		Non-fickian	
	0.64	5.5	0.576		0.9974		Non-fickian	
		7.5	0.562		0.9979		Non-fickian	
S9		1.2	0.506		0.9974		Non-fickian	
	0.70	5.5	0.540		0.9974		Non-fickian	
		7.5	0.513		0.9969		Non-fickian	

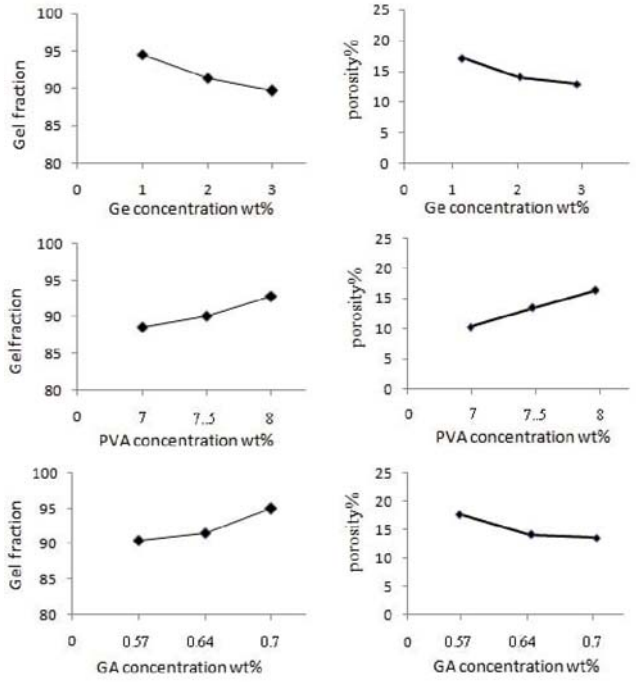

Figure 8. Gel fraction and % porosity of PVA/Ge hydrogel with different concentration of Ge, PVA and GA

Ge hydrogel and PVA/Ge drug loaded hydrogel. The FTIR of pure PVA showed a broad peak at 3440 cm^{-1} because of -OH groups stretching. Peak at 2911 cm^{-1} indicates –C-H stretching vibration and at 1145 cm^{-1}, the peak indicates C-O stretching. FTIR of Ge showed peak of -NH stretching of secondary amide at 3446 cm^{-1}, the peak at 1655 cm^{-1} is due to C =O stretching and at 2922 cm^{-1}, the peak indicates C-H stretching. The spectra of PVA/Ge hydrogel indicated the main changes in the region of 1200–1800 cm^{-1} and 2900–3500 cm^{-1} which is evidence of interaction between them. Figure 8 shows FTIR spectra of PVA/Ge hydrogel where the intensity of the broad peak at 3450 cm-1 is decreased as compared

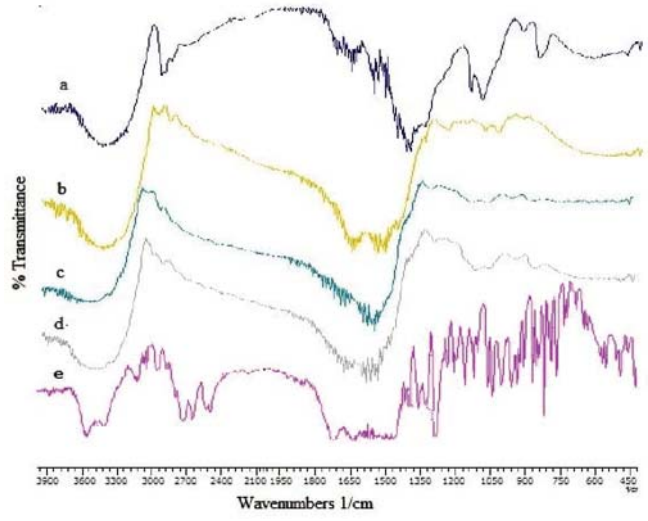

Figure 9. FTIR spectra of (a) PVA, (b) Ge, (c) PVA/Ge hydrogel, (d) drug loaded PVA/Ge hydrogel and (e) ciprofloxacin HCl

to the spectra of pure PVA and Ge, which indicates the consumption of these groups in hydrogel formation. Figure 8 also showed that the peak intensity at 2911 cm^{-1} in PVA/Ge hydrogel spectra is low as compared to the peak of pure PVA, which indicates the presence of gelatin in hydrogel. The peak at 1605 cm^{-1} is due to the formation of amine bond –C=N by amino group of Ge and aldehyde group of GA.

X-ray diffraction (XRD) study

Figure 10 shows XRD patterns of pure drug ciprofloxacin HCl, drug loaded PVA/Ge hydrogel and PVA/Ge hydrogel. XRD of the pure drug revealed several sharp peaks but after loading ciprofloxacin HCl into PVA/Ge hydrogel, the sharpness of the drug peaks decreased which indicates that ciprofloxacin HCl was dispersed at molecular level in the PVA/Ge hydrogel and decreased the crystalline form of drug.

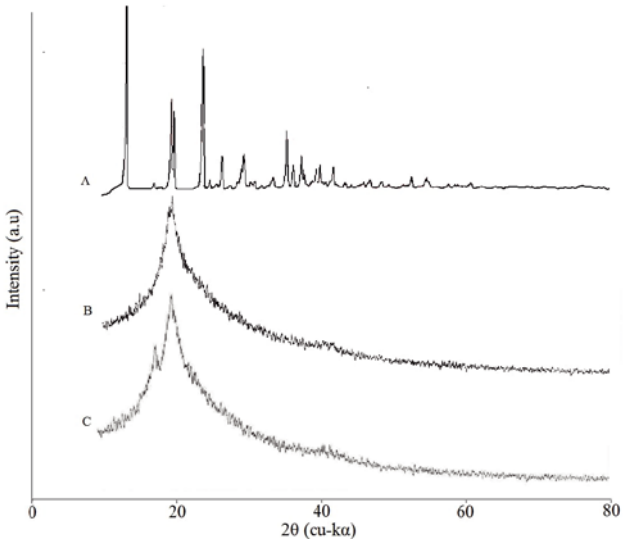

Figure 10. XRD patterns of a) ciprofloxacin HCl, b) ciprofloxacin HCl loaded PVA/Ge hydrogel and c) PVA/Ge hydrogel

CONCLUSIONS

In the present work, hydrogel based on polyvinyl alcohol (PVA) and gelatin (Ge) were prepared using glutaraldehyde (GA) as a crosslinking agent. The prepared hydrogels were characterized by FTIR and XRD to investigate the structure and crystallinity of hydrogel respectively. Furthermore, dynamic and equilibrium swelling studies and drug release from the prepared hydrogel was investigated. It was observed that swelling increases by increasing PVA concentration while swelling decreases with increased concentration of Ge and glutaraldehyde. High swelling ratio was observed at pH 1.2, 6.5 and 7.5 as compared to pH 5.5. Water diffusion coefficient, solvent interaction parameters, molecular weight between crosslinks and crosslinked density were measured to study the swelling behavior of the hydrogel. It was also observed that the porosity and gel fraction of PVA/Ge hydrogel increased with increase in PVA concentration while decreased with increase in Ge concentration. Increasing the concentration of GA resulted in increased gel fraction and decreased porosity. The results also showed that drug release from PVA/Ge hydrogel increased with increase in PVA concentration and drug release decreased with increased concentration of Ge and GA. Drug release from the hydrogel followed first-order release. The results suggest that PVA/Ge hydrogel has the potential to be used as a sustained drug delivery system for hydrophilic drugs.

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