 doi:10.20944/preprints202009.0510.v1

Targeted Delivery of Methotrexate through pH-sensitive Hydrogel: to treat Colon Pathology

Muhammad Akhlaq[1], Inam Ullah, Asif Nawaz[1], Muhammad Safdar[1], Abul Kalam Azad[2], Syed Atif Abbas[3] and ABM Helal Uddin[4]

[1] Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan. (dr.akhlaq@gu.edu.pk; asifnawaz676@gmail.com; safdarlaghari10@gmail.com)
[2] Advanced Drug Delivery Laboratory, Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan 25200, Malaysia. (azad2011iium@gmail.com)
[3] Faculty of Pharmacy, Quest International University Perak, Malaysia.(syed.atif@qiup.edu.my)
[4] Analytical and Bioanalytical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia, Kuantan 25200, Malaysia. (abmhelal@iium.edu.my)
*Correspondence: azad2011iium@gmail.com; syed.atif@qiup.edu.my; abmhelal@iium.edu.my; Tel.: +60-1116754350.

Abstract

The purpose of current research work was to formulate and typify gelatin and poly(vinyl) alcohol **(**Gel/PVA) hydrogel which would be highly pH-responsive and can able to accomplish targeted delivery of methotrexate in order to treat the colo-rectal pathologies. The primed gel/pva hydrogel discs were subjected to various physicochemical techniques i.e. swelling, diffusion co-efficient, sol-gel analysis and porosity using three altered sorts of pH (1.2, 6.8 & 7.4) phosphate buffer solutions for assessment/evaluation, and their characterization was done through Fourier transform infrared spectroscopy (FTIR) and thermal gravimetric analysis (TGA). Shape alteration and controlled methotrexate of release of Gel/PVA hydrogel have been done using three type of pH (1.2, 6.8 & 7.4) phosphate buffer mediums. Methotrexate was loaded through *in-situ* drug loading method due to hydrophobicity. Different kinetic models (first order & zero order kinetic), Higuchi model and Krosmere peppas model/Power law were applied to manipulate the drug release data. Physicochemical evaluation tests and drug release profile results were found insignificant ($p< 0.05$) in various pH mediums and dependent upon polymers concentration pH of medium and cross-linker amount. Kinetic model disclosed that release of methotrexate from Gel/PVA hydrogel follow non-Fickian diffusion method. It became concluded from this research work that release of methotrexate Gel/PVA hydrogel in targeted colon area can be achieved for treating colo-rectal disorders.

Keywords: Hydrogel, pH-responsive, colon, targeted delivery, methotrexate

 doi:10.20944/preprints202009.0510.v1

1. Introduction

Chronic diarrhea, chronic inflammatory bowel disease, crohn's disease and ulcerative colitis, may leads to more severe case i.e. cancer called colon cancer or colorectal cancer. Every year above ten lac/one million cases of the colo-rectal cancer are newly diagnosed in worldwide [1]. Colitis associated with cancer is a subtype of colo-rectal cancer that is associated with the IBD, which is difficult to treat, and high mortality rate. Among the IBD patients more than 20% of them may get colo-rectal cancer within 30 years of the onset of the disease, and of them 50% die due this CRC [2]. There are some diseases like Crohn's disease, colorectal cancer, amebiasis, and ulcerative colitis, which are related to colon that can be treated by providing a large concentration of that specific drug to the colon [3]. In order to deliver the drug moiety to targeted site, proximal area of the colon is most suitable [4]. Along with formulations being used and developing new drug moiety, researchers are doing efforts also to prepare such dosage formulations that can overcome the drawbacks of the currently available dosage formulations in market and fulfill the need of targeted delivery. Among such formulation's hydrogels are most popular dosage form in pharmaceutical and biomedical fields [5]. So various stimuli like pH [6], temperature [7], light [8], enzyme [9] and glucose [10] etc., sensitive hydrogels are prepared to meet the need of the targeted delivery of the drug to the targeted region of the colon through by-passing the harsh acidic environment of the stomach [11]. These hydrogel formulations having stimulus sensitivity properties totally dependent on their crosslinking materials and polymers. This system mostly depends on the cross-linkers and polymers types; from which it is formulated. They are usually sensitive to the external stimulus like pH and temperature of the biological medium or physiological fluid [12]. The hydrogel in other words are also called as intelligent or smart gel [13]. They are actually three-dimensional structures, which have polymeric networking and are hydrophilic in nature [14].

Methotrexate is {(2S)-2-[[4-[(2,4-diaminopteridin-6-yl)-methyl-ethylamino] benzoyl] amino] pentanedioic acid)}, antagonist to folic acid, by inhibiting enzyme dihydrofolate reductase thus blocks conversion of dihydrofolate to tetrahydrofolate and thus stop DNA synthesis in the tumor cell [15]. Developed in 1948, clinically first used in leukemia, also in treatment of autoimmunological disorders and various type of cancer [16], also used in psoriasis, cronh's disorder, (IBDs) irritable/ inflammatory bowel disease, ulcerative colitis (UC), rheumatoid arthritis (RA), and wegeners disease for nearly 60 years [17]. Used in treatment of lymphoma, leukemia [18], head and neck cancer [19], osteogenic sarcoma [20], choricarcinoma [21], like rheumatoid arthritis [22], psoriasis [23], drug of choice for curative treatment of acute lymphocytic leukemia in children [24], prevention of occurrence of graft-versus host diseases after organ transplantation in human body [25]. The purpose of drug targeting to the desired organ system (area) of the body is not to deliver a drug only for increasing its therapeutic effects there, but also to lower its toxic effects in low dose range during that therapy [26] and improve the efficacy and reduce the side effects [27]. Due to various reasons the drug targeting to specific organ or tissue is required, such as to save/protect the drug moiety from the harsh environment, to minimize the adverse effect and improve the therapeutic effect of the drug in that targeted organ and to protect also the other organs or tissues from the harmful/adverse effect of that targeted drug substance [28].

In the present research work, the drug methotrexate was loaded on gelatin and polyvinyl alcohol hybrid hydrogel in order to deliver drug to the specific target area in controlled manner. For this purpose, first of all Gel/ PVA hybrid hydrogel samples with different polymer concentrations were prepared, then methotrexate loaded samples were prepared and subjected to different physicochemical evaluation test and *in-vitro* methotrexate release profile. No previous study has been done on loading of methotrexate on Gelatin/PVA hybrid hydrogel. The gelatin/PVA hydrogel samples might have goodness in sense of methotrexate release in controlled manner at targeted site following zero order kinetic. The method of drug loading (*in-situ* method) become advantageous for the drugs which are hydrophobic (water insoluble) like methotrexate.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

2. Materials and methods

2.1. Materials

Active methotrexate was received as a gift sample from Wilson's Pharma. Polyvinyl Alcohol (PVA) & Gelatin (Fluka Biochemika) were used as polymers. Glutaraldehyde (Sigma Aldrich, Germany) used as cross-linker, Hydrochloric acid (Sigma Aldrich, Germany), potassium phosphate monobasic (Sigma Aldrich, Germany) and potassium chloride (Sigma Aldrich, Germany), Sodium Hydroxide (Icon Chemicals) and distal water (Merck, Germany), all these chemicals were of analytical grade and provided by Department of Pharmaceutics, Faculty of Pharmacy, Gomal University, D.I Khan, Khyber Pakhtunkhwa, Pakistan.

2.2. Methods

2.2.1. Preparation of Gel/PVA hybrid hydrogel

For preparation of Gel/PVA hybrid hydrogel, two separate solutions (A & B) were prepared with slight modifications [29]. For preparation of solution 'A', a weighed amount (1.5, 2 & 2.5 gm) of polymer "poly vinyl alcohol" added in 20ml distal water in a 50ml beaker along with magnet bar and placed that on a magnetic stirrer. The polymer and solvent mixture then heated from 60˚C to 70˚C up to 30 minutes with continuous stirring at 200 rpm, until a clear solution formed. The final volume was made by adding specific quantity of distal water. When a clear solution prepared on complete dissolution of polyvinyl alcohol, then it was placed aside from stirrer. Then to prepare solution 'B', a weighed amount of polymer "gelatin" (9, 8, 7.5 & 7 gm) was added in 25ml distilled water in separate 50ml beaker along with magnet bar and placed that on a magnetic stirrer. The polymer and solvent mixture were then heated from 30˚C to 35˚C for 25 to 30 minutes with continuous stirring at 150 to 200 rpm, until a clear solution was prepared. The final volume was made by adding specific amount of distal water. Then the two solutions A & B were mixed in such a way, that solution "B" was added drop wise into solution "A" with continuous stirring at 150-200 rpm up to 25-35 minutes at 30˚C for complete mixing of the two solutions. A total of nine (n=9) formulations were prepared, among which in three of them glutaraldehyde was added in drop wise manner as 2, 3 & 4 drops. When hybrid gel formulations of Gelatin/ PVA polymers were prepared with various concentration as planned, then they are poured in test tubes and placed at room temperature during daytime and at about 8˚C during night for 5 days until the hydrogel was congealed completely. Then the congealed gels were removed from test tubes and then cut into 5 mm discs. Such discs were placed in petri dishes and again they were placed at room temperature and again at 25˚C re-dried in oven for few hours until they got constant weight.

2.2.2. Swelling studies (dynamic and equilibrium swelling)

The dried hydrogel discs are first weighed and then they are dropped into buffer medium of varying pH 1.2, pH 6.8 and pH 7.4. They got swelled. This swelling was studied in two different forms i.e. dynamic swelling and equilibrium swelling [30].

Accurately weighed dried hydrogel discs were immersed in buffer solution of pH 1.2, 6.8 & 7.4, they got swelled and then they were reweighed after specific interval of time of 0.5, 1, 1.5, 3, 5, 6, 7, and 8 hours. The dynamic selling at each specific interval of time was determined according to the following equation:

$$S = \frac{Ww}{Wd} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. eq. 1$$

"S" represents dynamic swelling, "Wd" represent the weight of the dry disc & "Ww" represent the weight of the wet disc.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

For getting average dynamic swelling the sum of the total number of dynamic swelling values were divided by the whole number of formulation sampling. The following equation (2) was used.

$$S(Avg) = \frac{S1 + S2 + \cdots \ldots \ldots \ldots \ldots \ldots \ldots \ldots + S8}{Total\ number\ of\ samples} \ldots \ldots . eq. 2$$

"S(Avg)" represents average dynamic swelling.

After completion of dynamic swelling study, the already immersed hydrogel discs of Gel/PVA were left in buffer medium, mostly about 24-hours, until they attained constant/equilibrium weight. The following equation (3) was used.

$$S(Eq) = \frac{We}{Wd} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . eq. 3$$

"S(Eq)" represent the equilibrium swelling, "We" represent the weight of swelled discs after 24 hours swelling, "Wd" represents the weight of the dry disc before swelling.

2.2.3. Diffusion Co-Efficient

Diffusion co-efficient represent the quantity of the solvent that diffuses through a unit area of the gel in unit time through concentration gradient [31]. Following parameters depends on the partial mobility of the solvent, that can be determined by the following equation (4):

$$D = \pi[\frac{h\theta}{4} . q(eq)]^2 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . \ldots \ldots eq. 4$$

"D" represents the diffusion co-efficient, "$q_{(eq)}$" represents the equilibrium swelling of hydrogel disc, "θ" represent slope of linear part of the swelling curve while "h" represents the height (distance between the top and bottom) or thickness of the hydrogel discs.

2.2.4. Sol-Gel Analysis

Sol-gel is the production of solid substances from small sol molecules. Sol acts as a predecessor for the preparation of the gel. Actually, by this method we determine the % gel fraction and % sol fraction of the materials. In this method we take discs and weighed them and then placed that disc in tap water in a beaker and leave them for 72 hours. Then after 72 hours we remove that vary discs from beakers and placed them in open air at room temperature for few days until they dried completely. Then they were weighed again, and then the % sol fraction and % gel fraction were determined with the following equations (5 & 6) [32].

$$Sol\ fraction(\%) = \frac{W_1 - W_2}{W_1} \times 100 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots . \ldots eq. 5$$

$$Gel\ fraction(\%) = 100 - Gel\ fraction \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots eq. 6$$

"W_1" represent weight of disc prior to immersion in the water and "W_2" represents the weight of the disc after 72 hours when dried again after removal from water.

2.2.5. Porosity Measurement

For determination of porosity of Gel/PVA hydrogel discs, each disc was measured before immersion and then each one was immersed in absolute ethanol for 24 hours. After that each disc was removed and excess

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

ethanol solvent present on the surface was removed with help of tissue paper and then weighed again. The % porosity of each disc was determined by applying following equation (7) [33].

$$\%Porosity = \frac{M_2 - M_1}{pV} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots eq.7$$

"M_2" represents the mass of the disc after removal from ethanol solvent, "M_1" represents the mass of the disc before immersion in the ethanol solvent, "ϱ" represents the density of the absolute ethanol and "V" represents the volume of the hydrogel disc.

2.2.6. Method of Drug Loading (*In-situ* method)

To load a drug on the discs of the Gel/PVA hydrogel, *In-situ* drug loading method was followed. In this method Gelatin/PVA hybrid hydrogel solutions were prepared in different concentration and then the drug (methotrexate) was added in each solution and was stirred on a magnetic stirrer for 40 minutes. Then drug loaded formulations were congealed, removed, cut in discs and dried with same procedure as used for drug un-loaded formulations.

2.2.7. Drug Release

In order to determine the release of the drug from the hydrogel discs of varying formulations, the dissolution apparatus of paddle method (Pharma-Test, Hamburg, Germany) and UV-VIS spectrophotometer (SHIMADZU UV-1800) were used. First, 0.2M USP buffer solutions of different pH (1.2, 6.8 & 7.4) were prepared. Then the release of drug was checked in each buffer for 12 hours. During dissolution process, 500ml buffer medium was added in each/every flask/cup of dissolution apparatus and then each disc was immersed in each flask. The paddle rotation method was adopted. The speed of the paddle rotation was set at 100rpm and temperature was kept up to about 37°C of the dissolution apparatus. Then at specific time interval (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12 hours) about 5ml of the sample was withdrawn from each flask and then fresh 5ml same buffer solution was added in that flask to maintain the total 500ml volume of the flask constant. The drug release was determined by UV-VIS spectrophotometer at λmax of 325, at which the methotrexate showed maximum absorbance. All the experiments were carried out in triplicate, mean ± standard deviation of each sample was taken.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded between 4000–400 cm−1, with a resolution of 1 cm−1 with ambient air as a background using PerkinElmer Spectrum 100 spectrophotometer (Perkin Elmer Corp., Norwalk, CT, USA). The spectra were processed using the SpectraGryph 1.2 spectroscopy software [34].

2.3.2. Thermal gravimetric analysis (TGA)

Both polymers (gelatin and polyvinyl alcohol), methotrexate & hydrogel both (loaded & unloaded) were conducted.

2.4. Statistical Analysis

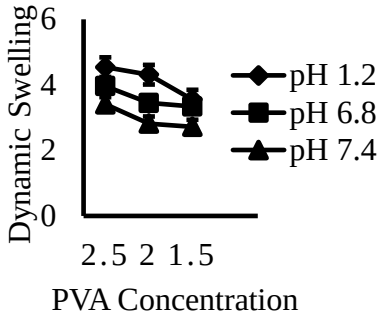
Through SPSS of version 20 the analysis was performed. During statistical analysis mean and standard deviation of all the formulations/samples were done/calculated. These calculations were performed in triplicate. ANOVA and t-test were also applied. P-values occurred less than 0.01 of a result were considered of significant difference [28].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

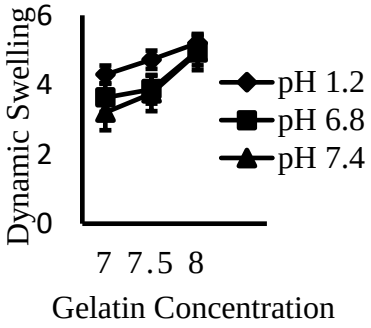
3. Results

3.1. Swelling study results

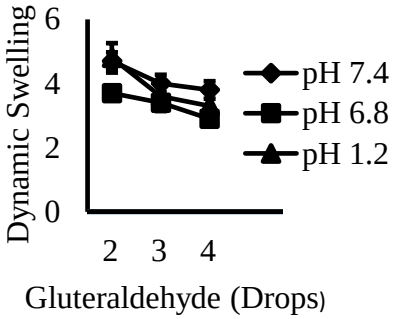
Both dynamic and equilibrium swelling study results were found insignificant (p<0.05) because in both acidic and basic mediums of phosphate buffer they showed swelling but having different affinities with varying solvent's pH. From sample A1 to A3 the concentration of gelatin was kept constant while concentration of polyvinyl alcohol was varied as (1.5gm, 2gm & 2.5gm) and their formulations were evaluated in order to determine the effect of decreasing concentration of the polyvinyl alcohol on swelling. Sample A1 showed increased dynamic swelling 4.54 ± 0.23 at pH 1.2, while 3.41 ± 0.10 at pH 7.4 and highest equilibrium swelling 9.35 ± 0.35 at pH 1.2 as compare to A2 and A3. Sample A2 showed highest equilibrium swelling 9.10 ± 0.27 at pH 1.2, while minimum at pH 7.4. Sample A3 showed minimum dynamic and equilibrium swelling at all pH as compare to A1 and A2 with decreasing the polyvinyl alcohol concentration and vice versa. The swelling ratio was as A1>A2>A3 as shown in graph (a) of figure 1. The same result was found with polymer gelatin. When PVA concentration maintained constant and gelatin concentration increased gradually from sample A4 to sample A6, there occurred increase in swelling profile of the Gel/ PVA hydrogel samples. With increased gelatin concentration sample A6 showed maximum both equilibrium swelling 10.09 ± 0.18 and dynamic swelling 5.20 ± 0.13 at all pH as compared to both sample A5 and A4. The swelling ratio was as A4<A5<A6 shown in graph (b) of figure 1. From sample A7 to A9 the effect of glutaraldehyde concentration (cross-linker) was investigated by keeping constant concentration of both PVA and gelatin polymers. Graph (c) of figure 1 showed the effect of the increasing amount of glutaraldehyde drops and decreasing the swelling profile of the Gel/ PVA hydrogel formulations. Sample A9 showed maximum equilibrium swelling 7.80 ± 0.49 at pH 1.2 and 3.10 ± 0.50 at pH 7.4. while sample A 7 showed maximum equilibrium swelling 7.98 ± 0.14 at pH 1.2 and 4.09 ± 0.45 at pH 7.4. while maximum equilibrium swelling shown by sample A8 was 7.92 ± 0.78 at pH 1.2 and 3.92 ± 0.31 at pH 7.4.



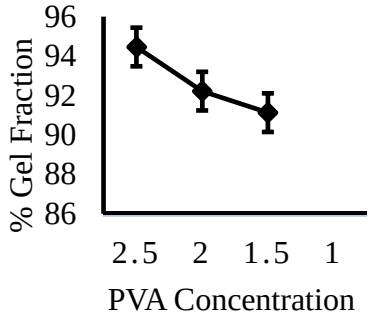
(a)



(b)



(c)



(d)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

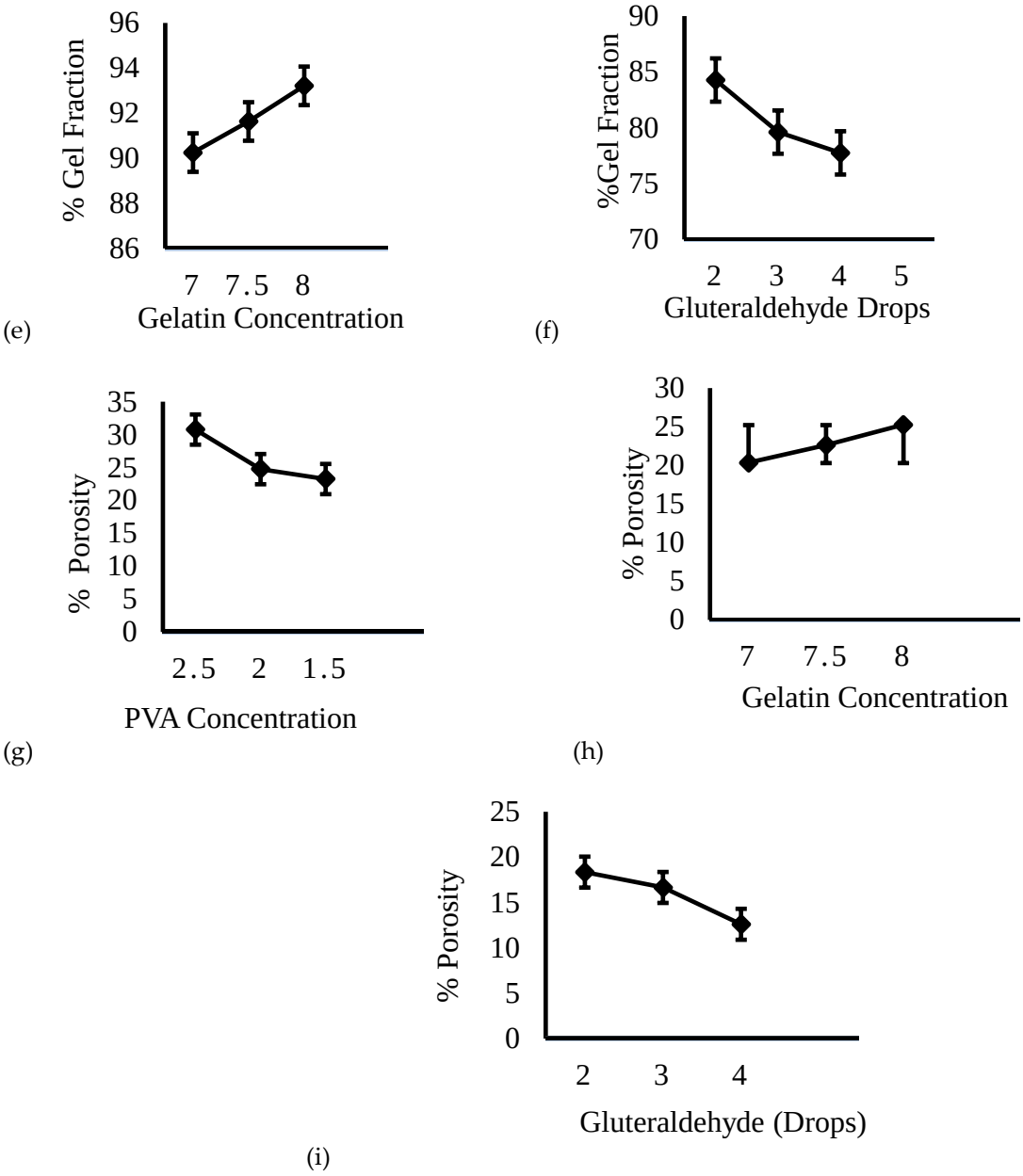


Figure 1. dynamic swelling of Gel/PVA Hydrogel with (a) decreasing concentration of PVA (b) increasing concentration of Gelatin (c) increasing quantity of glutaraldehyde, % gel fraction of Gel/PVA Hydrogel with (d) decreasing concentration of PVA (e) increasing concentration of Gelatin (f) increasing quantity of glutaraldehyde, % porosity of Gel/PVA Hydrogel with (g) decreasing concentration of PVA (h) increasing concentration of Gelatin (i) increasing quantity of glutaraldehyde.

The swelling profile ratio was as A7>A8>A9 as shown in figure 1(c). Sample A6 showed highest equilibrium swelling 10.09 ± 0.18 and dynamic swelling 5.20 ± 0.13 among the swelling ratio profile at pH 1.2 of all formulated Gel/PVA formulations. The dynamic swelling ratio for sample A9 was occurred 2.14 ± 0.15 at pH 7.4 and equilibrium swelling ratio for sample A1 was occurred 5.83 ± 0.35 at pH 7.4. Thus,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

sample A6 showed significantly different (p<0.01) degree of swelling profile among all formulated samples for release of methotrexate in controlled manner.

3.2. Sol-Gel Analysis

To determine of the un-crossed portion of the polymer in the formulation, sol-gel method was adopted. Sol-gel portion is directly proportional to the concentration of the polymers i.e. PVA and gelatin, and inversely proportional to the quantity of the cross-linker added to the formulations. The percent sol-gel fraction was occurred 94.46 ± 0.30% for A1, 92.22 ± 0.30% for A2, 91.13 ± 0.53% for A3, 90.25 ± 0.39% for A4, 91.63 ± 0.59% for A5, 93.21 ± 0.40% for A6, 84.27 ± 0.59% for A7, 79.61 ± 0.48% for A8, 77.75 ± 0.12% for A9. Among all 9-fomulations the highest degree of sol-gel fraction was found for sample A1, then was found for sample A6, while minimum degree of sol-gel fraction was found for sample A9. Graph (d) of figure 1 showed that decreasing the concentration of polyvinyl alcohol polymer result in decreasing of the % sol-gel fraction i.e. maximum sol-gel of sample A1 (94.46 ± 0.30%) and minimum for A3 (91.13 ± 0.53%). Graph (e) of figure 1 showed that as the concentration of gelatin increases there also occurred increase in % sol-gel fraction i.e. maximum sol-gel of sample A6 (93.21 ± 0.40%) and minimum for A4 (90.25 ± 0.39%), while graph (f) showed that increasing the cross-linker (glutaraldehyde) quantity also result in decreasing the % sol-gel fraction i.e. very low sol-gel fraction for sample A9 (77.75 ± 0.12%).

3.3. Diffusion Co-Efficient

It was the process of determination of the quantity of the solvent that diffuses through a unit area in a unit time through a unit conc; gradient. For sample A1 diffusion co-efficient value occured 0.0148 ± 0.0061 (cm^2/sec), for sample A2 diffusion co-efficient value occured 0.0119 ± 0.0081 (cm^2/sec), for sample A3 diffusion co-efficient value occured 0.0102 ± 0.0015 (cm^2/sec), for sample A4 diffusion co-efficient value occured 0.0096 ± 0.0031 (cm^2/sec), for sample A5 diffusion co-efficient value occured 0.0105 ± 0.0017 (cm^2/sec), for sample A6 diffusion co-efficient value occured 0.0176 ± 0.0021 (cm^2/sec), for sample A7 diffusion co-efficient value occured 0.0195 ± 0.0014 (cm^2/sec), for sample A8 diffusion co-efficient value occured 0.0161 ± 0.0042 (cm^2/sec) and for sample A9 diffusion co-efficient value occured 0.0107 ± 0.0037 (cm^2/sec). So it was cleared from their calculated diffusion co-efficient values, that when the concentration of polyvinyl alcohol decreased, there also occurred a decrease in diffusion co-efficient values as from sample A1 to A3 and when the concentration of gelatin was increased, there occurred an increase in diffusion co-efficient values as shown from sample A4 to A6, while increasing the glutaraldehyde concentration resulted in decreasing the diffusion co-efficient values as shown from sample A7 to A9. So, it became cleared that diffusion co-efficient is directly proportional to the polymer concentration and inversely proportional to concentration of cross-linker (glutaraldehyde).

3.4. Measurement Porosity

After formulating various formulation of GEL/PVA hydrogel, the effect of the different concentration of the polymers (gelatin & polyvinyl alcohol) and of the cross-linker on the hydrogel formulations can be investigated from the amount of the porosity of that vary sample. All the formulations of Gel/PVA hydrogel were evaluated for porosity and data of the results was calculated in triplicate and then their mean and standard deviation of the results were also calculated. Percent (%) porosity of sample A1 was (30.76 ± 1.350), sample A2 was (24.75 ± 1.327), sample A3 was (23.32 ± 1.054), sample A4 was (20.36 ± 1.388), sample A5 was (22.63 ± 1.108), sample A6 was (28.27 ± 1.387), sample A7 was (18.35 ± 1.083), sample A8 was (16.66 ± 1.4512) and sample A9 was (10.60 ± 0.450).

It was evident that when the concentration of the PVA decreases and gelatin was kept constant from S1 to S3 there also occurred significant decrease in the porosity of the hydrogel formulations. The maximum % porosity occurred for sample A1 i.e. 30.76±1.350, moderate for sample A2 i.e. 24.75±1.327 and minimum for sample A3 i.e. 23.24±1.054 as shown in graph (g) of figure 1. S4 to S6 the gelatin concentration

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

was gradually increased, while PVA was kept constant, then there occur significant gradual increase in the % porosity of the hydrogel formulations was as; A6 had increased degree of porosity i.e. 28.27±1.387, moderate for A5 i.e. 22.63±1.108 and lower for A4 i.e. 20.36±1.388 as shown in graph (h) of figure 1 and when polymers concentration became placed constant while increased the cross-linker (glutaraldehyde) quantity, there occurred a decrease in % porosity of hydrogel formulations from A7 to A9 i.e. % porosity of sample A7 was 18.35±1.083, for A8 was 16.66 ± 1.4512 and for A9 was 10.60±0.450 as shown in graph (i) of figure 1.

3.5. Amount of drug loaded

After calculation the amount of the methotrexate drug loaded on each formulated disc was approximately 0.50mg of sample A1, 0.34mg of sample A2, 0.58mg of sample A3, 0.53mg of sample A4, 0.51mg of sample A5 and 0.52mg of sample A6. The drug methotrexate was loaded by *in-situ* drug loading method in first six formulations.

3.6. Percentage of Drug release profile

The drug release profile was investigated by the amount of the drug released from the hydrogel formulation after 12 hours and the impact of the pH, polyvinyl alcohol & gelatin on drug (methotrexate) release was determined. Sample A1 to A3 the gelatin concentration i.e. (9gm) was kept constant, while that of PVA was decreased gradually i.e. (2.5gm, 2gm and 1.5gm) to determine its concentration effect on methotrexate release from Gel/PVA hydrogel formulations at different pH values of phosphate buffer mediums. The percent drug release profile results showed that as the concentration of the PVA decreased, the drug (methotrexate) release was also decreased especially at pH 7.4 and vice versa. Sample A1 showed higher % methotrexate release 94.30 ± 1.24 at pH 1.2, 83.21 ± 0.19 at pH 6.8, 82.32 ± 0.48 at pH 7.4 as shown in graph (j) of figure 2, while sample A2 showed % drug (methotrexate) release 89.67 ± 0.12 at pH 1.2, 77.53 ± 0.98 at pH 6.8, 66.54 ± 0.37 at pH 7.4 as shown in graph (k) of figure 2 and sample A3 showed % drug (methotrexate) release 85.28 ± 0.96 at pH 1.2, 71.38 ± 0.41 at pH 6.8, 58.03 ± 0.62 at pH 7.4 as shown in graph (l) of figure 2.

Sample A4 to A6 the PVA concentration i.e. (2gm) was kept constant, while increased the gelatin concentration gradually i.e. (7gm, 7.5gm and 8gm) to investigate its concentration effect one methotrexate release from Gel/PVA hydrogel formulations at different pH values of phosphate buffer mediums. The percent drug release profile results showed that as the concentration of the gelatin increased, the drug (methotrexate) release was also increased especially at pH 1.2 and vice versa. Sample A4 showed % drug (methotrexate) release 79.89 ± 0.56 at pH 1.2, 72.03 ± 0.12 at pH 6.8, 61.63 ± 0.07 at pH 7.4 as shown in graph (m) of figure 2, while sample A5 showed % drug (methotrexate) release 89.96 ± 0.10 at pH 1.2, 76.66 ± 0.63 at pH 6.8, 73.39 ± 0.56 at pH 7.4 as shown in graph (n) of figure 2 and sample A6 showed % drug (methotrexate) release 93.75 ± 0.13 at pH 1.2, 81.40 ± 0.59 at pH 6.8, 75.65 ± 0.75 at pH 7.4 as shown in graph (o) of figure 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

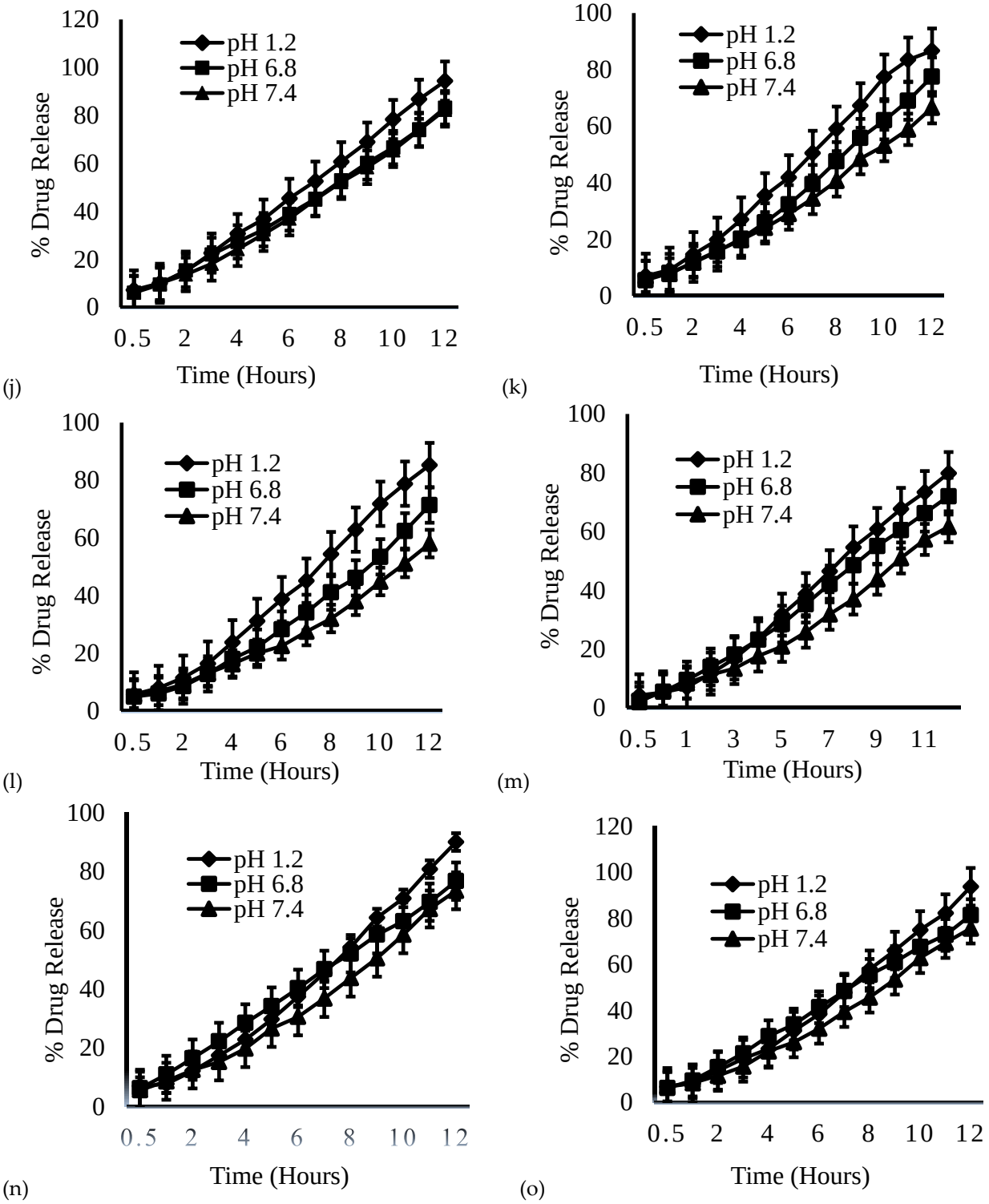
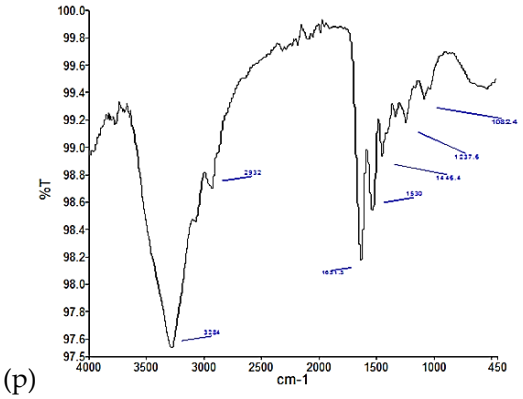


Figure 2. % methotrexate release graphs from Gel/PVA Hydrogel with polymer concentrations (j) GEL/PVA (9/2.5 gm), (k) GEL/PVA (9/2 gm), (l) GEL/PVA (9/1.5 gm), (m) GEL/PVA (7/2.5 gm), (n) GEL/PVA (7.5/2 gm), (o) GEL/PVA (8/2 gm) at pH 1.2, pH 6.8 and pH 7.4 at 37˚C.

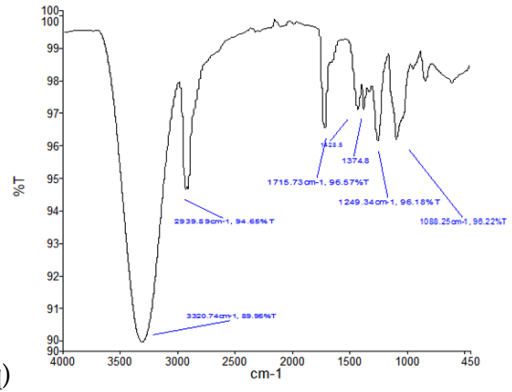
3.7. FT-IR (Fourier Transform Infrared Spectroscopy)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

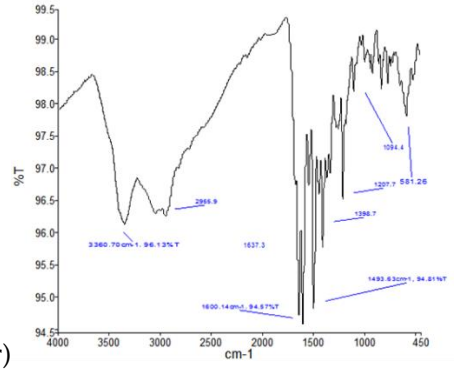
The peaks of gelatin polymer shown in the FTIR spectra indicate the following chemical groups. The peak occurs at 3284 cm^{-1} indicate the stretching of -N-H group of secondary amides, the peak occur at 2932 cm^{-1} indicates C-H stretching group, the peak of 1631.3 cm^{-1} indicates the stretching of C=O, amide I, and C-N stretching groups, the peak at 1530 cm^{-1} indicate -N-H bending, the peak at 1237.6 cm^{-1} indicate the amide -III [28] as shown in figure 3(p). The FTIR spectra of the pure PVA shown a peak of broad band at 3320.74 cm^{-1} is belong to the O-H stretching groups which occur due to inter and intra molecular hydrogen bond. A peak at 2939.89 cm^{-1} shows the vibrational bend due to stretching of C-H bond from the alkyl groups. The peak at 1715.73 cm^{-1} indicate C=O due to the strong carboxylic group. The peak at 1485 cm^{-1} and 1342 cm^{-1} indicate scissoring -CH2 group and vibrational bending -OH group [29] as shown in figure 3(q). The peak at 3360.70 cm^{-1} indicate the stretching of the O-H from the carboxyl group, the peak at 2955.9 cm^{-1} indicate N-H stretching of primary amine, the peaks at 1670cm^{-1} to 1600 cm^{-1} -C=O stretching of (amide group and carboxylic group), peak at 1493.63 cm^{-1} indicate the -N-H bending from of amide group, the peak appear at 1400cm^{-1} to 1200 cm^{-1} indicate the stretching of -C-O from carboxylic group (Fulias et al, 2014) as shown in figure 3(r). As the functional chemical groups of the gelatin and polyvinyl alcohol are occurring in the same peak regions, due which they are overlapping over one another and so there occurred no significant chemical interaction as shown in figure 3(s). The methotrexate drug was water insoluble showed no chemical interaction with the hydrogel, which is evident from the FTIR spectra of the methotrexate loaded gelatin/PVA hydrogel sample as shown in figure 3(t).



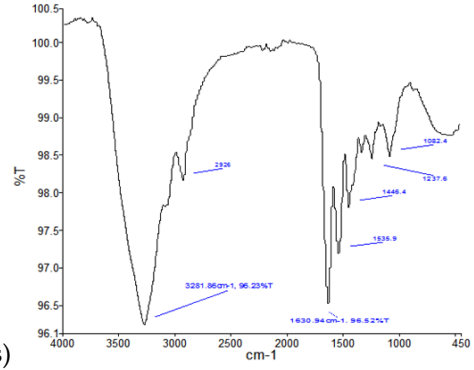
(p)



(q)



(r)



(s)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

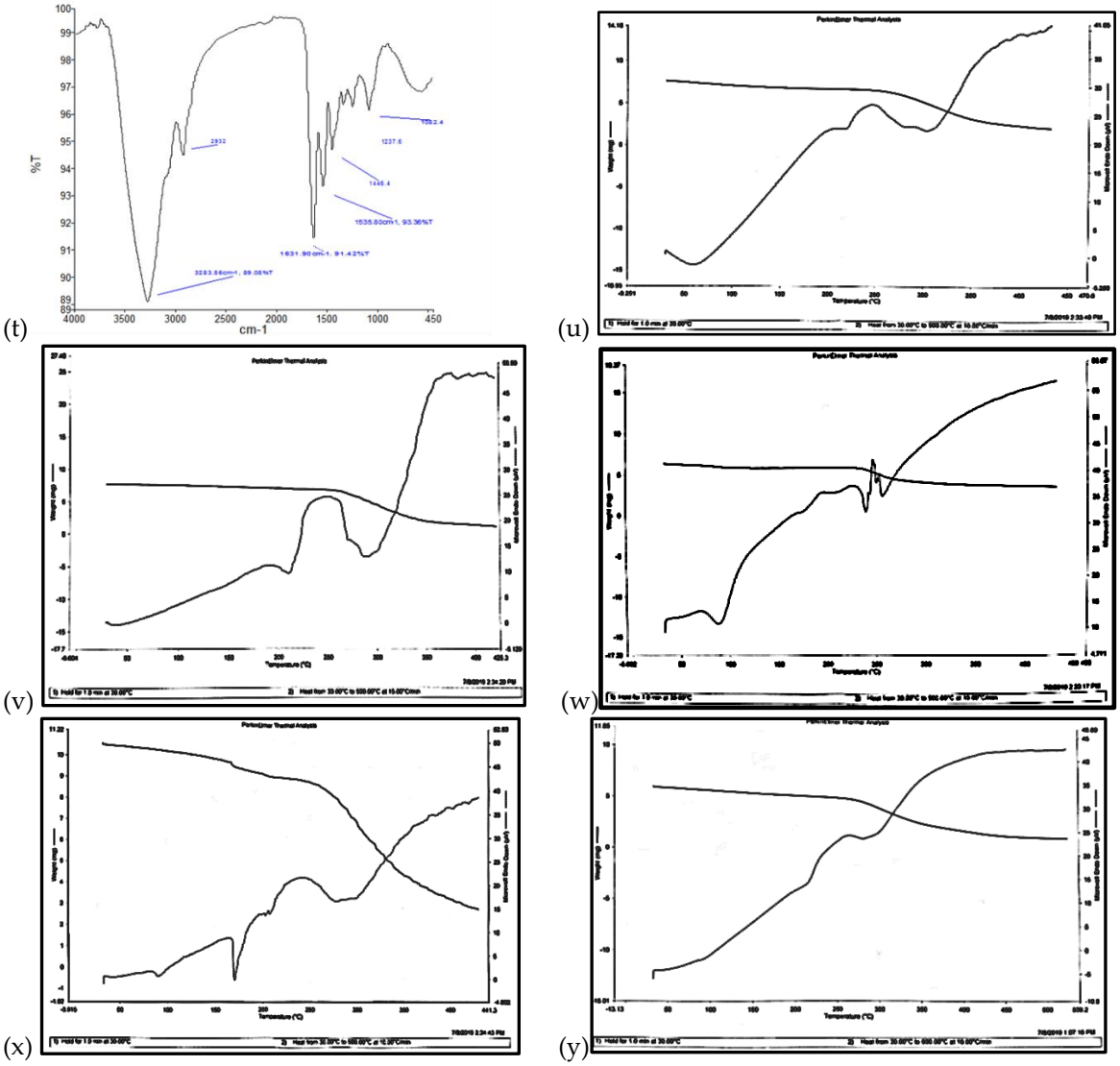


Figure 3. FTIR spectra of (p) pure PVA, (q) pure gelatin, (r) methotrexate, (s) unloaded Gel/PVA hydrogel, (t) methotrexate loaded Gel/PVA hydrogel, TGA spectra of (u) pure gelatin, (v) pure PVA, (w) methotrexate, (x) unloaded Gel/PVA hydrogel, (y) methotrexate loaded Gel/PVA hydrogel.

3.8. Thermal gravimetric analysis (TGA)

An endothermic peak at 180˚C indicate melting point of the amino acid. Broad endotherm at 250˚C indicate decomposition of the protein. TGA shows approximately 15% weight loss up to 150˚C due to loss of water. A steep change in weight of the sample over a temperature range of 250˚C to 350˚C characterized by 35% weight loss is related thermal decomposition of the protein as shown in figure 3(u). Thermogram of PVA shows an endothermic event at 180 ˚C which is related to the glass transition of the polymer. Another endotherm at 250 ˚C characterized by 30% weight loss in the TGA is linked to the decomposition of the samples as shown in figure 3(v). Thermogram of methotrexate demonstrate an endothermic peak at 100˚C which is ascribed to the dehydration of the sample. The second endothermic peak at 170 ˚C is due to the melting of the drug substance. A noisy endotherm at 240˚C is possibly linked to the thermal decomposition of the sample. TGA data also correlated to the weight loss at 100 ˚C due to dehydration and thermal degradation at 240 ˚C as shown in figure 3(w). The DSC thermogram indicate an endothermic peak at 80˚

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

C is the dehydration of the sample. Sharp endothermic peak at 170 ˚C indicate melting of the formulation. a broad endotherm at 250 ˚C to 350 ˚C show thermal decomposition of the sample. TGA results indicates a low gradient of weight change at up to 150 ˚C due to removal of water. A small yet abrupt change in the sample weight at 170 ˚C matches with the enthalphic shift due to melting of the sample. Likewise, a 50% reduction in the sample weight over a temperature range of 250 ˚ C and onward refer to thermal decomposition of the mass as shown in figure 3(x). DSC profile shows an endothermic peak at 220 ˚C due to fusion while the second endotherm at 260 ˚ C is due to decomposition of the sample. TGA profile compliment the DSC and small changes in weight are evident up to 200 ˚ C. thermal decomposition is recorded at exceeding 200 ˚C reflecting thermal stability of the sample as shown in figure 3(y).

4. Discussion

The pka values of the of the acidic components of the polymers and the pH of the buffer medium/solution used for swelling of the hydrogel, might play significant role in determination of the swelling behavior of the hydrogel. Swelling properties of the hydrogel's polymer network may greatly have affected by changing the pH of surrounding buffer medium. During studying the parameter swelling kinetic of the Gel/PVA hydrogel, it is observed that the primary amino groups of the polymers get ionized at lower pH (acidic pH) of the buffer medium below the pka value of the polymers. Then swelling might be occurred due to the protonation of the polymer's amino groups. After protonation of the –NH2 groups, +vely charged $–NH3^+$ groups may be distributed in network of hydrogel polymers. When the concentration of these positively charged groups increases, they lead to create a difference in the osmotic pressure of the inner and outer environment of the hydrogel polymer network. These positively charged groups then started repulsion among the polymer's chains of the gel network, which could lead to more swelling of the hydrogel network [35]. The degree of swelling both dynamic and equilibrium were observed to increase in acidic medium pH 1.2, which may be due to $–NH_2$ groups of the gelatin polymer in the PVA/gelatin hydrogel and actually the amino groups are basic in nature and thus ionized (protonated) at pH lower to pkb value of gelatin, which can result to produce positive charged groups $–NH3^+$, that repel each other and may result in increased swelling of hydrogel at lower pH [36]. At pH value of medium, higher than the acidic pH value, there occur increase in swelling of hydrogel network. The increasing equilibrium swelling of Gel/PVA hydrogel at basic pH of the dissolution medium may be due to –OH groups of the polymer in the gel network [37]. With the increase of polyvinyl alcohol concentration, there can be occur an increase in swelling of the hydrogel. The functional groups of polyvinyl alcohol i.e. amino group ($–NH_2$) and hydroxyl group (–OH) may be actually responsible for swelling of the hydrogel because these functional groups are hydrophilic in nature [38]. The PVA is water soluble polymer, so when its concentration increases, there occur equilibrium swelling very early, because when the PVA quantity increases its chains are hydrated highly due to their hydrophilic nature [39]. During studying swelling kinetics of PVA/Gelatin hydrogel, it was observed that both the dynamic and equilibrium swelling were decreased in both acidic and basic pH mediums, as the concentration of the glutaraldehyde increases, due to crosslinking of a greater number of polymer chains. When the concentration of the glutaraldehyde increases in gel formulation, the –OH groups of the PVA and more $–NH_2$ groups of the gelatin consumed in crosslinking reactions, in which the aldehyde group of the cross-linker react with the –OH group of the PVA to form acetal group and $–NH_2$ group of gelatin for a Schiff base. After these cross-linking reactions, the PVA hydroxyl groups diminished to form hydrogen bonding with water molecules and thus result in decrease of the swelling parameters of the hydrogel formulation. These findings can be correlated with the results founded by [37]. When the PVA concentration increased in the formulation as compare to gelatin, then such formulation may be showing more swelling in basic pH medium as compared to acidic pH medium because of providing large number of $–COO^-$ groups thus might be result to swell more at basic pH of the medium [40]. For the determination of the un-crossed linked polymer fraction of the gel/PVA hydrogel

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 September 2020 doi:10.20944/preprints202009.0510.v1

formulation, % sol-gel fraction analysis method was adopted. After taking results of all the samples, it was observed that by increasing the concentration of the PVA, the % gel fraction was increased and vice versa [41]. By increasing the polymer 'gelatin' concentration, there can be occur an increase in the % gel fraction and decrease in the sol fraction [28]. Adding or increasing concentration of glutaraldehyde in the formulations may also results in increasing of the % gel fraction, because cross-linker will cause increase entanglement of the more polymer chains of the gel formulation [42]. The entering of solvent of the buffer medium into pre-existing pores or the spaces formed thermodynamically between the chains of polymer of the gel formulation is diffusion co-efficient. So, diffusion co-efficient may also directly proportional to the amount of polymer concentration. Increasing the polymer concentration either PVA or gelatin in hydrogel formulations may result to increase in the diffusion co-efficient and vice versa. While diffusion co-efficient is inversely proportional to glutaraldehyde quantity [43]. Porosity of the Gel/PVA hydrogel increases, when the concentration of the gelatin or poly (vinyl alcohol) polymer increases. The reason might be attributed to the increasing of the polymer concentration may increase the viscosity of the gel solution. During preparation of that solution there also formed bubbles in the solution, and due its viscous nature the bubbles are entrapped in the formulation, which acts as an interconnected pore channels for the hydrogel, that results in increasing of porosity of the gel. Increasing the glutaraldehyde cross-linker concentration will lead to decrease the spaces among the polymer chains by cross-linking them and thus results in decrease of the spaces and pores and thus the % porosity of the gel [44].

The methotrexate drug release from Gel/PVA hydrogel formulations may also follow swelling controlled release mechanism. During swelling the hydrogel discs might swell up and can change it shape with time in buffer solution. The shape transition of hydrogel discs would also depend on pH of the swelling medium, diffusion co-efficient, quantity of polymers (PVA and gelatin) and porosity. The solvent of the buffer medium may enter the hydrogel disc and thus the disc swells up and releases the drug to its surrounding environment [28]. The FTIR spectra of the drug unloaded hydrogel (graph 4.33) may show no definite change (significant chemical interactions) from the gelatin and poly (vinyl alcohol) spectra. The reason could be that the concentration of the PVA polymer is lesser in quantity, so will not affect the drug unloaded hydrogel FTIR spectra [44] and most functional chemical groups occurring in the same peak regions and also have no cross-linker in drug loaded samples (A1-A6), so they may only overlap upon each other. While the FTIR spectra of the drug loaded hydrogel graph 4.34 may also show no any significant variation from the spectra of graph 4.33, because the methotrexate in water insoluble, so may not interact with the water soluble polymers (either gelatin or PVA), these can be correlated with observations of [14].

5. Conclusion

During this research work, Gel/PVA hydrogel formulations with different concentration of gelatin and polyvinyl alcohol were formulated using glutaraldehyde as a cross-linker for targeted delivery of drug in controlled manner. The formulations were also pH sensitive. So, for this purpose 0.2M phosphate buffer solutions with different pH values i.e. 1.2, 6.8 and 7.4 were prepared to evaluate each formulated Gel/PVA hydrogel of different concentrations by their swelling profile, porosity, sol-gel fraction, diffusion co-efficient and % percent drug release. All the formulations release the drug at higher rate at pH 1.2, moderate at pH 6.8 and least at pH 7.4. so, all the were able to release the drug in controlled manner to the targeted site. In-situ drug loading method was adopted fpr proper loading because of drug hydrophobicity. Their characterization was done by FTIR and TGA methods, which confirm that Gel/PVA polymer hydrogel can be formulated and without cross-linker can also be prepared, so can make the formulation safe from harsh (adverse) effects of chemical cross-linkers. So, from the overall study it can concluded that these Gel/PVA hydrogel formulations were highly pH sensitive, having characteristic of time dependent drug release in controlled way to the specific target organ site. Also, it can be concluded from this study, that one can also

prepare more colon targeted specific release hydrogel formulation by increasing concentration ratio of polyvinyl alcohol than gelatin during formulation process.

Author Contributions

Conceptualization, M. A.; I. U. and A. N. M. S; Methodology, M. A.; I. U. and A. N. M. S; Software, M. A.; I. U. and A. N. M. S; Validation, M. A.; I. U. and A. N. M. S; Formal Analysis, M. A.; I. U. and A. N. M. S; Investigation, M. A.; I. U. and A. N. M. S; Resources, M. A.; I. U. and A. N. M. S.; Data Curation, M. A.; I. U.; A. N. M. S and A.K.A.; Writing—Original Draft Preparation, M. A.; I. U. and A. N. M. S and A.K.A.; Review & Editing, A.K.A.; Visualization, A.K.A.; Supervision, M. A.; I. U. and A. N. M. S.; Project Administration, M. A.; I. U. and A. N. M. S.; Funding Acquisition, ABM. H. U. and S. A. A. All authors have read and agreed to the published version of the manuscript.

Funding

This work was funded by the Research Management Centre, International Islamic University Malaysia for grant RPDF19-002-0012.

Acknowledgments

The authors would like to thank Gomal University, Pakistan for technical and facilitation support.

Conflicts of Interest

Authors declared no conflict of interest with other form of study.

References

1. Tenesa, A. Dunlop, M. G. (2009). New insights into the etiology of colorectal cancer from genome-wide association studies. *Nat Rev Genet* **2009**, *10*, 353-358
2. Terzic, J., Grivennikov, S., Karin, E., Karin, M. Inflammation and colon cancer. *Gastroenterology* **2010**, *138*, 2101-2014.
3. Chourasia, M. K. Jain, S. K. (2004). Polysaccharides for Colon Targeted Drug Delivery. *Drug Delivery* **2004**, *11*, 129–148.
4. Sinha, V. R., Mittal, B. R., Buhtani, K. K. Rachna, K. Colonic drug delivery of 5-fluorouracil: an in vitro evaluation. *Int J Pharm* **2004**, *269*, 101–108.
5. Schmaljohann, D. Thermo and pH responsive polymers in drug delivery. *Adv Drug Deliv Rev* **2006**, *58*, 1655-1670.
6. Sharpe, A. L., Daily, M. A., Horava, D. S. Peppas, A. N. Therapeutic applications of hydrogels in oral drug delivery. *Expert Opin Drug Deliv* **2014**, *11*, 901-915.
7. Liu, X.M., Wang, L.S., Wang, L., Huang, J. He, C. The effect of salt and pH on the phase-transition behaviors of temperature-sensitive copolymers based on N-isopropylacrylamide. *Biomaterials* **2004**, 25, 5659-5666.
8. Zhang, H., Guo, S., Fu, S. Zaho, Y. A Near-Infrared Light-Responsive Hybrid Hydrogel Based on UCST Triblock Copolymer and Gold Nanorods. *Polymers* **2017**, *9*, 1-9.
9. Silva, L. P. D., Jha, A. K., Correlo, V. M., Marques, A. P., Reis, R. L. Healy, K. E. Gellan Gum Hydrogels with Enzyme-Sensitive Biodegradation and Endotheileal Cell Biorecognition Sites. *Adv Healthcare Mater* **2018**, *7*, 686-700.

10. Valuev, I. L., Vanchugova, L. V. Valuev L. I. Glucose-Sensitive Hydrogel Systems. *Polymer Science* **2011**, *53*, 385-389.
11. Bawa, P., Pillay V., Choonara, YE., Toit c du, L. Stimuli responsive polymers and their applications in drug delivery. *Biomed Mater* **2009**, *4*, 022001.
12. He, C., Kim, S. W. Lee, D. S. In-situ gelling stimuli-sensitive block copolymer hydrogels for drug delivery. *J Control Release* **2008**, *127*, 189-207.
13. Gupta, P., Vermani, K., Garg S. hydrogels: from controlled release to pH-responsivedrug delivery. *Drug Discov Today* **2002**, *7*, 569-579.
14. Nawaz, S., Khan, S., Farooq, U., Haider, M. S., Ranjha, N. M., Rasul, A., Nawaz, A., Arshad, N., Hameed, R. Biocompatible hydrogel for the controlled delivery of the anti-hypertensive agent: development, characterization and in vitro evaluation. *Des Monomers Polym* **2018**, *21*, 18-32.
15. Vadia, N. Rajput, S. Study on formulation variables of methotrexate loaded mesoporous MCM-41 nanoparticles for dissolution enhancement. *Eur J Pharm Sci* **2012**, *45*, 8–18.
16. Ciekot, J., Goszczynski, T. Boratynki, J. Methods for methotrexate determination in macromolecular conjugates drug carrier. *Acta Pol Pharm* **2012**, *69*, 1342-1346.
17. Herfarth, H. H., Osterman, M. T., Isaacs, K. L., Lewis, J. D. & Sands, B. E. Efficacy of Methotrexate in Ulcerative Colitis: Failure or Promise. *Inflamm Bowel Dis* **2012**, *16*, 1421-1430.
18. Imanishi, H., Okamura, N., Yagi, M., Noro, Y., Moriya, Y., Nakamura, T., Hayakawa, A., Takeshima, Y., Sakaeda, T., Matsuo, M. Okumura, K. Genetic polymorphisms associated with adverse events and elimination of methotrexate in the childhood acute lymphoblastic leukemia and malignant lymphoma. *J Hum Genet* **2007**, *52*, 166-171.
19. Stewart, J. S., Cohen, E. E., Licitra, L., Van Herpen C. M., Khorprasert, C., Soulieres, D., Vodvarka, P., Rischin, D., Garin, A. M., Hirsch, F. R., Varella-Garcia, M., Ghiorghiu, S., Hargreaves, L., Armour, A., Speake, G., Swaisland, A. Vokes, E. E. Phase III study of gefitinib compared with intravenous methotrexate for recurrent squamous cell carcinoma of the head and neck. *J Clin Oncol* **2009**, *27*, 1864-1871.
20. Norman, J. Osteosarcoma: Review of the Past, Impact on the Future. The American Experience. Pediatric and Adolescent Osteosarcoma **2009**, *152*, 239-262.
21. Terakawa, T., Miyake, H., Muramaki, M., Takenaka, A. Fujisawa, M. Salvage chemotherapy with methotrexate, etoposide and actinomycin D in men with metastatic noneminomatous germ cell tumors with choriocarcinoma component: A preliminary report. *Int J Urol* **2010**, *17*, 881-885.
22. Szekanecz, E., Andras, C., Sandor, Z., Antal-Szalmas, P., Szanto, J., Tamasi, L., Kiss, E., Szekanecz, Z. Malignancies and soluble tumor antigens in rheumatoid arthritis. *Auto Immun* Rev **2006**, *6*, 42–47.
23. Kalb, E.R., Strober, B., Weinstein, G. Lebwohl, M. Methotrexate and Psoriasis: 2009 National Psoriasis Foundation Consensus Conference. *J Am Acad Dermatol* **2009**, *60*, 824-837.
24. Nash, R. A., Jhonston, L., Parker, P., Mccune, J. S., Storer, B., Slattery, J. T., Furlong, T., Anasetti, C., Appelbaum, F. R., Lloid, M. E., Deeg, H. J., Peterkiem, H., Martin, P. J., Schubert, M. M., Witherspoon, R. P., Forman, S. J., Blume, K. G. Strob, R. A Phase I/II Study of Mycophenolate Mofetil in Combination with Cyclosporine for Prophylaxis of Acute Graft-versus-Host Disease after Myeloablative Conditioning and Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* **2005**, *11*, 495-505.
25. Kannagi, R., Izawa, M., Koike, T., Miyazaki, K., Kimura, N. Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. *Cancer Sci* **2004**, *95*, 377-384.
26. Muller, R. H., Keck, C. M. Challenges and solution for the delivery of biotech drugs- a review of drug nanocrystal technology and lipid nanoparticles. *J Biotechnol* **2004**, *113*, 151-170.
27. Koo, O. M., Rubintein, I. Onyuksel, H. Role of nanotechnology in targeted drug delivery and imaging: a concise review. *Nanomed-Nanotechnol* **2005**, *1*, 193-212.

28. Akhlaq, M., Maryam, F., Elaissari, A., Ullah, H., Adeel, M., Hussain, A., Ramzan, M., Ullah, O., Danish, M. Z., Iftihar, S. Aziz, N. Pharmacokinetic evaluation of quetiapine fumarate controlled release hybrid hydrogel: a healthier treatment of schizophrenia. *Drug Deliv* **2018**, *25*, 916-927.
29. Ranjha, N. M., Hanif, M., Afzal, Z. Abbas, G. Diffusion coefficient, porosity measurement, dynamic and equilibrium swelling studies of acrylic acid/polyvinyl alcohol (aa/pva) hydrogels. *Pak J Pharm Sci* **2015**, *1*, 48-57.
30. Mudassir, J. Ranjha, N. M. Dynamic and equilibrium swelling studies: cross-linked pH sensitive methyl methacrylate-co-itaconic acid (MMA-co-IA) hydrogels. *J Polym Res* **2008**, *15*, 195-203.
31. Shabir, F., Erum, A., Tulain, U. R., Hussain, M. A., Ahmad, M. Akhter, F. Preparation and characterization of pH sensitive crosslinked Linseed polysaccharides-co-acrylic acid/methacrylic acid hydrogels for controlled delivery of ketoprofen. *Des Monomers Polym* **2017**, *20*, 485-495.
32. Lin, W. J. Lu, C. H. Characterization and permeation of microporous poly (ε-caprolactone) films. *J Membr Sci* **2002**, *198*, 109-118.
33. Costa-Junior, E. D. S., Pereira, M. M. Mansur, H. S. Properties and biocompatibility of chitosan films modified by blending with PVA and chemically crosslinked. *J Mater Sci Mater Med* **2009**, *20*, 553-561.
34. Azad, A. K., Al-Mahmood, S. M. A., Chatterjee, B., Wan Sulaiman, W. M. A., Elsayed, T. M., Doolaanea, A. A. Encapsulation of Black Seed Oil in Alginate Beads as a pH-Sensitive Carrier for Intestine-Targeted Drug Delivery: In Vitro, In Vivo and Ex Vivo Study. *Pharmaceutics* 2020, 12, 219.
35. Kim, S. J., Lee, K. J., Kim, I. Y. Kim, S. I. Swelling kinetics of interpeneterating polymer hydrogels composed of poly (vinyl alcohol)/chitosan. *J Macromo Sci A* **2003**, *40*, 501-510.
36. Saarai, A., Sedlacek, T. Kasparkova, V. Characterization of sodium alginate/gelatin –based hydrogels for wound dressing. *J Appl Polym Sci* **2012**, *126*, E79-E88.
37. Bahrami, S. B., Kordestani, S. S., Mirzadeh, H. Mansoori, P. Poly (vinyl alcohol)-chitosan blends: preparation, echanical and physical properties. *Iran Polym J* **2003**, *12*, 139-146.
38. Khan, S. U. Ranjha, N. M. Effect of degree of crosslinking on swelling and on drug release of low viscous chitosan/poly (vinyl alcohol) hydrogel. *Polym Bull* **2014**, *71*, 2133-2158.
39. Shukla, S., Bajpai, A. K. Kulkarin, R. A. Preparation, characterization, and water-sorption study of poly (vinyl alcohol) based hydrogels with grafted hydrophilic and hydrophobic segments. *J Appl Polym Sci* **2005**, 95, 1129-1142.
40. Tareq, A. Z., Hussein, M. S. Mustafa, A. M. Synthesis and characterization of PVA-Gelatin hydrogel membranes for controlled delivery of captopril. *Int Res J Pure Appl Chem* **2016**, *12*, 1-10.
41. Sung, J. H., Hwang, M. R., Kim, J. O., Lee, J. H., Kim, Y. I., Kim, J. H., Chang, S. W., Jin, S, G., Kim, J. A., Lyoo, W. S., Han, S. S., Ku, S.K., Yong, S. H. Choi, H. G. Gel characterization and in *vivo* evaluation of minocycline-loaded wound dressing with enhanced wound healing using poly (vinyl alcohol) and chitosan. *Int J Pharm* **2010**, *392*, 232-240.
42. Chen, S., Liu, M., Jin, S. Wang, B. Preparation of ionic-crosslinlked chitosan-based gel beads & effect of reaction conditions on drug release. *Int J Pharm* **2008**, *349*, 180-187.
43. Yin, L., Fei, L., Cui, F., Tang, C. Yin, C. Superporous hydrogels containing poly (acrylicacid-co-acrylamide)/O-carboxymethyl chitosan interpenetrating polymer networks. *Biomaterials* **2010**, *28*, 1258-1266.
44. Priscila, M. A. A., Rosemarya. C., Izabel, C. F. M., Carla, G. L., Ana Monica, Q. B. B. Paulo, J. A. S. (2011). Development of films based on blends of gelatin and poly (vinyl alcohol) cross linked with glutaraldehyde. *Food Hydrocoll* **2011**, *25*, 1751-1757.