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Original Article

Fabrication and Evaluation of Physically Crosslinked Stimuli Sensitive Polymeric Blend of Pva-Gelatin as Drug Delivery System by Freeze Thaw Cycles

[a]Deepanjan Datta, [a*]Trishna Bal, [a]Sabyasachi Swain, [b]Sudipta Goswami

[a]Department of Pharmaceutical sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India, [b]Department of Chemical Engineering and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

Abstract

Vacuum dried Metronidazole hydrochloride (MTZ) loaded Crosslinked polymeric blend of Poly (vinyl) alcohol (PVA) and Gelatin (GE) in different ratios by 30 freeze/thaw cycles (FTC) tested for stimuli sensitivity and crosslinking at various pH, temperature, ionic concentration and oscillatory test along with dye absorption test showed maximum swelling in alkaline pH and decreased swelling with increased ionic concentration. Digital scanning calorimetry (DSC) thermo grams of the blank crosslinked sample showed a sharp endothermic peak at 160.57 ^{0}C and MTZ loaded samples showed two sharp endothermic peaks at 159.95 ^{0}C and 324.74 ^{0}C indicating the entrapment of drug in the polymeric network. Scanning electron microscopy (SEM) showed the rough polymeric texture. Fourier Transformation Infrared Spectroscopy (FTIR) confirmed the presence of new peaks in the cross-linked and drug-loaded sample. *In-vitro* drug release studies showed 98.253% ± 0.363 and 92.248% ± 0.244 releases in the first 6 hours. Biodegradability and bactericidal studies of the blank film indicated that the crosslinked sample is biodegradable and does not inhibit any microbial growth.

Key words: Crosslinked polymeric blend, Freeze-thaw cycle, Gelatin, Poly (vinyl alcohol), Stimuli-Sensitive, Metronidazole Hydrochloride

Corresponding Author: Trishna Bal, Department of Pharmaceutical sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India
Tel: 09470141136
E-Mail: twinkisengupta@gmail.com
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1. Introduction

Hydrogels, in the form of three-dimensional cross-linked polymer networks, can absorb a large amount of solvent (water) without dissolution. These polymers exist as glassy in a dehydrated state and become soft elastic after swelling in water up to a certain temperature. Depending on the nature of

functional groups present in the matrix polymer, hydrogels can be responsive to various external stimuli such as pH, temperature, ionic strength, light, magnetic field, etc. [1].

In combination with the stimuli-responsive character their high absorbency, soft consistency, elasticity and good water retention capacity have made such materials as the choice in modern medical industries for prostheses development, soft tissue replacement, wound dressing, targeted drug transporting and delivery devices [2].Hydrogels based on Poly (vinyl) alcohol (PVA) networks have been developed using freeze/thaw cycle to avoid the contamination problem associated with chemical crosslinking. Blending PVA with another water-soluble natural polymer e.g. chitosan, alginate etc. have been tried by some researchers for various applications [3]. However, PVA/gelatin combination, through physical crosslinking, is not yet been popular. The solubility parameter of PVA and gelatin being very close [4, 5] is expected to form a miscible blend and therefore better properties may be exhibited. Gelatin (GE), a biopolymer obtained from animal tissue, is well known as a biocompatible material too. The form of PVA/natural polymer blends vary from soft and tacky hydrogels to solid non-tacky thin films which are suitable to be loaded with the drug and carry the same into the general systemic circulation when administered into the animal body. Also, the hydrogels are capable to release the required dosage at a particular target site/organ through the action of enzymes or by differential swelling in response to changes in pH, temperature or of molarity etc. [6, 7]. However, the organ specificity during the drug delivery is still haunting and new approaches are being continuously designed and tried to achieve success in this direction. In the present study, the hydrogels are produced by physical-cross linking method i.e., Freeze-Thaw processing. The advantage of this method is that it is experimentally straight forward, and avoiding chemical cross-linkers for the preparation of the films/ hydrogels may improve the quality of the product as the chance of unwanted reactants entrapment is excluded. The mechanism of the freeze-thaw method is driven by phase separation due to the freezing of the solution and simultaneous rejection of the polymer from the growing ice crystallites. This process is continued with repeated cycling. The size of the dispersed domains (crystallites) increases with the increase in the number of cycles. The resultant gels are mainly seemed to be composed of puddles, where the ice has melted, and being surrounded by a polymer skeleton [8]. Previously studies were being conducted for hydrogel preparation with the combination of gelatin (GE) and Poly(vinyl)alcohol (PVA) utilizing freeze-thaw cycles but only up to 3-6 cycles [9,10]. In the view of above, in the present study, an attempt has been made to prepare a crosslinked polymeric blend of PVA and GE using 30-freeze-thaw cycles loaded with metronidazole hydrochloride

(MTZ) and thereby study their release. The prepared crosslinked blend has been characterized by FTIR, DSC, SEM, stimuli sensitivity, swelling studies, and drug release studies. Biodegradability and bactericidal effects of the blank crosslinked blend have also been carried out.

2. Materials and Methodology

2.1. Solution Blending of Polyvinyl Alcohol (PVA) and Gelatin (GE)

White powder of Poly (vinyl alcohol) (PVA) was dissolved without any further purification in Millipore water at 95 0C, under magnetic stirring until a clear solution of 10% (w/v) concentration was obtained. Similarly concentrated (15% w/v) Gelatin (GE) solution was prepared by adding yellow granules of gelatintoMilliporewaterat50˚C under magnetic stirring for 2h [11, 12]. After cooling at room temperature PVA solution was mixed with gelatin solution in calculated proportions e.g. 60:40, 50:50 and 40:60 (w/w) till the clear mixture is prepared.

2.2. Preparation of PVA/GE Blend Films

The PVA/GE solution blend was transferred immediately into a covered Petri dish and allowed to dry at room temperature (27˚C) for 2-3 days and vacuum dried at 30˚C. The solid films prepared in this way were termed as PG-1 (50:50), PG-2 (40:60), and PG-3 (60:40). The ratio enclosed in the parenthesis here indicates the weight ratio of PVA to gelation in the corresponding mixture. The dry samples were used for various experiments to characterize them.

2.3. Crosslinking of PVA in PVA/GE Blend by Freeze-Thaw Cycle

Vacuum dried PVA-Gelatin (PG) blend films were then subjected to physical cross-linking by Freeze-thaw cycles, which underwent repeated freezing and thawing respectively for 30 cycles. Each freeze/ thaw cycle meant freezing at 0 ˚C for 9 hours followed by thawing at 37˚C for another 9h and then repeated the same for 30 times. After completion of 30 cycles, the samples appeared too transparent, hard and could be ground to fine powder.

2.4. Characterization

2.4.1. Swelling Studies

Swelling tests with the dry samples in de-ionized water at two different temperatures (25˚ and 37˚C) and pH medium were carried out. Equilibrium Degree of Swelling (EDS) was calculated using the equation 1 as stated below [13].

EDS (%) = [(WS - WD)/(WD) * 100]--- -(1)

Where WS is the swollen weight and WD is the dry weight of the films at time “t”.

2.4.2. Water Swelling Studies

The samples were soaked in Millipore water for 2 hours. Its weight was checked every 10 minutes until the constant value of final weight (Ws) reached.

2.4.3. pH & Temperature Sensitivity

The samples (≤ 0.1gm) were soaked in solutions of different pH of values 1.2, 7.4 and 9.2 [14] to check the degree of swelling

in presence of the specified pH. The resistivity against the dissolution of the samples was checked with a change in pH of the medium. This test mimics the in-vivo swelling of the polymeric delivery systems in various parts of the body where variable pH is found e.g. in GI tract the pH= (6.5-7.4) in the small intestine, in pancreas pH= (7.1-8.2) etc. So, by this test pH sensitivity of the hydrogels was determined.

Similarly, to check the temperature sensitivity, the samples (≤0.1gm) were soaked in Millipore water of pH =7.0 at two different temperatures (25^{o}C and 37^{0}C). The samples, in each case, were kept in water for a stipulated period and taken out till the equilibrium degree of swelling was reached [13]. The two-specified temperature used here was to mimic the body condition as well as room condition.

2.4.4. Ionic Strength Swelling Test

The Ionic Strength Swelling Test was carried out to check the efficiency of the device to dissolute the drugs in a cationic concentration [13, 14]. From the literature it is evident that the sodium ion (Na+) content increases from the stomach toward the small intestine, starting from 0.04mol/L and reaching blood level at 0.14 mol/L [15]. To see the effect of variation of ionic strength on the swelling of samples these were soaked in sodium chloride (LR grade) solutions with different molar concentrations ranging from 0.005 to 0.5M till the EDS reached.

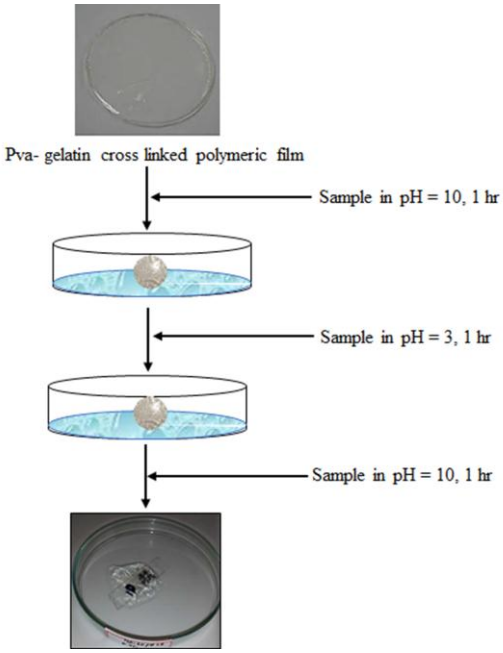

Figure 1. Flow diagram of oscillatory swelling test for pva -gelatin cross linked polymeric film.

2.4.5. Oscillatory Swelling Test

The samples were soaked in a pH=10 buffer for first 1 hour and were then transferred to a pH 3 buffer solution for the next 1 hour and were again transferred to buffer solution of pH 10 for the next hour. Swollen samples from the (pH=10) medium were taken out to see the weight change and then dipped in the solution (pH=10) again [14]. Now the swollen sample was taken out at every 10minutes interval to note down the weight change.

The flow diagram (Figure 1) shows the steps:

1. Sample in pH = 10 (1 hour)

↓

2. Sample pH = 3 (next 1 hour)

↓

3. Sample in pH = 10 (next 1 hour)

↓

4. Samples from (pH=10)

The samples were finally taken out time to time to see the weight changes and then dipped in the same buffer solution (pH=10) to note the change in weight till the EDS reached.

2.4.6. Dye Absorption Test

For dye absorption test, 0.1mM solution of Congo red was prepared. The absorbance of the pure dye solution was measured using a UV/Vis Spectrophotometer. Prepared samples PG-1, PG-2& PG-3(≤0.1gm) were soaked in the prepared Congo red solution and left for 24 hours. After a span of 24hours, the swelled polymer sample was taken out and the absorbance of the solution was measured [14].

2.4.7. Swelling Kinetics for Stimuli Response

2.4.7.1. Zero-Order Kinetics

The zero-order kinetics (Eq. 1) describes the systems where the release rate is independent of its concentration [16, 17].

$$C = K_0\, t \text{ …….. (2)}$$

WhereK_0 is zero-order proportionality rate constant and t is the time.

2.4.7.2. First Order Kinetics

The first order kinetics as proposed by Quintana et al., states that the rate of swelling at any time t is directly proportional to the water content and that the hydrogel must obtain before the equilibrium water content W∞ is reached [18]. The swelling is then expressed as

$$dw/dt = k(W\infty - W) \text{ …… (3)}$$

Where, W is the water content of the hydrogel at time "t", and k is the proportionality constant between the swelling rate and the unrealized swelling capacity W∞ - W

Upon integrating the Equation 2 between the limits t=0 to t and w=0 to w, the following expression can be obtained:

$$\text{In } W_{\infty}(W_{\infty} - W) = K_t \text{ …… (4)}$$

2.4.7.3. Second Order Kinetics

The second order kinetics, the rate of swelling at any time t is directly proportional to the water content of the hydrogel [18]. Therefore, considering second order kinetics, the rate at any time may be expressed as

$$dw/dt = k(W_{\infty} - W)^2 \text{ …… (5)}$$

Integrating the above Equation between the limits t=0 to t and w=0 to w, the following expression can be obtained:

$$t/w = 1/kW_{\infty} + 1/W_{\infty}\, t \text{ ……. (6)}$$

2.4.7.4. Swelling Power Number Determination

The values of initial swelling rate, swelling rate constant, maximum equilibrium swelling and diffusion coefficients of the crosslinked polymeric devices were calculated from the swelling kinetics studies by determining the swelling power number (n), applying the following equation [19, 20]:

$$(M_t - M_0)/M_0 = kt^n \text{ ……. (7)}$$

Where Mt and Mo are the mass of the swollen and dry sample at time t, K is the swelling constant, and n is the swelling exponent. Based on the values of n, which range generally between 0.60 and 0.72, the water uptake by the sample can be predicted.

2.5. Analytical Characterizations

The crosslinked polymeric blend of PVA and GE were characterized by FTIR, SEM, DSC techniques. FTIR spectra of MTZ, individual polymers, crosslinked polymeric blend were obtained by Shimadzu, FT-IR8400S Spectrophotometer. Surface topology was studied by Scanning electron microscope [Jeol JSM -6390 LV]. DSC was carried out with DSC: 60 [Shimadzu, Japan] from 20-3000C temperature at a heating rate of 10^0C/min in a nitrogen atmosphere.

2.6. Drug Loading onto the Polymer Matrix

The loading of MTZ onto the crosslinked polymeric blend was done by equilibrium swelling method [21]. The crosslinked polymeric blends were placed in known drug concentration in dark conditions at 370C and allowed to attain swelling equilibrium. The devices were obtained by drying at room temperature till equilibrium weight obtained.

2.7. Drug Loading Efficiency

The drug loading efficiency in the optimized film was determined either by spectrophotometrically or by weighing method. The drug-loaded film was placed in 5ml of buffer solution and sonicated for two hours to extract the drug from the film. The solution was then filtered and assayed at λmax of 277nm.The difference between the amount of drug initially employed and the drug content in the washings was taken as an

indication of the amount of drug entrapped. The drug loading efficiency in the Polymeric films can also be calculated from weighing method. The difference between the initial weight of the dry film and that of the dried drug loaded film can also be taken as an indication of the amount of drug entrapped.

The percentage of drug loaded and drug entrapped was calculated using the following equations [22, 23] as given below:

Percentage of drug loading = (weight of the drug-loadedfilm/weight of the film) * 100

Percentage of drug entrapped = (Mass of the drug present in the film / Theoretical mass of drug) * 100

Percentage Encapsulation Efficiency = (percentage actual loading / percentage theoretical loading) * 100

2.8. Biodegradability and Bactericidal Effect

A Minimal Agar Media (devoid of carbon source) used for biodegradation study [24] of crosslinked samples using *B.subtilis* and incubated at 25^0C for 15days to study the growth of microorganism on solidified media and weight loss of the crosslinked polymeric samples.

The Bactericidal effect of polymeric samples was carried out using *Escherichia coli* [25] in the media and incubated at 37^0C in a shaker for 45mins. The pH was maintained in the range 7.2-7.4. The growth of bacterial cells on the film was determined by U.V spectrophotometer considering control as blank.

2.9. In Vitro Release Study

The drug release study from stimuli-sensitive crosslinked polymeric films was carried out in phosphate buffer (pH 7.4) [26] at 319nm and drug release kinetics for each matrix was analyzed by assessing the fitting

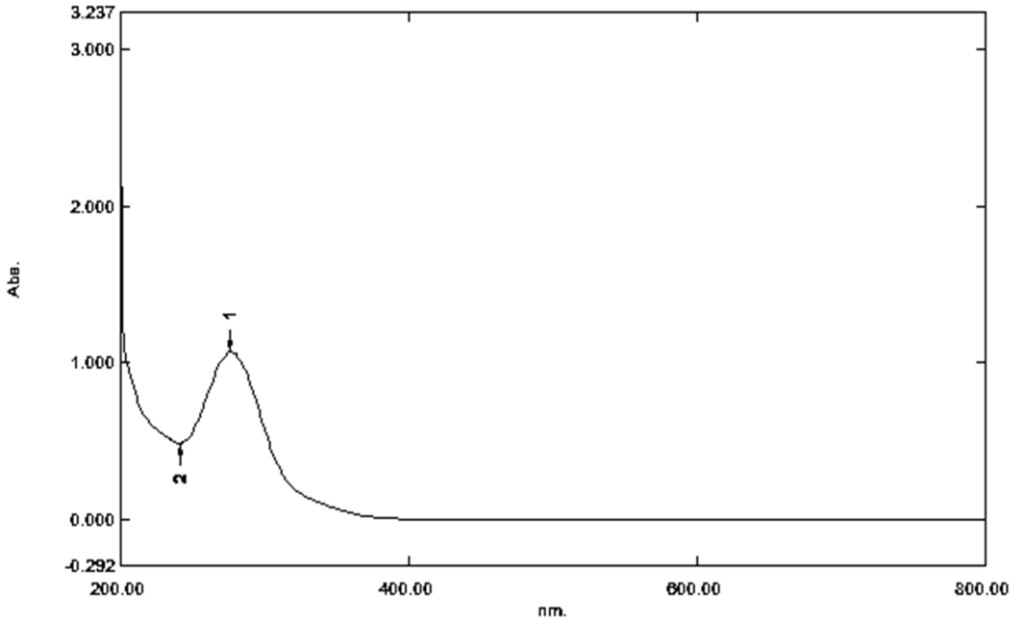

Figure 2. Absorption maxima of drug (MTZ) at pH 1.2.

of the release data to each of the following models [27].The drug-loaded polymeric films were immersed in a beaker containing 50ml of the stated buffer solution and after every stipulated interval 1ml of samples were withdrawn and analyzed in UV-Vis spectrophotometer for determination of the absorbance which was used to calculate the drug release from the system by plotting in the standard curve of the drug.

The standard curve of the drug (MTZ) was determined by accurately weighing MTZ (10mg) and transferring in a 100ml volumetric flask containing a buffer of pH1.2 to obtain a concentration of 100µg/ml as the stock solution (Stock –I). From this Stock-I solution, 10 ml of solution was withdrawn and transferred to a 100ml volumetric flask and made up the volume with the same to obtain a concentration of 10µg/ml (Stock-II). This Stock-II Solution was scanned in UV-Spectrophotometer, ranging 200-400nm (UV-1800, Shimadzu to determine the absorption maxima. The maximum absorbance was observed at 277nmas shown in Figure 2 and 3.

3. Results and Discussion

3.1. Swelling Tests

3.1.1. Water Swelling

Figure 4 represents the water swelling behavior of the crosslinked polymeric blend with respect to time. Inclusion of an amorphous polymer gelatin (GE) into the crystalline polymer poly (vinyl) alcohol (PVA) matrix might have reduced the packing of the molecules of the later in the solid film. Therefore, swelling in water is highest with the composition of 40:60 (PG-2) and lowest with the 60:40 (PG-3). This is probably due to the presence of an important quantity of ionizable groups in the gelatin. Moreover when the polymeric chains have ionizable groups there are hydrostatic repulsion between the groups which increases the volume between polymeric chains thereby enhancing the swelling capacity of the crosslinked polymeric blend [14] and this

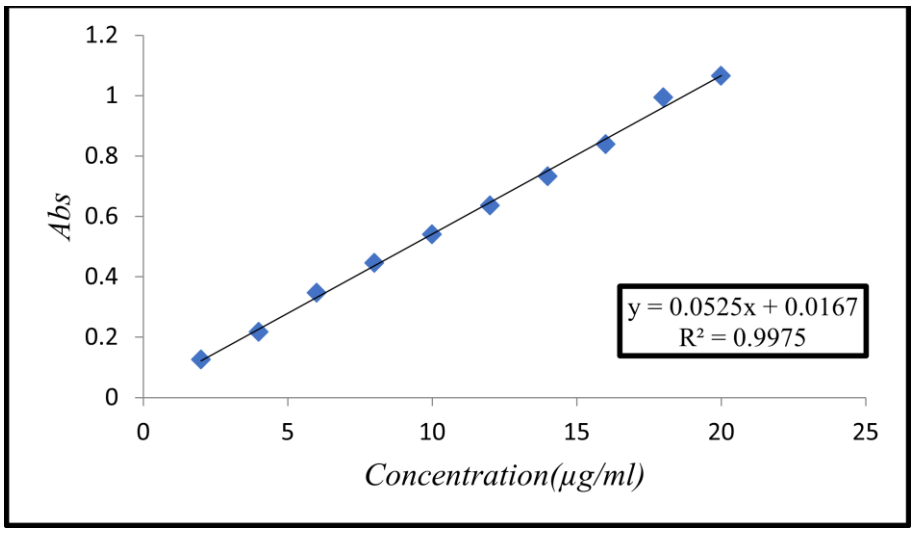

Figure 3. Calibration plot of Metronidazole drug at pH 7.4.

reason probably justifies the more swelling capacity of PG-2 than in comparison to PG-1 and PG-3, but at the same time PG-1 and PG-2 after a particular time period starts deswelling probably due to less concentration of PVA .Whereas in case of PG-3, the increased amount of PVA results in increased crystalline networks thereby rendering more

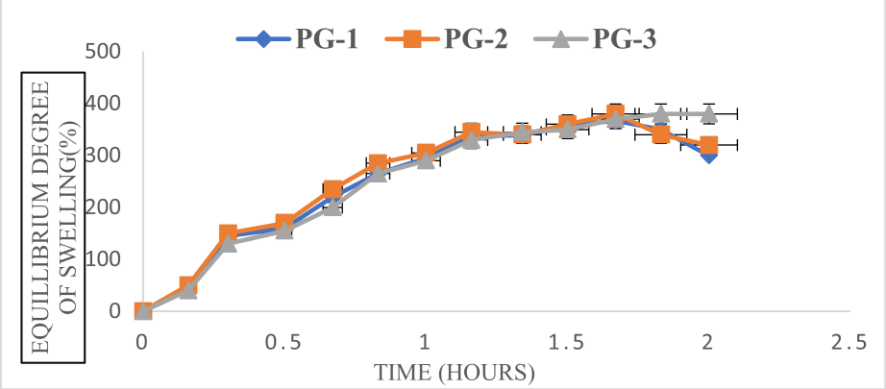

Figure 4. Water swelling behavior of PVA/GE blend films with respect to time.

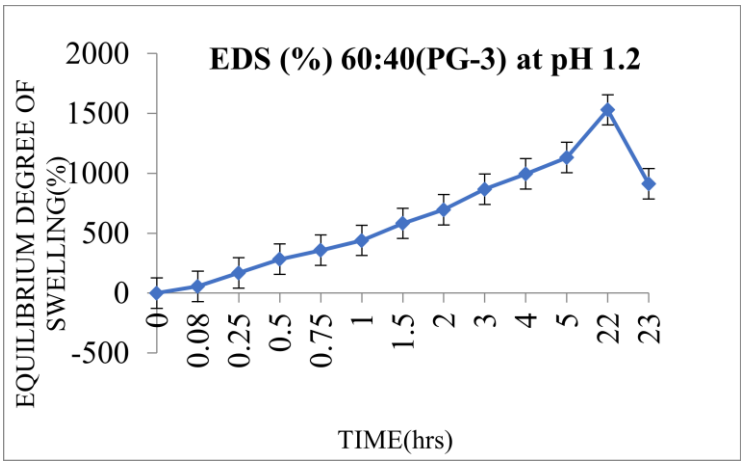

Figure 5 (a). EDS of PVA/GE blend 60:40 (PG-3) at pH 1.2.

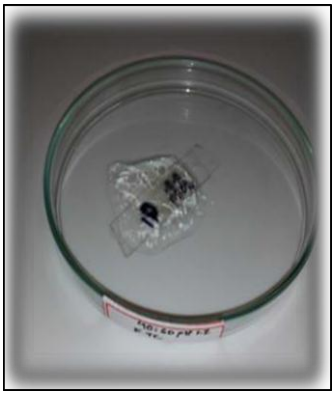

Figure 5 (b). Integrity of PG-3 film at pH 1.2.

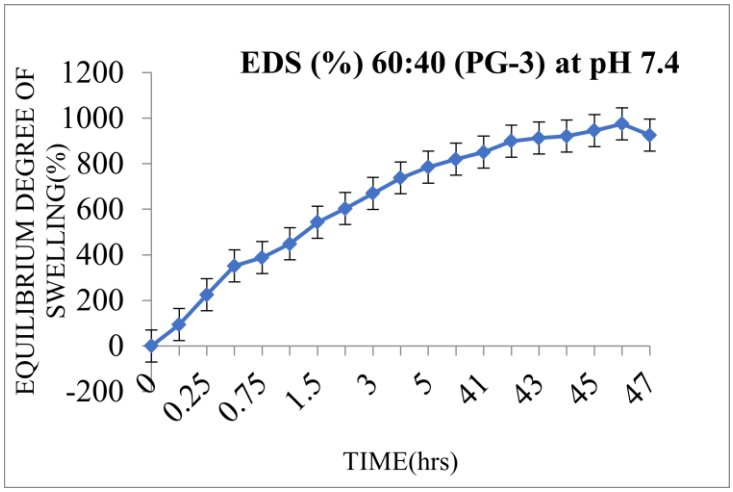

Figure 6 (a). EDS of PVA/GE blend 60:40 (PG-3) at pH 7.4.

Figure 6 (b). Integrity of PG-3 film at pH 7.4.

strength to the system and thus the swelling continues without the system being disrupted. Thus PG-3 was selected for further studies.

3.1.2. pH & Temperature Sensitivity

Figures 5-7 shows equilibrium degree of swelling (EDS) as a function of time of sample60:40 (PG-3) at pH1.2, 7.4 and 9.2 and figures 5a, 6a, and 7a show the integrity of the polymeric film at the same pH respectively. It was also noted that the sample had better swelling capacity compared to other blended ratios i.e., 50:50(PG-1) and 40:60(PG-2) even at high pH. The sample PG-3 shows the integrity at pH1.2 even after maximum swelling which is almost double quantitatively.

Figure 8a and 8b show the effect of temperature on the swelling behavior of the prepared crosslinked polymeric blends. Samples with higher percentage of gelatin (GE) PG-1 and PG-2 although showed more selling but may have undergone ionic-dissociation in water, hence dissolved

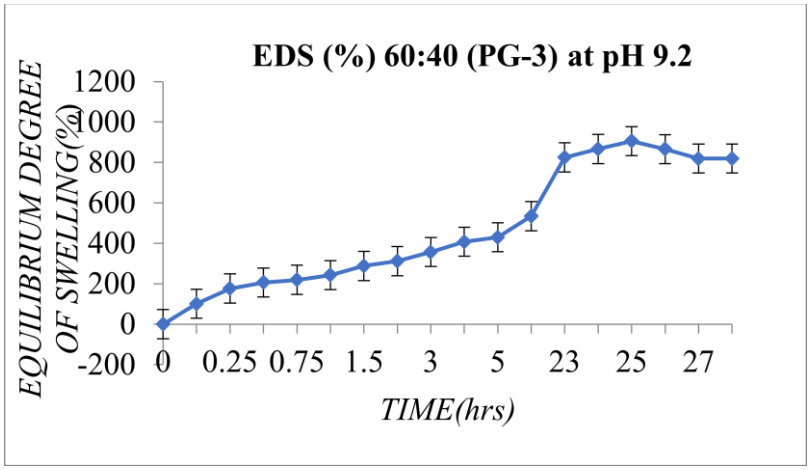

Figure 7 (a). EDS of PVA/GE blend 60:40(PG-3) at pH 9.2**.**

Figure 7 (b). Integrity of PG-3 film at pH 9.2.

immediately after adding them into the solvent, and the sample blended in the ratio PG-3 showed resistance in water even after increase in temperature up to 37^0C as the PVA content is more.

3.1.3. Ionic Strength Swelling Test

Figure 9 shows the ionic swelling behavior as a function of time of the polymeric blendPG-3at 0.005M, 0.05M, 0.1M, 0.2M, 0.3M, 0.4M and 0.5M sodium chloride solutions. It shows that as the concentration of sodium chloride is increased, the swelling ratio dropped drastically [14].

Figure 10-12 show the EDS of polymeric blend PVA/GE 50:50 (PG-1), 40:60 (PG-2) and 60:40 (PG-3) at different molar concentrations of sodium chloride (NaCl). Gelatin, with charges distributed over the surface, should be capable of combining with a neutral salt as with an acid or base, and any such protein-salt compounds, since they involve at least one strong electrolyte, would be expected to be dissociated [28].

Thus:

$$\overset{-+}{\text{protein}} + \overset{+-}{\text{NaCl}} = \overset{+}{\text{Na}}\ \overset{-+}{\text{protein}} + \overset{-}{\text{Cl}}$$

$$\overset{-+}{\text{protein}} + \overset{+-}{\text{NaCl}} = \overset{-+}{\text{protein}}\ \overset{-}{\text{Cl}} + \overset{+}{\text{Na}}$$

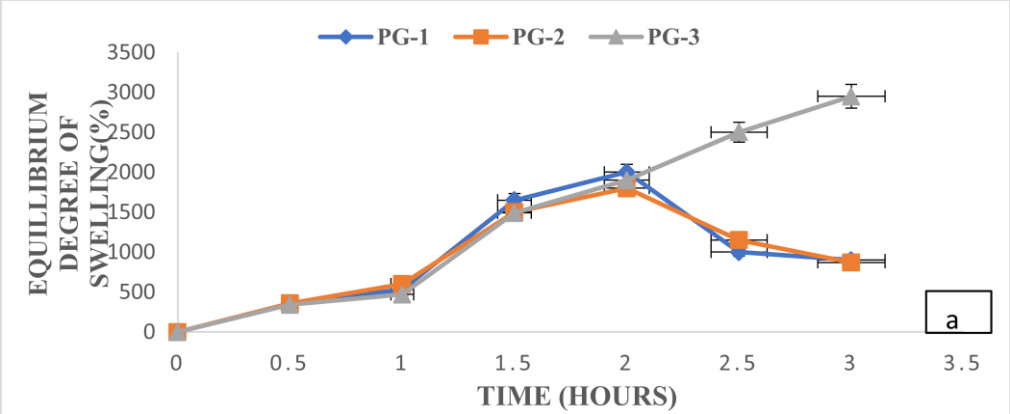

Figure 8 (a). Variation of EDS of PVA/GE blends in with time at 25˚C.

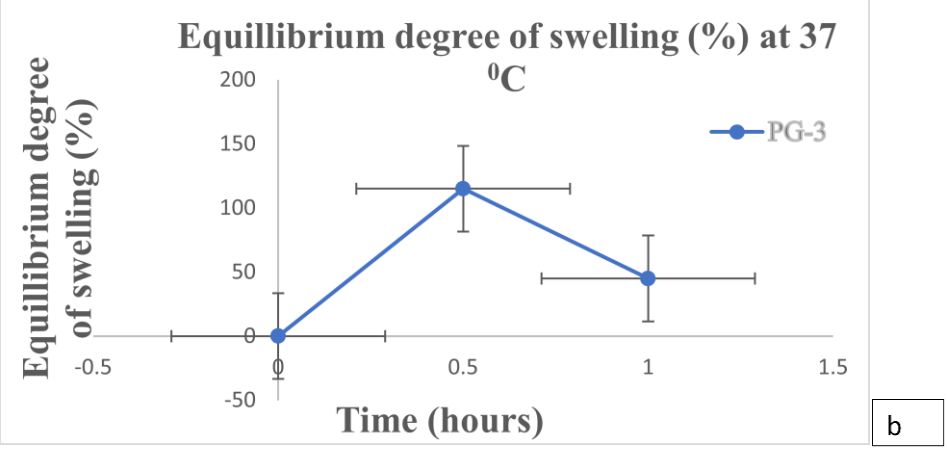

Figure 8 (b). Variation of EDS of PG-3 with time at 37˚C.

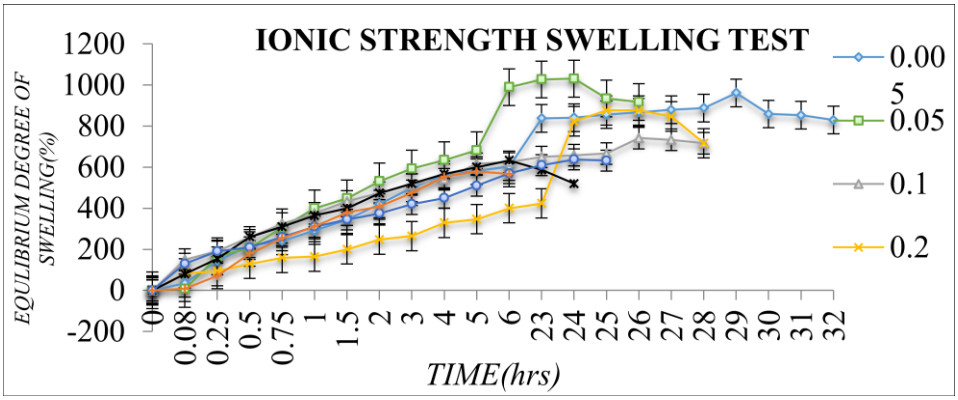

Figure 9. EDS (%) vs .time of PG-3 in0.005M,0.05M,0.1M,0.2M,0.3M, 0.4M and 0.5(M) NaCl solution.

3.1.4. Oscillatory Swelling Test

This test determines the ability of the crosslinked polymeric system to respond to pH pulses [14]. Also figure 13 shows the sample PG-3 of ratio 60:40was able to respond quickly to the pH pulses showing a

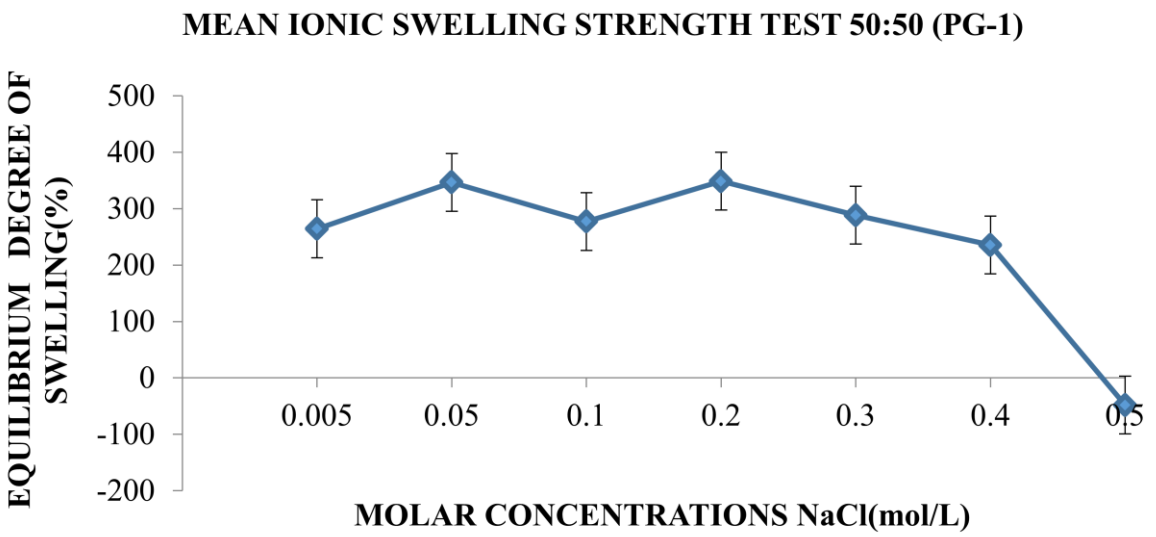

Figure 10. Mean Ionic Swelling behaviors of PVA/GE blend PG-1 in different molar concentrations of common salt solution.

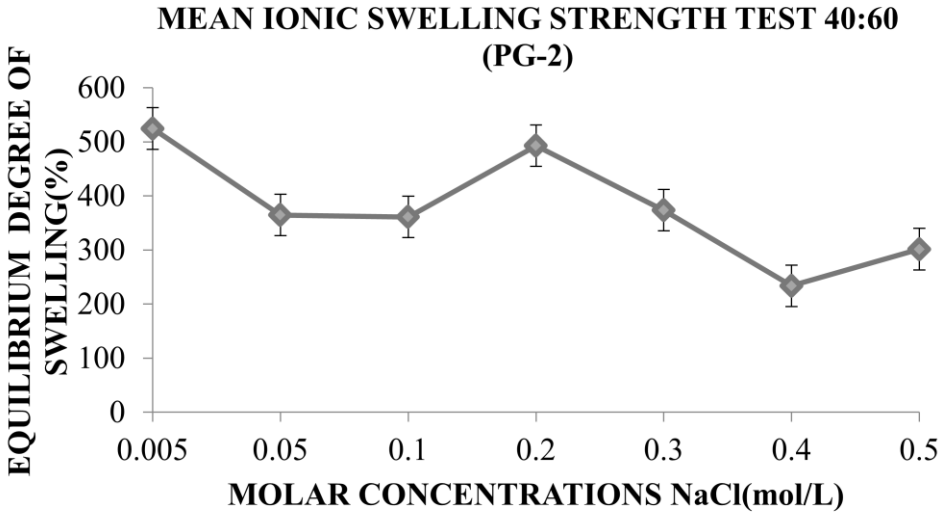

Figure 11. Mean Ionic Swelling behavior of PG-2 in different molar concentrations of common salt solution.

superior curve for a prolonged period compared to the other two blended ratios PG-1 and PG-2.

3.1.5. Dye Absorption Test

Congo red is the sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid; as existing as anions in solution is attracted strongly to the positively charged amine groups of the polymeric blends. Figure 14 shows the change in the absorbance verifying that the crosslinked polymeric blends can act as a potential purifying agent for waste contaminated with dye [14]. It was found that the blended ratio 60:40(PG-3) has the better absorption capacity than PG-1 and PG-2 as the dye solution showed maximum reading with PG-1 and PG-2, which confirms that PG-3 can prove to be a potential purifying agent. Figure 15 shows the dye

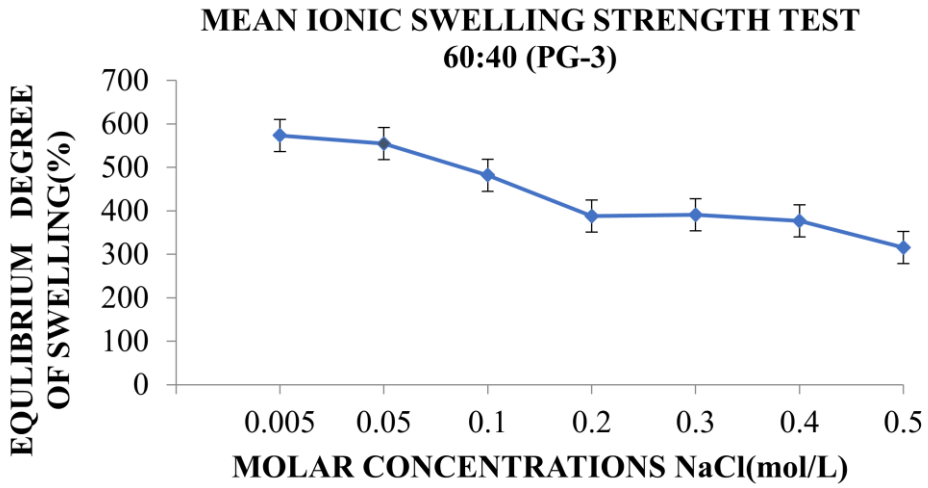

Figure 12. Mean Ionic Swelling behavior of PG-3 in different molar concentrations of common salt solution.

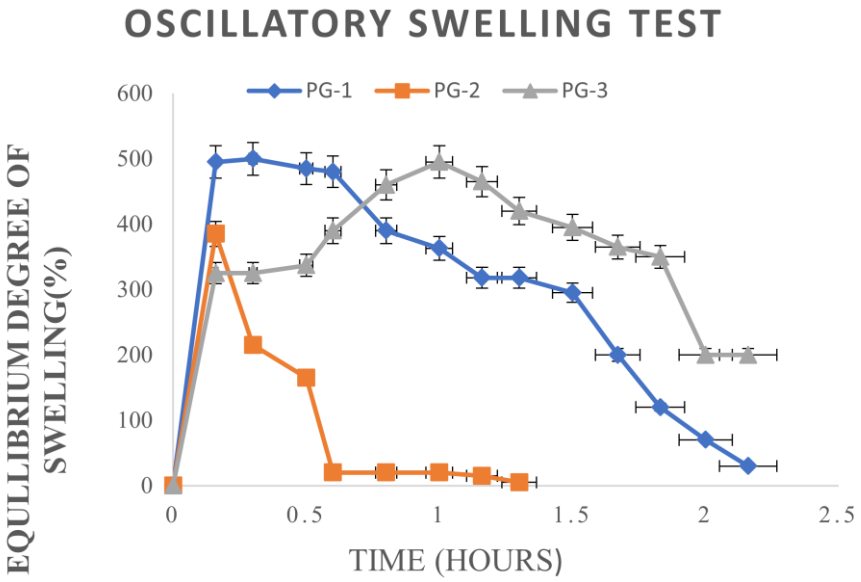

Figure 13. Variation in EDS of PVA/GE blends with time during Oscillatory swelling test.

absorbed polymeric films.

3.2. Swelling Kinetics

As seen from figure 16 a, b and c, for zero order kinetics and from Figure 16 d, e, and f, for first-order swelling kinetics, the regression coefficient is not nearing to one in either of the two swelling kinetics models. Thus, for more exhaustive results, the second order kinetics calculation was done to find out the exact kinetics followed by the optimized polymeric system. For extensive swelling of the polymers, the following second-order kinetics can be used:

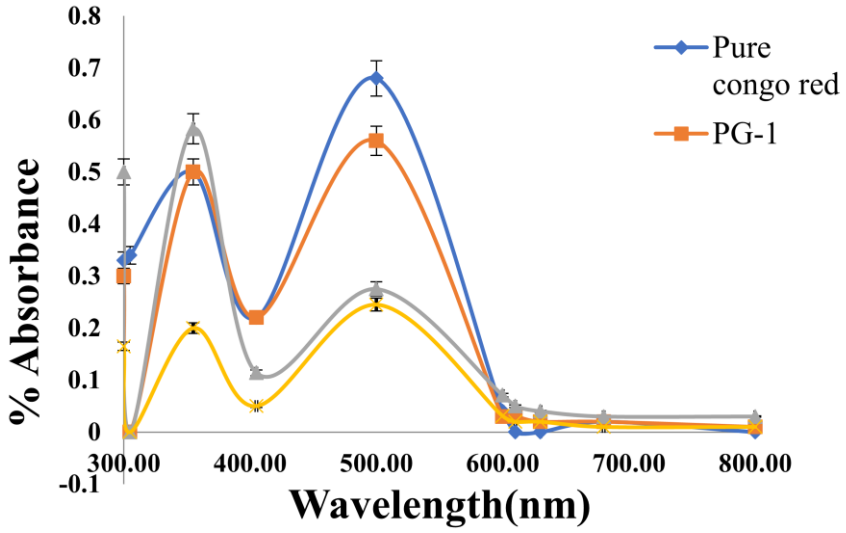

Figure 14. Plot of UV Absorbance of dye solutions with wavelength after swelling of polymer blends.

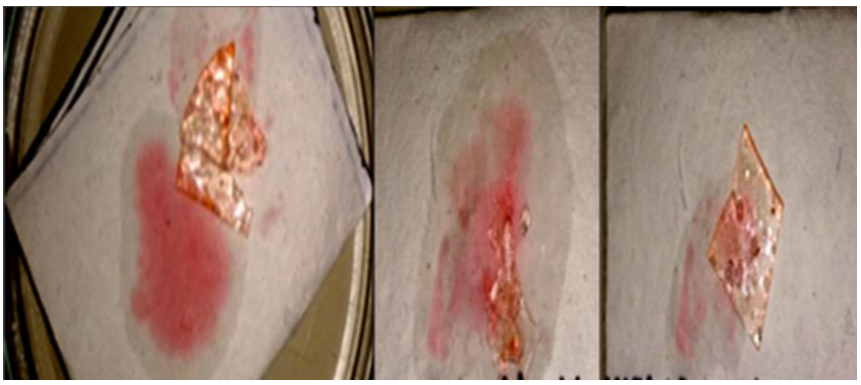

Figure 15. Photograph of dye absorbed PVA/GE films 50:50(PG-1), 40:60(PG-2) and 60:40(PG-3).

$$\frac{dW}{dt} = k\,(W\infty - W)^2 \text{---------- (9)}$$

Integrating the above Equation between the limits t=0 to t and w=0 to w, the following expression can be obtained:

$$\frac{t}{W} = \frac{1}{KW\infty} + \frac{1}{W\infty}t \text{--------- (10)}$$

All the mathematical kinetics which were so carried out for each individual ratio of blended crosslinked polymeric devices, PG-3 seem to best fit the second-order kinetics from the established fact that this particular polymeric blend has the best response towards all the stimuli-sensitive parameters as stated in

Table 1. Swelling rates (W_∞), correlation coefficient (R^2) and Swelling constants (K) of the Polymeric blend PG 3 under different conditions of swelling.

Parameters			
Second order kinetics	W∞(mg)	R^2	K
PG-3 water swelling test	97.087	0.9698	$5.99*10^{-4}$
PG-3 oscillatory swelling test	126.582	0.9966	0.069
PG-3 pH sensitivity (pH7.4)	1111.111	0.9962	$1.88*10^{-5}$

Table 1. Figure 16 g, h and i, show the second-order kinetic curves with respect to time of polymeric blends water-swelling test, oscillatory-swelling test and pH sensitivity at pH 7.4 respectively.

According to the equation no. 10, the swelling data must fit the straight line with the slope of 1/W∞ and an ordinate intercept of 1/KW2∞. The variation of t/W against time is plotted in figure 16g, h and i, for the so prepared crosslinked polymeric filmPG-3 and it was found that the swelling data for all the above gives a straight line. Therefore, the swelling behaviors of the best prepared polymeric blend PG-3 obey second-order kinetics which is according to other combination of Chitosan-g-poly (Acrylamide) which also follows second-order kinetics [29].

3.3. Analytical Characterizations

3.3.1. FTIR Spectroscopy

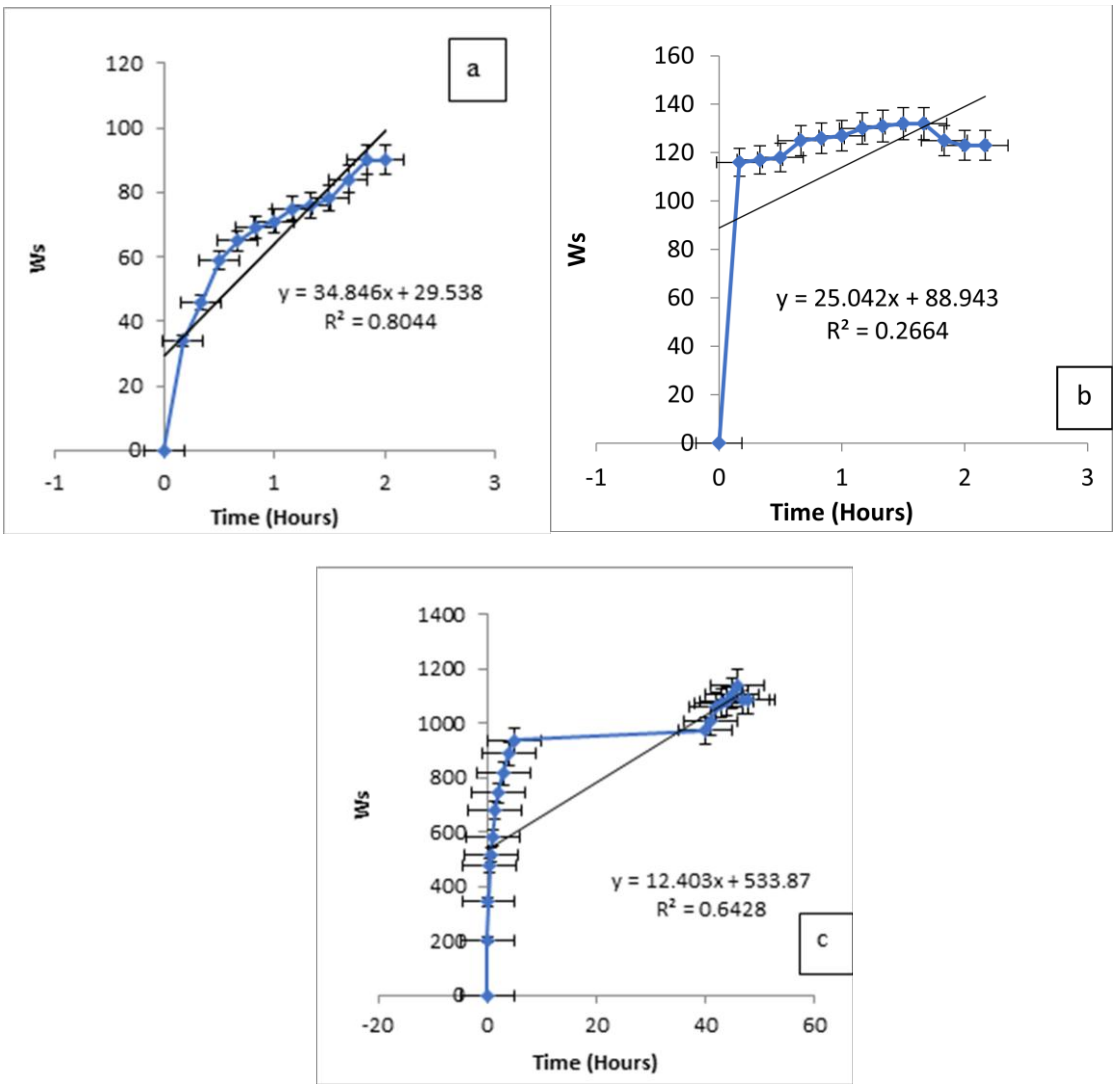

Figure16 a, b & c. Plot of Zero-Order Kinetics (W_s vs. Time) for PG-3 in (a) Swelling in water (b)Oscillato Swelling Test and (c) pH sensitivity test at pH7.4.

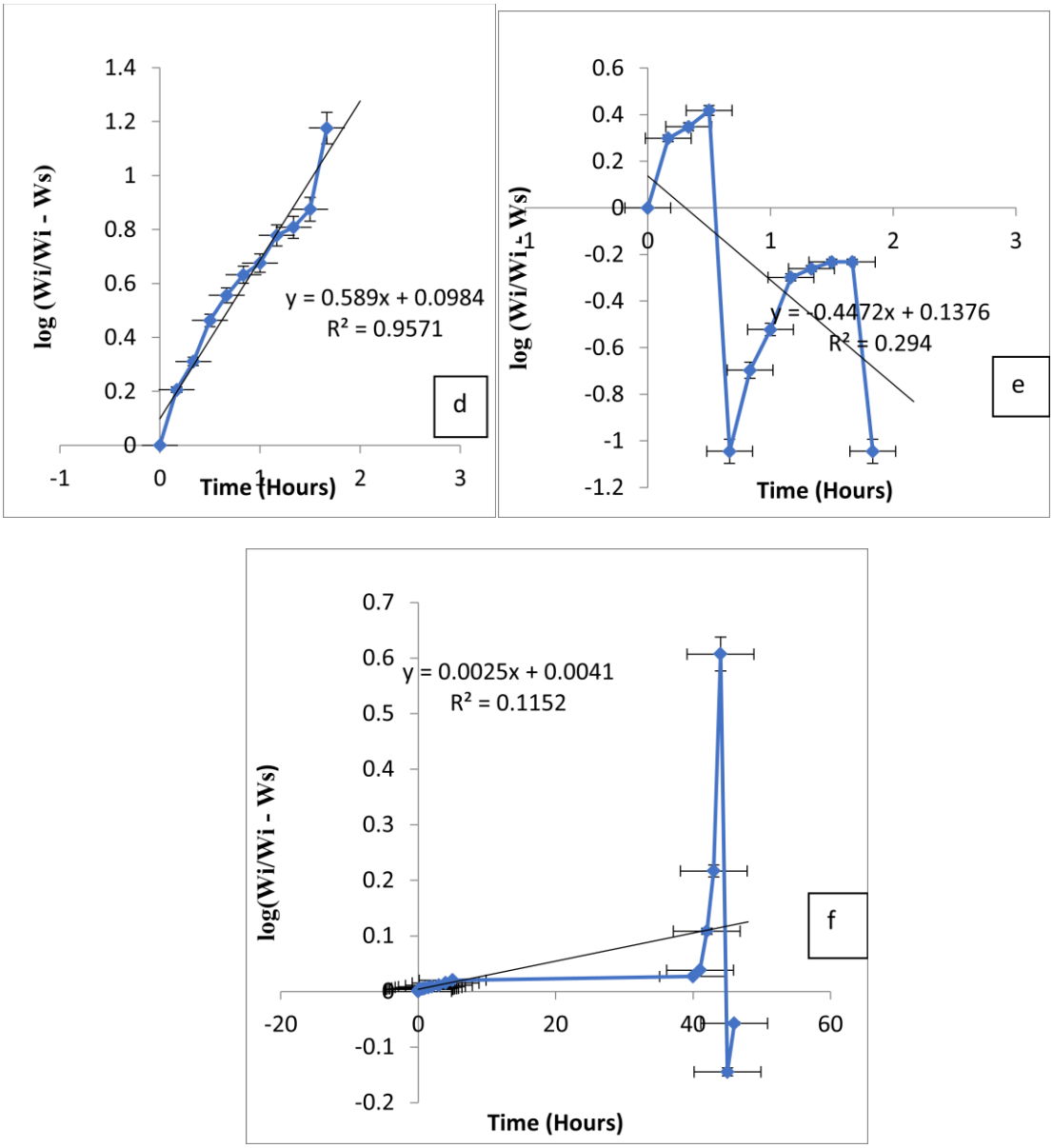

Figure 16 d, e & f. Plot of First-Order Kinetics (Log (Wi/Wi – Ws) vs. Time) for PG-3 in (d) Swelling in water (e) Oscillatory Swelling Test and (f) pH sensitivity test at pH7.4.

IR spectra of pure drug MTZ, physical mixture of pure drug with gelatin and poly (vinyl) alcohol (PM), uncross linked polymeric blend (PG-4), drug-loaded cross linked polymeric blend (PG-3), pure polymers poly (vinyl) alcohol (PVA) and gelatin (GE) are presented in figure 17. The spectra of drug metronidazole (MTZ) shows characteristics peaks of O-H intramolecular hydrogen bonding with stretching vibration at around 3221.23cm^{-1}, C-H (alkanes) stretching vibration at around 2958.90 and 2847.03cm^{-1}, C=N (amines) stretching vibration at around 1267.27cm-1. The other peaks such as N=O (nitro group) symmetrical and asymmetrical stretching vibration at around 1371.47cm^{-1} and 1535.39cm^{-1}. –CH2 (methylene group) bending vibration at around 1485.24cm^{-1}, =C-H (alkenes) stretching vibration at around 3101.64cm^{-1}. –CH3 (methyl group) bending vibration at around 1431.23cm^{-1}. C-N (tertiary

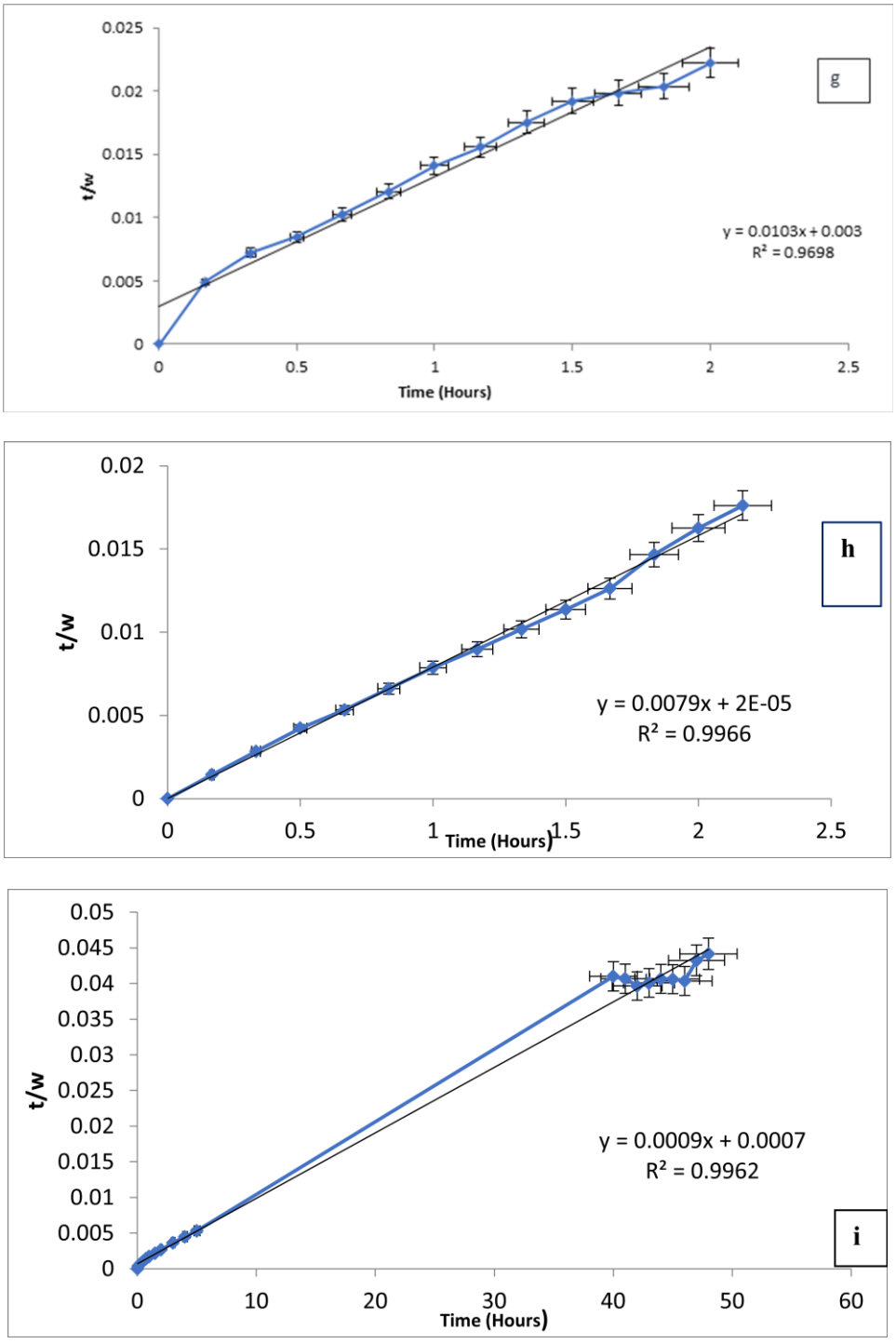

Figure 16 g, h & i. Plot of t/w vs. time for PG-3 in (g)Swelling in Water (h)Oscillatory Swelling Test and (i) pH sensitivity test at pH7.4pH sensitivity test at pH7.4.

amine group) stretching vibrations at around 1188.19cm^{-1}. C-C (alkanes) stretching vibration at around 907.54cm^{-1}, C=H (alkenes) bending vibration (out of plane) at

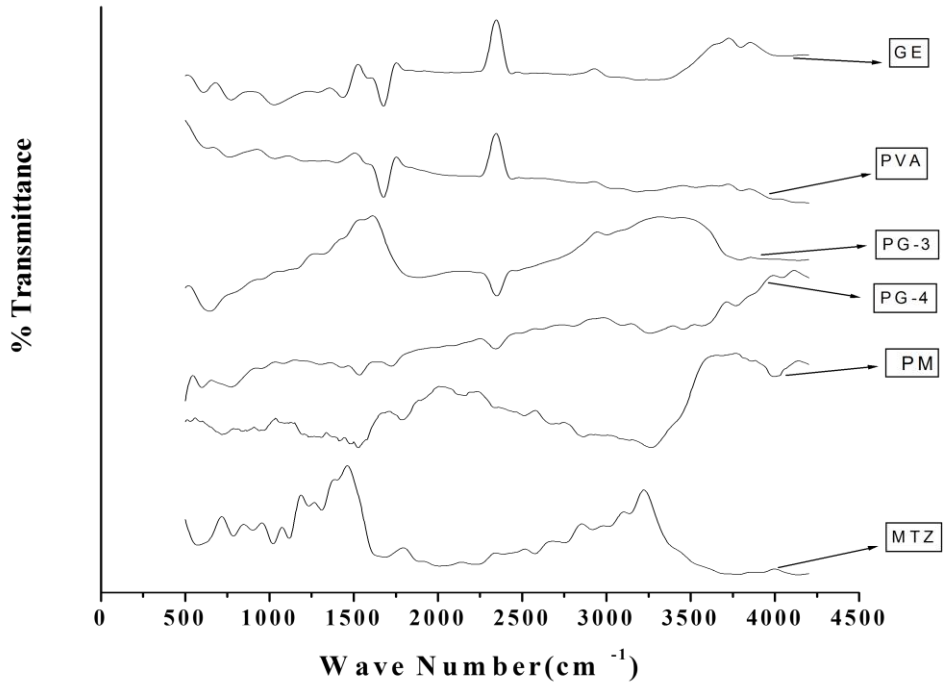

Figure 17. FTIR spectra of Pure drug (MTZ), blank un crosslinked sample (PG-4), drug-loaded crosslinked polymeric blend (PG-3), Physical mixture (PM), individual polymers (PVA; GE).

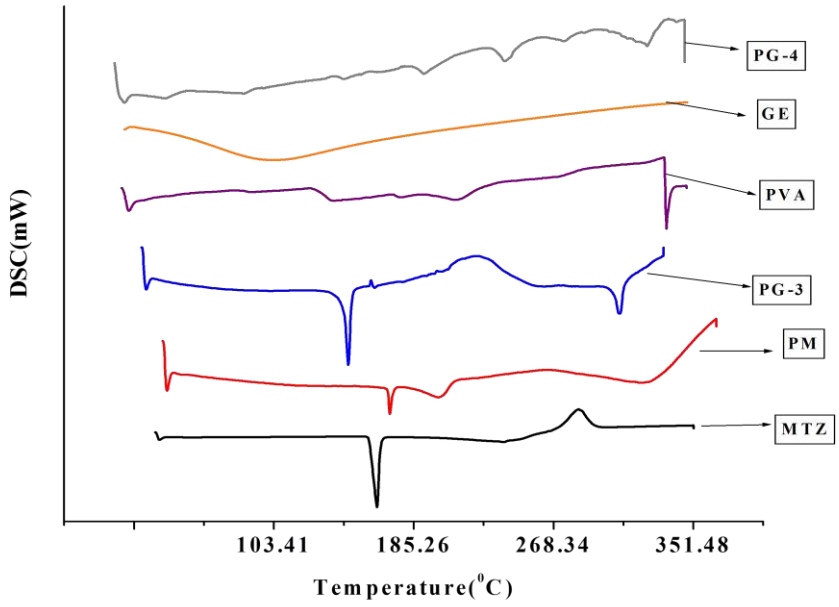

Figure 18. DSC thermogram of Pure drug (MTZ), Physical mixture of drug with polymers (PM), Drug load cross linked sample (PG-3), Poly(vinyl)alcohol (PVA), Gelatin (GE) and un crosslinked blank sample (PG-4).

around 1000- 647.14cm^{-1} and C-O group stretching vibration at around 1075.35cm^{-1} respectively were also noticed. Whereas in spectra of the physical mixture, PM, i.e.,

(PVA and Gelatin with the drug), the same characteristics peaks related to the drug were noticed with slight variations. This ruled out the drug-polymer interactions, hence the drug is stable in the formulation. FTIR spectra for individual polymers i.e., Poly (vinyl alcohol) and Gelatin, as well as that of the PG-3, exhibits bands at 2928cm^{-1}and 1537cm^{-1}corresponding to carboxylic and amine groups. The IR spectra of the uncrosslinked blank PG-4 sample show almost the same spectra as that present in both the polymers. FTIR spectral analysis of drug loaded crosslinked polymeric blend PG-3 was compared with that of MTZ and it was found that the characteristic peak of the drug was prominent in the drug-loaded film. PG-3 showed O-H with stretching vibration at around 2928.01cm^{-1}, N-H with bending vibration at around 1658.81cm^{-1}, CC with stretching vibration at around 1510.31cm^{-1}, C-O with stretching vibration at around 1108.08cm^{-1}, C-N with stretching vibration at around 1251.83cm^{-1}and same peaks are also seen in case of PG-3. The occurrence of the additional peaks may be co-related to that of the peaks of the pure drug metronidazole, such as the presence of O-H intramolecular hydrogen bond with stretching vibration at around 3221.23cm^{-1}, N=O (nitro group) symmetrical and asymmetrical stretching vibration at around 1371.47 and 1535.39cm^{-1}, C-O group stretching vibration at around 1075.35cm^{-1} and may be an indicator of crosslinking. Thus, the characteristic peaks of the drug are retained in both of physical mixture as well as the crosslinked polymeric entity indicating that the drug is mutually compatible.

3.3.2. Thermal Analysis

As shown in figure 18, the DSC thermogram of PG-4, the uncrosslinked sample, shows no such sharp peak indicating the absence of cross-linking. The thermogram of pure drug MTZ shows peak at 162.41^{0}C, whereas the DSC thermogram of drug loaded PG-3, shows two sharp peaks at 159.95^{0}C and 324.74^{0}C indicating the drug entrapment within the crosslinked polymeric matrix and the shift in the endothermic peak towards the higher temperature indicates the formation of more rigid polymeric network due to chain entanglements of PVA and GEL [25]. Since there is no change in the endothermic peak of the pure drug within the polymeric system, this indicates that the crosslinked polymeric network does not chemically interact with the drug [30] and they are compatible. Separately, the thermograms of the pure polymer PVA and GEL shows that there are no significant degradation peaks. The thermogram of the physical mixture (which contains drug and pure polymers), shows that the endothermic peak of the pure drug is retained in the mixture. This confirms that the polymers even in their physical state also have no chemical interaction with the drug and are therefore mutually compatible.

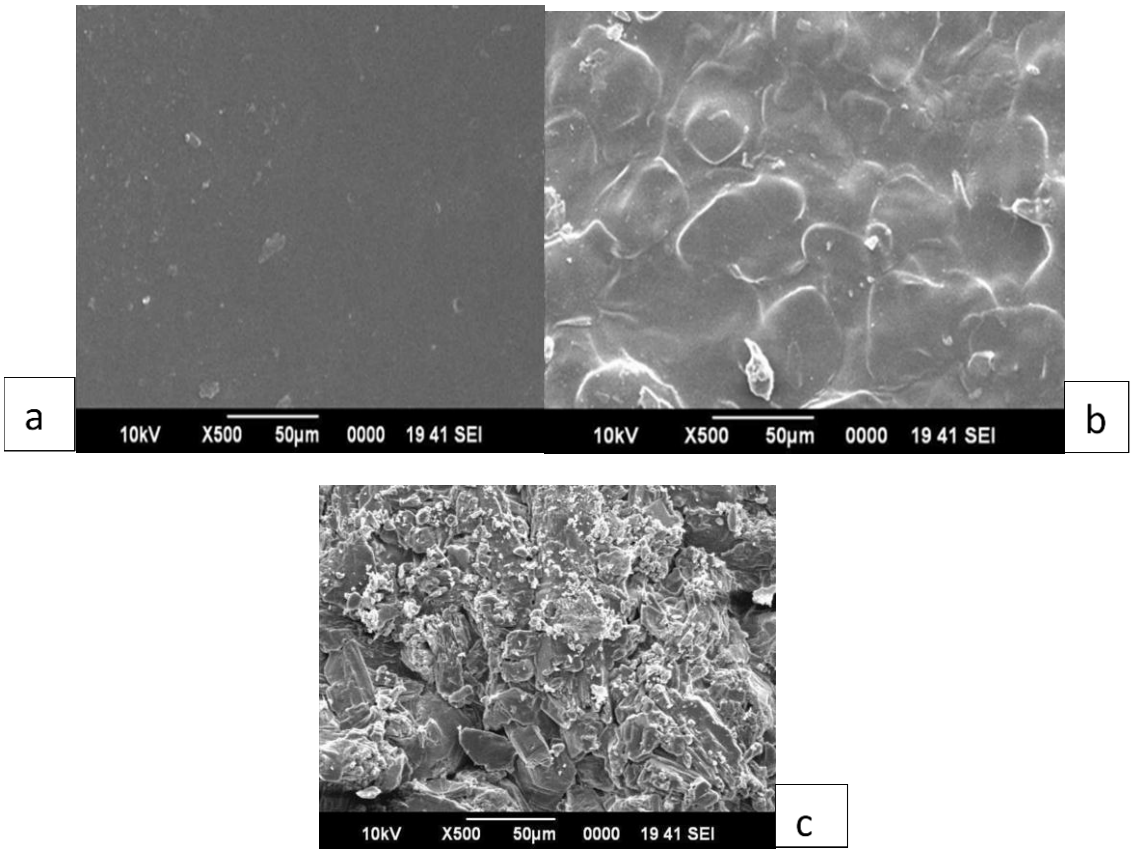

Figure 19. SEM micrographs of the surface sections of PVA/GE crosslinked polymeric blend (a) blank un crosslinked sample before freeze-thaw cycles (PG-4) (b) Blank crosslinked sample after freeze-thaw cycles (c Crosslinked drug loaded sample (PG-3).

Figure 20. Biodegradability images of PG-3 - (a) Control; (b) 1st day; (c)7th day and (d)14th day.

3.3.3. Surface Morphology by Scanning Electron Microscope (SEM)

In applied Freezing-thawing process, the structures of the PVA/GE polymeric films were determined by SEM. Figure 19 (a) and (b) show the scanning electron micrographs of transversal sections of the films before and after freeze-thaw cycles. Dual phase

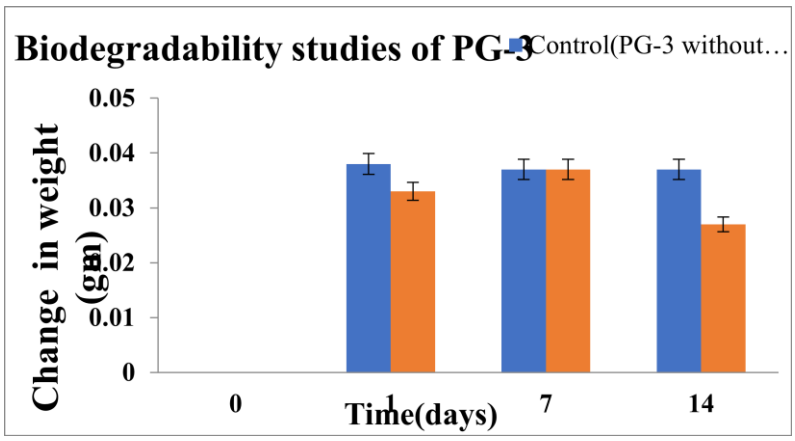

Figure 21. Change in weight of PG-3 in presence of microbes (Test) and in absence of microbes (Control).

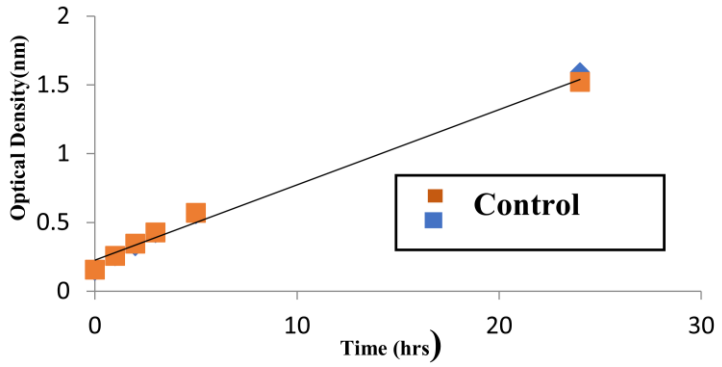

Figure 22. Bactericidal effect of the test sample (PG-3 in presence of bacterial cell suspension) and control sample (Pure mother culture) at different time intervals respectively.

morphology of PVA/GE blend is visible after carrying out freeze-thaw cycles (FTCs). These may be attributed to the formation of PVA cross-linked structure through hydrogen bonding during FTC. However, very small dispersed domains are visible for the uncrosslinked PVA/GE blend film suggesting miscibility of the two components. From figure 18 (c), it can be assumed that there is deposition of crystals within the polymeric matrix which are mainly attributed to the loading of the drug MTZ.

3.3.4. Biodegradability and Bactericidal Studies

The increase in initial weight of PG-3 on day 1 was mainly due to the soaking of water from the media. But decrease in the weight of the film for the rest of the 14 consecutive days clearly indicates the accessibility of the microorganism (*B. subtilis*) to the film i.e., degradation of the test film by the bacteria as

Table 2. Biodegradability test of Interpenetrating polymeric network film PG-3.

No. of Days	Weight of the sample (gm)	
	Control	**Test(T)**
1	0.038	0.033
7	0.037	0.037
14	0.037	0.027

Table 3. Percentage (%) Drug entrapment efficiency in the optimized film PG-3.

PG-3	**Mass of the drug in the film (W_s-W_d) (mg)**	**Theoretical mass of the drug(mg)**	**Drug entrapment (%)**
1	4.0	50	8.0
2	7.67	50	15.34
3	6.0	50	12.0
			Mean=11.78±3.67

shown in figure 20, figure 21 and table 2. No significant weight variations were notices for the film marked as Control.

Figure 22, explains the Bactericidal effect on the polymeric film PG-3. No significant changes in the optical density of bacterial culture were observed at ƛmax 600nm for Test sample (containing PG-3 with bacterial cell suspension) and Control sample (pure mother culture of bacterial cell suspension), which confirms that PG-3 does not inhibit bacterial growth [25].

3.4. Drug Loading Efficiency

Percentage (%) drug loading efficiency in the optimized film PG-3 was also calculated using the equation discussed in methodology and is shown in table 3.

So as from Table 3, the percent drug entrapment in the prepared device is less. This is expected due to the hydrophilic nature of drug MTZ possessing better compatibility with hydrophilic polymeric matrix [31].

3.5. In vitro Drug Release

Invitro release study was performed with PG-3 at pH 7.4. On exposure to aqueous fluid, hydrophilic matrices take up water and polymer starts hydrating to form a gel layer. A maximum of 98.253%± 0.363 and 92.248%±0.244 as shown in Figure 20, drug release occurred from the crosslinked polymeric device. Moreover, 50% of the drug released at the end of 2-3h high and this mainly depends upon the extent of cross-linking, the amount of drug loaded and concentrations of either PVA or GEL. With the increase in GE concentration, the drug release through the membranes was decreased, may be due to the formation of the dense network, which in turn decreases the drug release rate in the later hours [32]. An initial burst of soluble drug may have occurred due to surface leaching when a

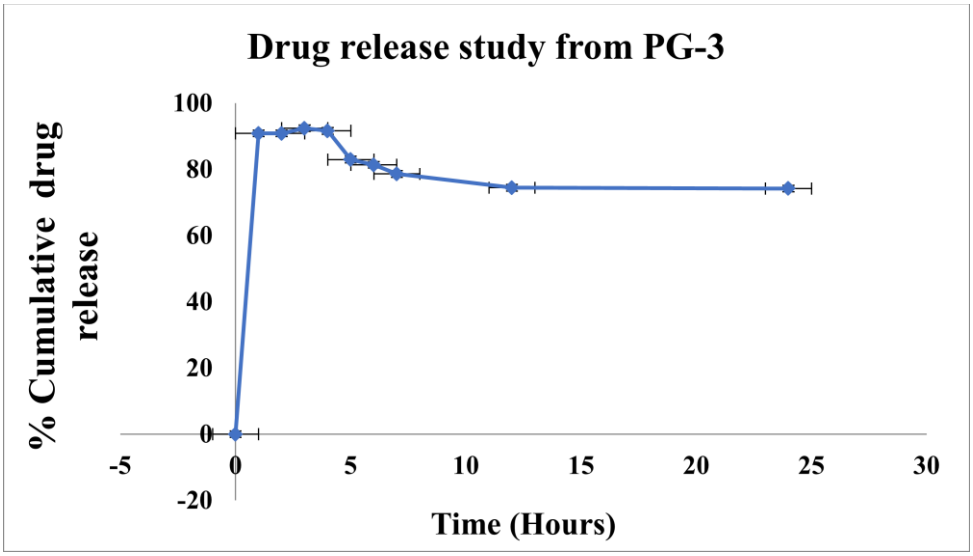

Figure 23. Drug release profile of PG-3 at pH7.4.

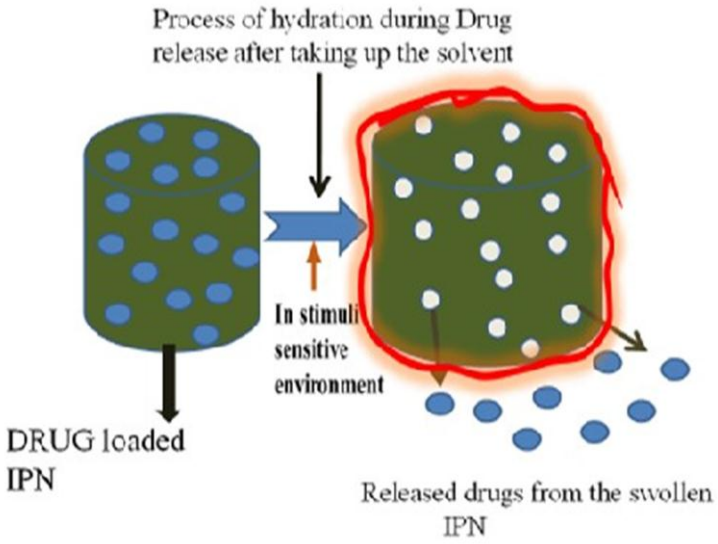

Figure 24. Drug release mechanism from the swollen Interpenetrating Polymeric Network.

matrix containing a swellable glassy polymer comes in contact with an aqueous medium, there is an abrupt change from a glassy to a rubbery state which is associated with swelling process with time, water infiltrator deep into the case increasing the thickness by the gel layer. Subsequently, the outer layer becomes fully hydrated and starts dissolving or eroding. When water reaches the center of the system and the concentration of the drug falls below the solubility value, the release rate of the drug begins to reduce. At the same

Table 4. Release kinetics modeling data.

Parameters	Values
R^2	0.902
n	0.131
Mechanism of drug release	KorsmeyerPeppas(Fickian)

time, an increase in thickness of the barrier layer with time increases the diffusion path length, reducing the rate of drug release [33]. The drug release data was found to be best fitted to Korsmeyer-Peppas release model indicating Fickian diffusion [34-39] as shown in figure 23 and table 4. Also, a scheme for the drug release from the stimuli-sensitive devices after being introduced in a particular medium is given in figure 24.

4. Conclusion

In the present work, an attempt has been made to study the Stimuli-sensitive Parameters of crosslinked polymeric blend polymeric films prepared by employing two polymers PVA and Gelatin using Freeze thaw of 30cycles as the physical cross-linking method.

- The blend containing the highest percentage of PVA (PG-3) showed a maximum degree of swelling in deionized water may be due to the ionization of gelatin and poor amorphousness of PVA through the inclusion of gelatin in its matrix.
- The polymeric films blended in the ratio 60:40(PG-3), with a greater concentration of gelatin show considerable sensitivity to alkaline solution i.e., pH=7.4 and pH=9.2 compared to that of the acidic medium i.e., pH=1.2.
- In case of temperature sensitivity test, the polymeric blend so prepared with the higher concentration of gelatin i.e., 40:60 (PG-2) disrupted after certain interval of time (≤ 2.5 hours) which may be accounted due to the breakdown of the hydrophilic/hydrophobic balance between the networks with increase in time or due to the immediate disintegration of the polymeric film at 250C due to ionic-dissociation. However, the blend with a lower concentration of gelatin 60:40 (PG-3) showed resistance even after the increase in temperature up to 370C.
- Ionic Swelling Test was also carried out for each of the individual samples i.e., PG-1 (50:50), PG-2 (40:60) and PG-3 (60:40). It was evident from the respective graphs that with the increase in the concentration of Sodium Chloride, there was a uniform drop in the swelling ratio as well. The reason being the presence of counterions at high ionic strength, which hindered the ionization of the amino group. The addition of neutral salt (NaCl) to gelatin solution increases the ionization of gelatin, i.e., that the gelatin, therefore, combines with the neutral salt to produce highly dissociated complexes.
- Oscillatory Test of the individual samples i.e., PG-1 (50:50), PG-2 (40:60) and PG-3 (60:40) were carried out which confirms that the polymeric film blended in the ratio PG-3

(60:40) was able to respond quickly to the pH pulses compared to the other two blended ratios PG-1(50:50) and PG-2(40:60) respectively and also showing superior curve for a prolonged period of time which may be attributed due to the formation of effective networks, achieved when was subjected to repeated freeze-thaw (30cycles) for a period of 30days.

- In case of Dye absorption test, all the polymeric blends so prepared were found to act as a potential purifying agent for waste contaminated with dye. The polymeric film blended in the ratio 60:40 (PG-3) has the better low absorbance curve compared to 50:50 (PG-1) and 40:60 (PG-2). This may be attributed due to the formation of dense network matrixes due to the presence of higher concentration of PVA.
- The swelling kinetics was calculated for each polymeric blend blended in the ratio 50:50 (PG-1), 40:60 (PG-2) and 60:40 (PG-3). The results showed that the polymeric blend 60:40 (PG-3) to follow a second-order kinetics with almost straight line and the data were also found to be close to the fitted regression line i.e., (R^2 = 0.9 - 1) in all the cases of swelling aspects (water-swelling test, oscillatory-swelling test and swelling test (pH 7.4) respectively.
- The scanning electron microscopic studies were also carried out for the cross-linked polymeric blend 60:40 (PG-3) and uncrosslinked polymeric sample (PG-4). The transverse section of the polymeric blend 60:40 showed a change in the network formation when compared to the uncross-linked sample due to the repeated freeze-thaw cycles (30days).
- Differential Scanning Calorimetric studies of the crosslinked sample PG-3 and the uncross-linked sample PG-4 reveals the occurrence of the sharp endothermic peaks in case of PG-3 due to the presence of the crosslinking and such sharp peaks are absent in case of PG-4.

Therefore, from the results obtained so far indicates that each polymeric network can retain its properties in the crosslinked systems. In addition to PVA/GE hydrogels can be used as a great water retainer for carrying some substances in aquatic fields in the pharmaceutical, agricultural, environment, and biomedical applications, or in the applications of immobilized biologically active molecules.

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