The Structural, Crystallinity and Thermal Properties of pH responsive Interpenetrating Gelatin/Sodium Alginate Based Polymeric Composites for the Controlled Delivery of Cetirizine HCl

[1]Samrin Afzal, [2]Samiullah Khan, [1]Nazar Mohammad Ranjha, [1]Aamir Jalil, [1]Amina Riaz, [1]Malik Salman Haider, [1]Shoaib Sarwar, [1]Fareha Saher, [1]Fahad Naeem

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan-60800 Pakistan

[2]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur 63100, Punjab, Pakistan

Address for correspondence

***Dr. Samiullah Khan**
Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur, Railway Road, Khawaja Fareed Campus, Bahawalpur,
PakistanTel: 0092-3339952522
Email: Sami_pharmacist99@hotmail.com

ABSTRACT

Objectives: The present work was aimed to design and synthesize pH-sensitive cross-linked Ge/SA hydrogels using different ratios of each polymer and to investigate the effect of each polymer on dynamic, equilibrium swelling and *in vitro* release pattern of cetirizine hydrochloride which was selected as a model drug.

Materials and Methods: These gelatin and sodium alginate hydrogels were prepared at room temperature by free radical polymerization technique using glutaraldehyde as a crosslinker. These polymeric composites were used as model systems to envisage various important characterizations. *In vitro* release pattern of drug was investigated in three different mediums (phosphate buffer solution of pH 1.2, 5.5, 7.5 whose ionic strength was kept constant. Various structure property relationships that affect its release behavior were determined like swelling analysis, porosity, sol-gel analysis, average molecular weight between crosslinks (Mc), solvent interaction parameter (χ), volume fraction of polymer ($V_{2,s}$) and diffusion coefficient. The structural, crystallinity and thermal stability was confirmed by FTIR, XRD and DSC analysis.

Results: These hydrogels showed maximum swelling at pH 1.2. Zero order, First order, Higuchi and Peppas models were applied to demonstrate the release pattern of drug. The release of drug occurred through non-fickian diffusion or anomalous mechanism. Porosity was found increased with an increase in concentration of both the polymers and when concentration of crosslinker was increased porosity decreased. Gel fraction increased with an increase in concentration of SA, Ge and glutaraldehyde.

Conclusion: The prepared pH sensitive hydrogels can be used as a potential carrier for the sustained delivery of cetirizine hydrochloride.

Key Words: pH responsive, Dynamic Swelling, Cetirizine HCl, *Invitro* release, Controlled delivery

INTRODUCTION

Modern research is oriented towards the site targeted and controlled release of the drug. Various peptides and proteins have been emerged in response to several advancements in genetics and biotechnology. Proper drug delivery systems for successful treatment are very necessary.[1] Hydrogels have earned much significance in this regard.

Hydrogels are 3D (three- dimensional networks) and have the ability to absorb considerable amount of water.[2] Water absorbing ability depends upon nature of aqueous environment and polymer composition.[3] Hydrogels have found extensive applications as drug delivery systems, contact lenses, wound dressings, artificial lung and artificial joint biomaterials. They are involved in catheterization and endoscopy to reduce the surface friction for comfort of patients.[4,5] Hydrogels have the ability to protect drugs from aggressive environments e.g. presence of certain enzymes and low pH of the stomach.

Natural polysaccharides play a vital role in developing solid dosage forms for drug delivery.[6] Mostly natural polymers are cheap and have attracted attention of researchers to prepare natural polymer based hydrogels.[7] In the present study gelatin and sodium alginate are used to prepare hydrogels. Both of these natural polymers are biodegradable and are employed for sustained release of drug as they are degraded within the human body.[8]

Gelatin is a product of protein prepared by hydrolyzing collagen (skin and connective tissues).[9] Amino acids including proline, glycine and hydroxy-proline are present in higher range in gelatin and others in lesser extent include aspartic acid, alanine, arginine and glutamic acid.[10] Gelatin contains ($-NH_3$) and (-COOH) as ionizing groups that swell at both lower and higher pH and this property of gelatin makes it a best option to develop hydrogels for sustained delivery of drug. Formation of thermo-reversible gels (100%) is also one of the properties of gelatin and it can be seen when gelatin is cooled below 35°C. Gels formed by gelatin are mostly stronger because of the presence of increase concentration of pyrrolidines in it. In order to form gel, gelatin has the capacity to absorb ten times its weight of water. Gelatin is insoluble in organic solvents (alcohol, CCl_4, ether and benzene). Gelatin can be used as a binding agent, a thickening agent and an encapsulating agent.

Sodium alginate (SA) belong to group of agents that were studied extensively and these agents were made of stiff linear polysaccharides. Sodium alginate belonged to the most commonly utilized gel forming agent obtained from seaweed. They contained residues of (β-1, 4-linked D-mannuronic acid) and (α-1, 4-linked L-glucuronic acid). They also have free –OH and –COOH groups for chemical modification. It can be used in the preparation of wound dressings because they have the ability to form gel when come in contact with moisture due to the formation of strong hydrophilic gel.[11] Hence, it is nontoxic, biocompatible and non-carcinogenic.[12, 13] These are the polysaccharides that can be utilized as chelators, emulsifiers and suspending agents and can also be used to prepare membranes. [14, 15]

Aldehydes with lower molecular weight such as formaldehyde and glutaraldehyde are used to harden gelatin [16] and crosslinking occurs through formation of Schiff bases. These bases are formed when (-NH_2) free groups in gelatin react with glutaraldehye.[17] Swelling of hydrogels is greatly affected by concentration of crosslinker. Hydrogels with higher degree of crosslinking will swell less than those with lower quantity of crosslinker.

Cetirizine dihydrochloride (CTZ HCl) is a potent second-generation histamine H_1 antagonist that is effective in the treatment of allergic rhinitis, chronic urticaria, and pollen-induced asthma. It is rapidly absorbed from the GI tract following oral administration with peak plasma concentrations achieved in about 1 hour. Unlike first-generation histamine H_1 antagonist, cetirizine is less able to cross the blood–brain barrier and induce drowsiness. However some serious side effects like somnolence, fatigue, dry mouth and Insomnia etc has been reported with Cetirizine HCl. So in order to control the toxicity associated with this drug, the delivery system need to be modified. Figure 1 indicates the chemical structure of Cetirizine HCl.

The main objective of the current study was to prepare pH sensitive Ge/SA hydrogels for sustained delivery of antihistaminic drug (Cetirizine HCl) to gastrointestinal tract (GIT). By developing these stimuli responsive polymeric hydrogel systems, the main idea was to provide the controlled delivery and metabolism of the drug and in turn to reduce the side effects associated with this drug. The prepared hydrogel samples were developed to obtain the following objectives; 1) To synthesize different hydrogel samples with different feed composition ratios and degree of crosslinking 2) To investigate the effect of composition and crosslinking ratio on dynamic and equilibrium swelling behavior in phosphate buffer solutions of variable pH values 3) To investigate

the effect of pH and composition on release of model drug in phosphate buffer solutions of variable pH values and to confirm the controlled delivery of model drug (Cetirizine HCl) 4) To evaluate sol-gel fraction analysis, porosity measurement, networking parameters and diffusion coefficient. 5) To evaluate the best release mechanism by applying various mathematical release models. 6) To confirm the network structure of hydrogels by various characterization tools like Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and differential scanning calorimetric (DSC) was used to look into the stability of the hydrogel samples respectively.

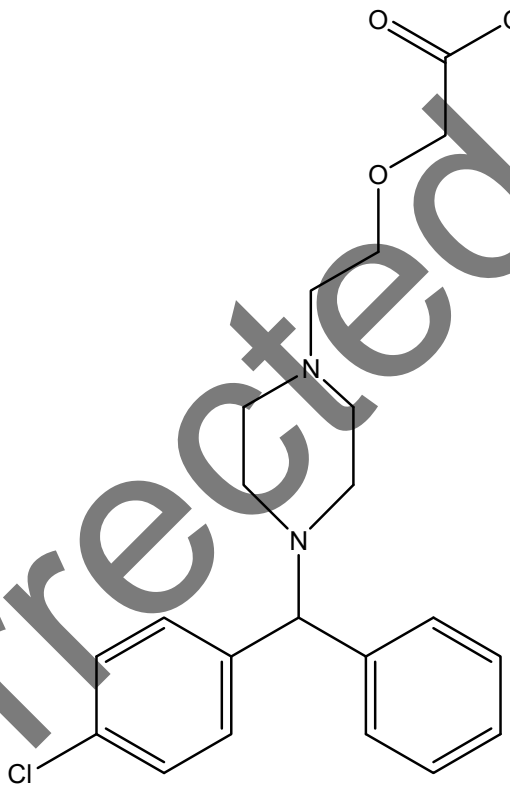

Figure 1. Structure of Cetirizine hydrochloride

MATERIALS AND METHODS

Materials

Gelatin type B from bovine skin (Ge) (Mw ~ 402.47 gmol−1) (Purity 98 %) (Merck, Germany) and Sodium Alginate (Merck, Germany) were used as polymers. Glutaraldehye (GA) was obtained from Merck-Schuchardt. Acetic acid used as catalyst was obtained from Merck-Schuchardt. Cetirizine HCl was gifted by Hamaz Pharma, Multan, Pakistan. Potassium bromide (KBr) of FTIR grade was purchased from Fisher Scientific (UK). Potassium dihydrogen phosphate, sodium hydroxide, sodium chloride were used as received. All chemicals used were of analytical grad. Double Distilled water was used for the preparation of hydrogels and buffer solutions.

Synthesis of pH sensitive Gelatin/Sodium Alginate (Ge/SA) Hydrogels

Ge/SA hydrogels crosslinked with glutaraldehyde (GA) was prepared at room temperature by free radical polymerization technique reported earlier with little modifications.[18] Briefly, Both the polymers were taken in different concentrations. Sodium Alginate solution was made by adding desired quantity of SA in bidistilled water at 60°C for 1 hr. The SA solution was then cooled down. Aqueous solution of gelatin was made by addition of weighed quantity of Gelatin in 3% solution of A.A (acetic acid) at a temperature of 40°C. Gelatin solution was placed at room temperature and then added to SA solution and was stirred for 45 minutes. Varying quantities of GA was added to this homogeneous mixture. Double distilled water was poured to make the final weight up to 50 grams. Prepared final homogeneous mixture was poured out in the test tubes (Pyrex) having 16 mm internal diameter and 150 mm length. The oxygen was removed from the glass tubes by nitrogen bubbling for 15 to 20 minutes. Oxygen can hinder the normal polymerization process. The tubes were capped and placed at room temperature for 72 hours. After complete polymerization and gel formation, hydrogel in cylindrical form was removed from tubes after 72 hours. Each cylinder was cut into 5mm length discs. These discs were dried at room temperature. After drying these discs were washed extensively with ethanol-water mixture (40:60) for complete removal of unreacted material. Throughout this time span the ethanol/water mixture was replaced every day until its pH becomes equal as pH of the water/ethanol mixture. Finally the synthesized Ge/SA discs were placed first at room temperature and afterwards in oven (vacuum) at 45°C until solid reached a stable mass. These prepared gelatin and sodium alginate hydrogels were stored in

vaccum desiccator for future usage [18]. Various formulations of SA/Ge hydrogels are given in Table 1. Figure 2 indicates the presumptive structure of Ge/SA hydrogels.

Table 1. Feed Composition of Various formulations of Ge/SA hydrogels.

Sample Codes	Gelatin (g) /100g solution	SA (g) /100g solution	Ge: SA	GA (g) /100g solution
S1	10.5	1.0	91.30/8.70	0.345
S2	10.5	1.5	87.5/12.5	0.360
S3	10.5	2	84/16	0.375
S4	10	2	83.33/16.67	0.360
S5	11	2	84.61/15.39	0.390
S6	12	2	85.71/14.29	0.42
S7	11	2	84.61/15.39	0.455
S8	11	2	84.61/15.39	0.487
S9	11	2	84.61/15.39	0.52

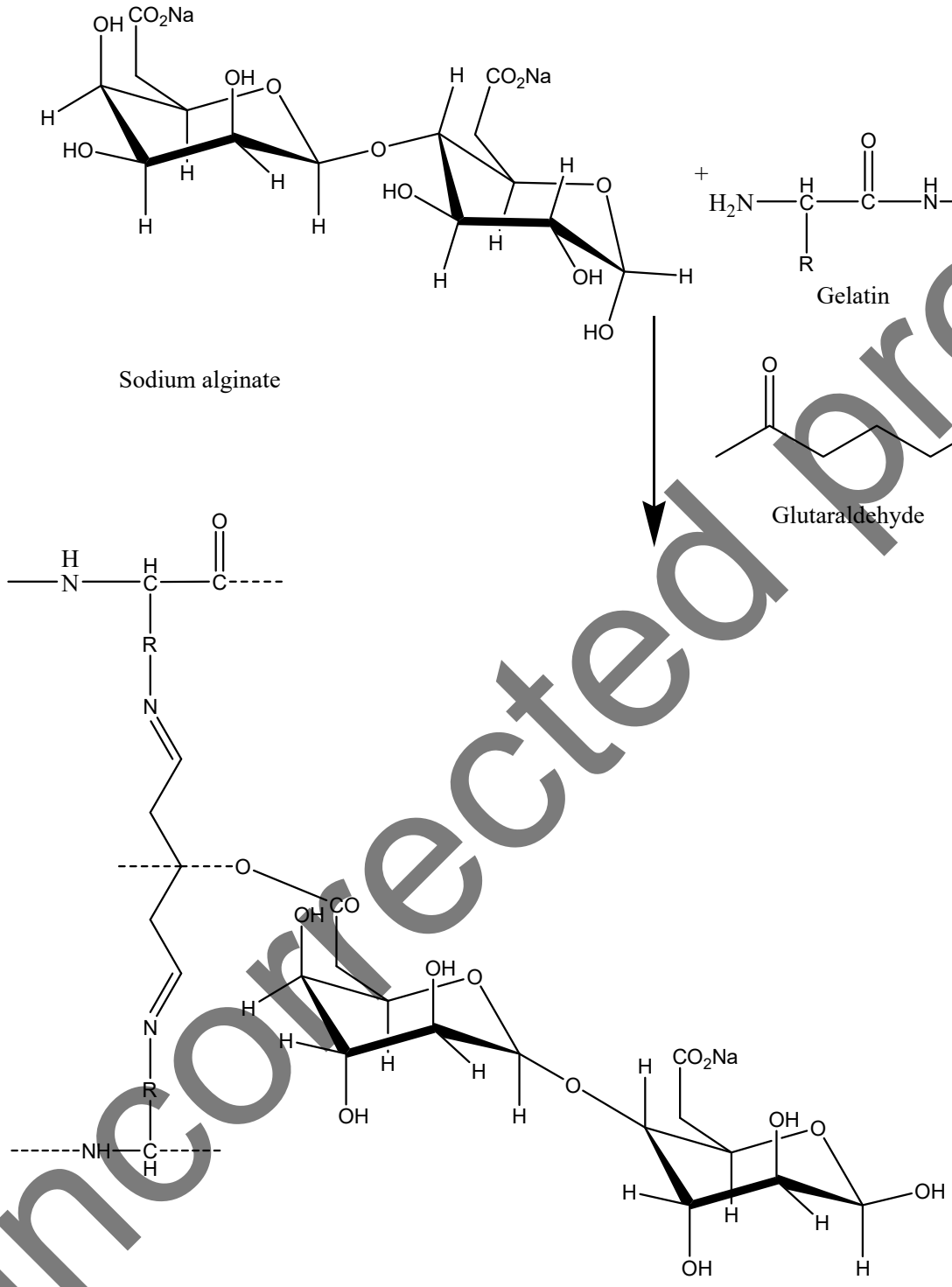

Figure 2. Proposed structure of Ge/SA hydrogel cross-linked with glutaraldehyde.

Swelling behavior of synthesized hydrogels

Preparation of buffer solutions

Phosphate buffer solutions of (pH 1.2, 5.5, 6.5 and 7.5) were prepared using Potassium Dihydrogen Phosphate (KH_2PO_4). Buffering agent concentration was 0.05 M. 0.2 M solution of HCL and NaOH was used to adjust the pH of these solutions. In order to maintain the ionic strength of these buffer solutions to I=0.65 M, NaCl was added.

Dynamic swelling studies

The swelling analysis was preceded in 100 ml USP PBS of pH 1.2, 5.5, 6.5 and 7.5. Pre-weighted dried hydrogel discs were allowed to swell in different pH solutions (1.2, 5.5, 6.5, 7.5) at room temperature i.e. 25-30°C. Swollen discs were taken out from the desired pH solutions at predetermined regular intervals of time. These discs were first blotted with filter paper to remove excess solution and were weighted and positioned in the similar bath solution. These studies were carried out for about 8 hours. The underlying relation denoted by (1) was used to determine the swelling ratio of the synthesized disc.[18, 19]

$$q = \frac{Wt - Wd}{Wd} \qquad (1)$$

Where W_t = mass of swollen gel at time t. W_d = original mass of dried hydrogel and q = the swelling coefficient.

Equilibrium swelling studies

Equilibrium swelling studies was conducted by allowing the hydrogel discs to swell till they attained a constant weight and reached equilibrium state. The discs at higher pH absorbed much water and became fragile. They must be handled carefully to avoid their breakage.

Following equation provided means to determine $Q_{eq.}$[19]

$$S_{(Eq)} = \frac{W_h}{W_d} \qquad (2)$$

W_h represents the mass of swollen gel at equilibrium, and W_d stands for original mass of dried hydrogel.

Diffusion coefficient

DC represents quantity of substance diffusing across a unit area through concentration gradient in unit time. (DC) is dependent on the amount and nature of chemicals in the polymer. Following equation was used for determination of DC. [20]

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

Where q_{eq} = swelling of gel at E_q , θ = slope of the linear part of the swelling curves, h=original thickness of gel before swelling and DC represents diffusion coefficient of the hydrogels.

Physicochemical characterization of Ge/SA hydrogels

For the evaluation of the structure and properties of hydrogel, we perform the underlying characterizations:

Volume fraction of the polymer

Polymer volume fraction is the quantity of fluid absorbed and retained by the gel in its swollen state.[21, 22] Following equation was used to determine the volume fraction of the polymer

$$V_{2s} = \left[1+\frac{d_p}{d_s}\left(\frac{M_a}{M_b}-1\right)\right]^{-1} \qquad (4)$$

Where, polymer density (gm/ml) is denoted with d_p and solvent density is denoted by ds. M_a and M_b are the masses taken in (g) of the swollen and dry hydrogels respectively. ($V_{2,s}$) units are ml/mol.

Determination of solvent interaction parameter (χ)

Solvent interaction parameter χ were calculated by means of Flory-Huggins theory .[23, 24] These parameters are used to determine whether the polymers are compatible with the molecules of the surrounding fluid. According to this theory, the underlying equation (5) was used to determine χ.

$$\chi = \frac{\ln(1 - V_{2,s}) + V_{2,s}}{V_{2,s}^2} \qquad (5)$$

$V_{2,s}$ = the volume fraction of swollen hydrogel in its equilibrium state.

Molecular weight between the crosslinks

Flory-Rehner theory was used to analyze this parameter. It can be predicted for Ge/ SA hydrogels by knowing the molecular weight between the crosslinks. It suggested that M_C values were amplified by increasing the swelling ratios of Ge /SA gels. Following relation was used to determine M_C.[24, 25]

$$M_c = \frac{d_p V_s (V_{2,s}^{1/3} - V_{2,s}/2)}{\ln(1 - V2,s) + V2,s + \chi V_{2,s}^2} \qquad (6)$$

Where, d_p denoted the density of the polymer and d_s represents the density of the solvent. $V_{2,s}$ = the volume fraction of the swollen gel and χ = Flory-Huggins parameters.

Cross-linked density (N)

It is defined as the number of links between two cross-linked chains. In order to measure N following equation was used.[26]

$$N = \frac{2M_c}{M_r} \qquad (7)$$

M_r =Molecular weight of the repeating unit and was determined using the underlying equation;

$$M_r = \frac{m_{SA}M_{SA} + m_{Ge}M_{Ge} + m_{GA}M_{GA}}{m_{SA} + m_{Ge} + m_{GA}} \quad (8)$$

Here m_{SA}, m_{Ge}, m_{GA} are the feed masses of sodium alginate, gelatin and glutaraldehyde respectively. M_{SA}, M_{Ge}, M_{GA} are the molar masses of sodium alginate, gelatin and glutaraldehyde.

Sol-gel analysis

Uncross linked polymer from the gel structure was determined by sol-gel analysis. For this purpose unwashed samples were cut into 3-4 mm size. The prepared sample discs were placed at room temperature for complete drying and afterwards in vacuum oven at 45°C to an invariable weight. Weighed discs were placed for Soxhelt extraction at 85°C minimum upto 4 hours. This process of extraction will remove the uncrosslinked polymer from the hydrogel. These extracted hydrogels were placed in the oven at 45°C for drying until constant weight is achieved. Gel fraction was measured by taking into consideration the initial dry weight (W_o) and extracted dry gel (W_i) weight by using underlying relation.[20, 26]

$$\text{Sol fraction (\%)} = \left[\frac{W_o - W_1}{W_o}\right] \times 100 \quad (9)$$

$$\text{Gel fraction (\%)} = (100 - \text{Sol fraction}) \quad (10)$$

Measurement of porosity

It is the measure of the presence of voids over the total volume of hydrogels between, 0-1 and in the form of percentage as 0-100%. These dried gelatin/SA hydrogels were dipped in absolute ethanol for a whole night and excess of ethanol was blotted with the help of filter paper. These blotted hydrogels were then weighed and porosity was determined by underlying relation.

$$\text{Porosity} = \frac{(M_2 - M_1)}{\rho V} \times 100 \quad (11)$$

Here, M_1= weight of hydrogel before placing in the absolute ethanol M_2 =weight obtained after immersion in ethanol. ρ stands for density of absolute ethanol. V = the volume of hydrogel.[20]

Preparation of drug loaded hydrogels

Loading and release studies of CTZ HCl were carried out in those Ge/SA hydrogel samples that had maximum swelling. These discs were loaded with drug by dipping them in 1% w/v aqueous solution of Cetirizine hydrochloride. The desired solution of cetirizine hydrochloride was made by dissolving the drug in water. Discs were allowed to remain in cetirizine hydrochloride solution till equilibrium swelling. Swollen hydrogels were taken out and first dried by placing them at room temperature followed by oven drying at 46°C to a consistent weight.[18]

Measuring the Cetirizine Hydrochloride loading

Three methodologies were used to measure the amount of cetirizine hydrochloride loaded in the Ge/ SA hydrogels.[20] Relation used to determine the amount of cetirizine loaded by weight method is as under:

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

$$\text{Drug Loading}\,\% = \frac{W_D - W_d}{W_d} \times 100 \quad (13)$$

In this relation W_d = weight of dry hydrogels before the loading of drug and W_D is the weight of drug loaded dried gels.

In the swelling method, weighted hydrogel disc was placed in cetirizine hydrochloride solution upto the equilibrium swelling. The hydrogels loaded with drug were taken out and weighted once more after blotting with blotting paper to determine the amount of absorbed drug solution. Difference in weight of the gels gave the volume of cetirizine hydrochloride loaded or entrapped in the Ge/SA hydrogels. Amount of cetirizine hydrochloride was calculated from the volume.

In the extraction method the drug was determined by extracting time after time the weighted amount of loaded gels in the presence of distilled water. In each turn 25 ml new distilled water was added until there was no drug left in the solution. Quantity of CTZ HCl was measured using spectrophotometer. Quantities of drug present in all parts of the extract were added and this provided means to measure the total drug loaded.

Release studies of Cetirizine hydrochloride

In-vitro dissolution test was used to determine the release of cetirizine hydrochloride, freely soluble in water which involved the use of dissolution apparatus 2 in association with UV-spectrophotometer (IRMECO, UV-Vis U2020). The pulsatile drug release profile of drug at equal intervals of time was obtained in dissolution medium comprised of various pH values (1.2, 5.5, and 7.5).The weighted Ge /SA gel discs were placed in 900 ml dissolution medium at 37 ± 2°C. The prepared medium was kept stirring at 100rpm to evenly distribute the released drug in the medium. Cetirizine hydrochloride release study was conducted at 229 nm up to 12 hours. Each time 5ml of dissolution medium was taken for UV analysis to verify the concentration of drug. The withdrawn solution was replaced with same amount of new 0.05 M PBS.[18]

Analyzing the pattern of drug release

In order to evaluate the drug release data zero order, first order, higuchi and korsmeyer-peppas model were employed. To achieve controlled release of drug it is necessary that the drug diffuses faster than the swelling of gel. Equations employed for the above mentioned models are:

Zero-order kinetics: [27] $F_t = K_o t$ (14)

F_t is the fraction of release of drug in time "t" and "K_o" is the zero-order release constant.

First-order kinetics: [27] $In\ (1-F) = -K_1 t$ (15)

Frepresents the fraction of drug release in time t and K_1 is the first-order release constant.

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

F represents fraction of drug release in time t and K_2 is the higuchi constant.

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

M_t is the mass of water absorbed at time t, M_∞ is the quantity of water at equilibrium, K_3 describes the swelling mechanism [28, 29] and n is the release exponent.

Characterization of Ge/SA hydrogels

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was done in the DSC unit (Netzsch DSC 200 PC Phox, Germany). The samples were heated in a close aluminum pan at temperature of 40°C/ minute. Nitrogen was used as a purge gas with a flow rate of 50ml/min.[20]

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) for drug loaded and unloaded hydrogel was performed using Bruker D8 Discover (Germany) apparatus. Measurement conditions included target (CuKα), voltage (35 KV), and current (35 mA). A system of diverging, receiving, and anti-scattering slits of 1º, 1º, 1º, 0.15º, respectively, was used. The percentage crystallinity was determined using the underlying equation .[20]

$$\% \text{ Crystallinity} = \text{Crystalline area} / \text{Total area} \times 100 \qquad (18)$$

Fourier Transformed Infra-Red (FTIR) Spectroscopic Analysis

Cross-linked hydrogel samples were crushed with pestle in an agate mortar. The crushed material was mixed with potassium bromide (Merck IR spectroscopy grade) in 1:100 proportions and dried at 40°C. The mixture was compressed to a 12 mm semitransparent disk by applying a pressure of 65 kN (Pressure gauge, Shimadzu) for 2 min. The FT-IR spectrum over the wavelength range 4,500-400 cm^{-1} were recorded using FTIR spectrometer (FT-IR 8400 S, Shimadzu).[18]

Statistical Analysis

For the statistical analysis of data, Student's t-test has been applied to compare the results and to determine the statistical significant/ non-significant interpretation at 95% confidence interval, P-value less than 0.05 was considered as significant difference in results. Data were displayed as mean ± SD (Standard deviation).

RESULTS AND DISCUSSION

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

Prepared hydrogels containing SA and Ge were used to investigate the pH responsive behavior in Phosphate buffer solution of various pH values. Those hydrogels that were sensitive to pH, their swelling was dependent on pK_a and pH of the swelling medium. These prepared hydrogels contained both NH_2 (amine) and -COOH (carboxylic group) and were referred as polyampholytic gels. These hydrogels showed maximum swelling in pH 1.2 solution and second highest swelling in pH buffer 7.5. Swelling of hydrogels was maximum at pH 1.2 because of protonation of $–NH_2$ groups and then these amine groups were ionized. Electrostatic repulsion of similar charges was main cause of swelling in these hydrogels.[30] At pH 7.5 (–COOH) groups present in both SA and Ge were changed to (COO^-) group and resulted in anion-anion repulsion which eventually increased the swelling of hydrogels. The results of swelling studies (dynamic and equilibrium swelling) are shown in Table 2 which showed that swelling of the hydrogels were decreased when pK_a value of the swelling medium was less than that of polymer. These synthesized SA/Ge hydrogels can swell at both acidic and basic pH. So, they can be used for the sustained delivery of drug.

For loading of cetirizine hydrochloride those hydrogel samples were selected that showed maximum swelling. Cetirizine Hydrochloride was selected as a model drug because of its solubility in water. Hydrogel samples prepared with different degrees of crosslinking agents (S_7-S_9) and with increased quantity of gelatin (S_4-S_7) were selected for the loading of drug. Table 3 shows the amount of loaded drug in selected samples.

In order to determine the effect of pH on cetirizine hydrochloride release, the hydrogel samples loaded with cetirizine hydrochloride were immersed in solutions of pH 1.2, 5.5 and 7.5. Dissolution apparatus was used to determine the release of drug. Maximum amount of drug was released when the hydrogels were immersed in pH solution of 1.2 and second highest release of drug was observed in pH solution of 7.5 and minimum amount of drug was released in pH solution of 5.5. Table 4 refers to the effect of pH of the dissolution medium on % drug release of Ge/SA hydrogels.

Table 2. Dynamic and equilibrium swelling values of Ge/SA hydrogels using GA as a crosslinker.

Sample code	Dynamic swelling coefficient				Equilibrium swelling coefficient			
	pH 1.2	pH 5.5	pH 6.5	pH 7.5	pH 1.2	pH 5.5	pH 6.5	pH 7.5
S_1	4.298	3.515	3.45	3.78	10.64	4.33	x	x
S_2	4.26	3.373	3.346	3.75	9.9	4.31	x	x
S_3	4.129	3.305	3.32	3.704	9.6	3.915	x	x
S_4	4.411	3.62	3.73	3.803	10.70	4.93	x	x
S_5	4.379	3.65	3.74	3.918	11.15	4.99	x	x
S_6	4.59	3.68	3.79	3.928	12.98	5.11	x	x
S_7	4.31	3.54	3.63	3.64	11.00	5.00	x	x
S_8	4.29	3.53	3.63	3.603	10.52	4.805	x	x
S_9	3.8	3.5	3.511	3.56	9.70	3.915	5.8	x

X= sample broken

Effect of Ge concentration on swelling and drug release from Ge/SA hydrogels

Gelatin is a natural polymer and its concentration was changed in different hydrogel samples, ranging from 10, 11 and 12 g/100g in Ge/SA hydrogels with glutaraldehyde as a crosslinker. Three samples with different concentrations of Ge (S_4 to S_6) were synthesized and used to analyze the effect of gelatin on dynamic and equilibrium swelling and on the release of cetirizine hydrochloride from the hydrogels. It was observed that with an increase in gelatin content an increase in drug release and swelling was noted. This increase is suggested to be due to the presence of ionizable (NH_2) and ($COOH^-$) groups which increase the spaces between the polymer chains and swelling of hydrogels was increased due to hydrostatic repulsion.[31] Figure 3 indicates the effect of different concentrations of Ge on dynamic swelling coefficient of Ge/SA hydrogels in PBS of various pH values. The quantity of drug release from these hydrogels was observed via dissolution. When quantity of gelatin was increased an increase in drug release was observed. The drug release increased from 78.15% to 81.28% in pH of 1.2, 49.86% to 52.31% in pH of 5.5 and 66.72% to 70.21% in pH of 7.5. Figure 9-11 indicates the effect of different concentration of Ge on *invitro* release of Cetirizine HCl as function of time from Ge/SA hydrogels in PBS of various pH values.

Table 3. Amount of Cetirizine Hydrochloride loaded in formulations of Ge/SA hydrogels

Sample codes	Amount of Cetirizine Hydrochloride loaded (g/g of dry gel)	
	By swelling	By extraction
S_4	0.078	0.0715
S_5	0.082	0.0798
S_6	0.083	0.0802
S_7	0.078	0.0750
S_8	0.073	0.0699
S_9	0.06225	0.0604

Sodium alginate effect on the swelling and on drug release from Ge/SA hydrogels

Concentration of sodium alginate used in the preparation of hydrogels ranged from 1, 1.5 to 2g/100g of the sample solution (S_1 to S_3) with constant amount of gelatin and glutaraldehyde. A decrease in swelling was observed with an increase in the SA content. This decrease was due to the presence of pores in the matrix of sodium alginate that hindered the diffusion of water into SA/Ge hydrogels. Bajpai *et al.*, also experienced the similar pattern for hydrogels prepared with sodium alginate.[32] Figure 4 refers to the impact of SA on dynamic swelling coefficient of Ge/SA hydrogels in PBS of variable pH values.

Effect of cross linker quantity on the swelling behavior and drug release from Ge/SA hydrogels

Extent of crosslinking was a major factor that affected swelling and CTZ HCl release properties of hydrogels. To highlight this factor, hydrogels prepared with different concentrations (S_7 to S_9) of glutaraldehyde (3.5%, 3.75% and 4%) keeping the quantity of both the polymers constant (SA/Ge = 2/11g). When increased quantity of crosslinker was used densed networks were produced with shrinked mesh size and a tighter structure. This resulted in minimum spaces for the entrance and accommodation of water and swelling decreased.[33] Figure 5 refers to the effect of

GA on dynamic swelling coefficient of Ge/SA hydrogels in PBS of variable pH values. Drug release from hydrogels is dependent on the swelling and samples with minimum amount of crosslinker showed maximum swelling and drug release. When the quantity of crosslinker was increased the drug release was decreased from 85.56% to 80.06% in pH of 1.2, 51.02% to 48.96% in 5.5 and 72.86% to 69.01% in pH solution of 7.5 respectively. Figure 12-14 indicates the effect of different concentration of GA on *invitro* release of Cetirizine HCl as function of time from Ge/SA hydrogels in PBS of various pH values.

Table 4. Cetirizine hydrochloride released (%) from various formulations of Ge/SA hydrogels.

Sample Codes	pH 1.2	pH 5.5	pH 7.5
S_4	78.15	49.86	66.72
S_5	79.52	50.21	67.87
S_6	81.28	52.31	70.21
S_7	85.56	51.02	72.86
S_8	83.85	49.53	70.99
S_9	80.06	48.96	69.01

Molecular weight between crosslinks (m_c) and solvent interaction parameters

Concentration of gelatin has direct relation with M_c values. An increase in gelatin concentration enhanced the Mc values. Higher swelling is due to the presence of (-COOH) and (-NH_2) groups in Ge. X and $V_{2,S}$ values increased by increasing the concentration of SA and crosslinker and decreased with an increase in concentration of gelatin. Mc value has direct relation with gelatin concentration and is inversely proportional to the SA and GA concentration. The values of the structural parameters are elaborated in Table 5.

Table 5. Flory-Huggins network parameters of Ge/SA hydrogel.

Sample codes	$V_{2,s}$	χ	M_c	M_r	q	$D\times 10^{-5}$ (cm^2sec^{-1})
S_1	0.01237	-0.5021	379.0811	327.0548	10.64	0.017051
S_2	0.01461	-0.5065	268.6011	323.353	9.9	0.015598
S_3	0.01627	-0.5069	208.9656	319.89	9.6	0.04494
S_4	0.01789	-0.5077	182.6242	317.1373	10.7	0.031071
S_5	0.01280	-0.5062	210.5662	322.4706	11.15	0.037747
S_6	0.00811	-0.5060	280.9112	327.1765	12.98	0.038721
S_7	0.01307	-0.5225	502.5351	315.7327	11	0.52161
S_8	0.01330	-0.5341	422.0532	312.5146	10.52	0.045609
S_9	0.01401	-0.5347	371.2564	309.3912	9.7	0.032161

Diffusion coefficient of polymers (D)

Diffusion coefficient is an indirect method to determine the amount of solute diffused in the polymer network of the hydrogel. It can be better measured using fick's law of diffusion. Diffusion coefficient was found to be decreased when increased concentration of SA and glutaraldehyde was used. Diffusion coefficient increased with increasing concentration of gelatin because swelling increases with increased concentration of gelatin. Table 5 indicates the values of D.

Sol-gel analysis

Technique of sol-gel analysis was carried out to determine the uncrosslinked polymer concentration in hydrogel. Different compositions of SA/Ge were used to determine the effect of polymers and degree of crosslinking on the gel fraction of hydrogels. Gel fraction increased with an increase in concentration of SA, Ge and GA and sol fraction was decreased. This mechanism was observed due to enhanced grafting and increased concentrations of polymers (SA and Ge) and crosslinker resulted in extensive crosslinking and this mechanism was not observed with lower concentrations of these agents. The similar findings were seen by Ranjha *et al.*, who prepared hydrogels composed of chitosan and acrylic acid.[27] Natural polymers showed increase in gel fraction at higher concentration. Table 6 indicates the gel fraction (%) of Ge/SA hydrogels.

Table 6. Gel fraction and Porosity % of various formulations of Ge/SA hydrogels.

Sample Codes	Amount of GA %	Gel fraction %	Sol fraction (%)	Porosity %
S_1	3.00	81.64	18.36	10.21
S_2	3.00	82.50	17.50	12.01
S_3	3.00	83.23	16.77	13.65
S_4	3.00	87.10	12.90	14.66
S_5	3.00	89.90	10.10	21.45
S_6	3.00	91.81	8.91	28.25
S_7	3.50	89.96	10.04	24.66
S_8	3.75	91.12	8.88	20.10
S_9	4.00	93.30	6.70	10.54

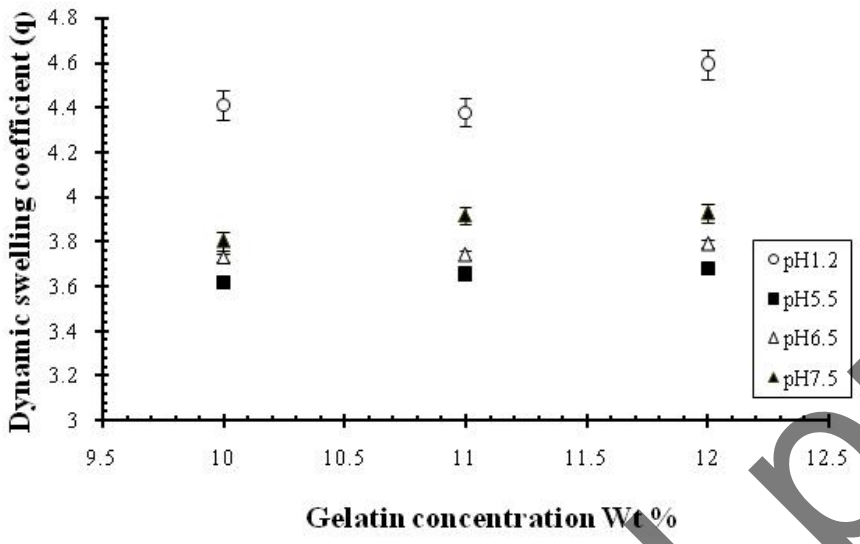

Figure 3. Dynamic swelling behavior of SA/Ge hydrogels with different concentrations of Ge (S_4-S_6), keeping concentration of SA and GA constant in solutions of various pHs (1.2, 5.5, 6.5 and 7.5).

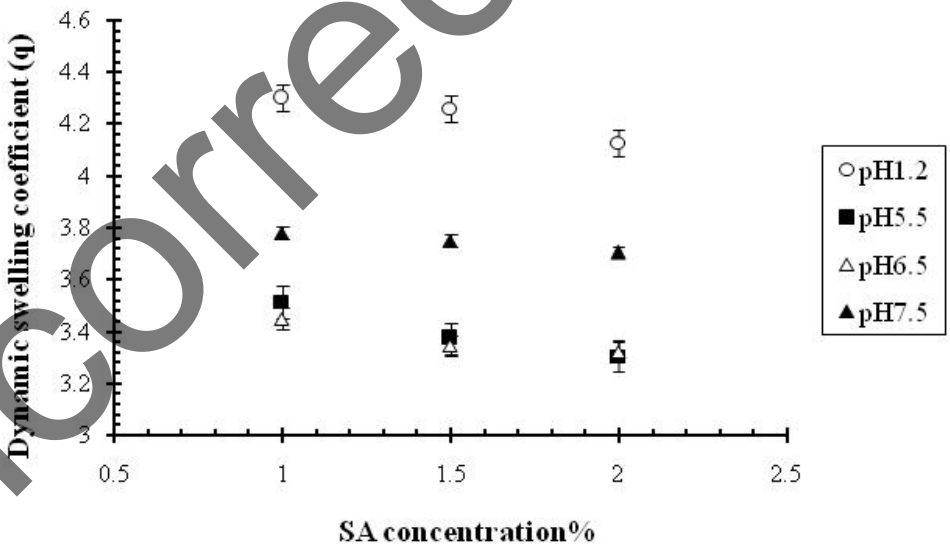

Figure 4. Dynamic swelling behavior of SA/Ge hydrogels with different concentrations of SA (S_1-S_3), keeping concentrations of Ge and GA constant in various pH solutions (1.2, 5.5, 6.5 and 7.5).

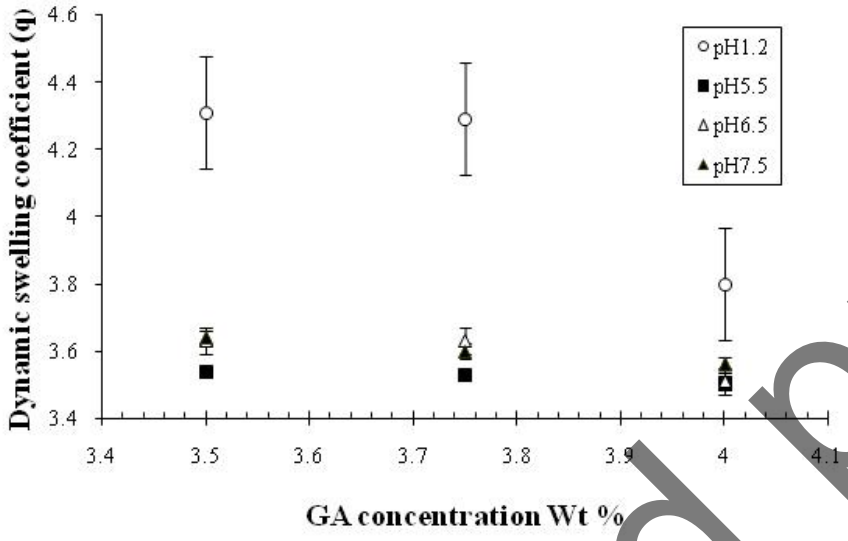

Figure 5. Dynamic selling behavior of SA/Ge hydrogels with different concentrations of GA (S_7-S_9) keeping SA and Ge constant at solutions of various pH (1.2, 5.5, 6.5 and 7.5).

Porosity

It was analyzed that by increasing the concentration of sodium alginate and gelatin in the hydrogel, the porosity increases. Porosity increases because both SA and Ge increased the viscosity of the resulting solution. Viscous solutions had the capability to prevent the escape of bubbles from the solution. Viscous solutions also limited the movement of free radicals and resulted in impaired polymerization and as a result porosity increases.

By increasing the concentration of GA, porosity deceases. Increased crosslinker concentration causes the shrimkage in mesh size of the resulting gels, lesser pores were formed and eventually porosity decreases. Figures (6-8) indicates the effect of variables on porosity % of Ge/SA hydrogels. Ranjha et al., used chitosan to prepare hydrogels and results showed that chitosan formed a viscous solution and entrapped the bubbles in it which lead to voids in the hydrogel matrix. Table 6 indicates the Porosity (%) of Ge/SA hydrogels.

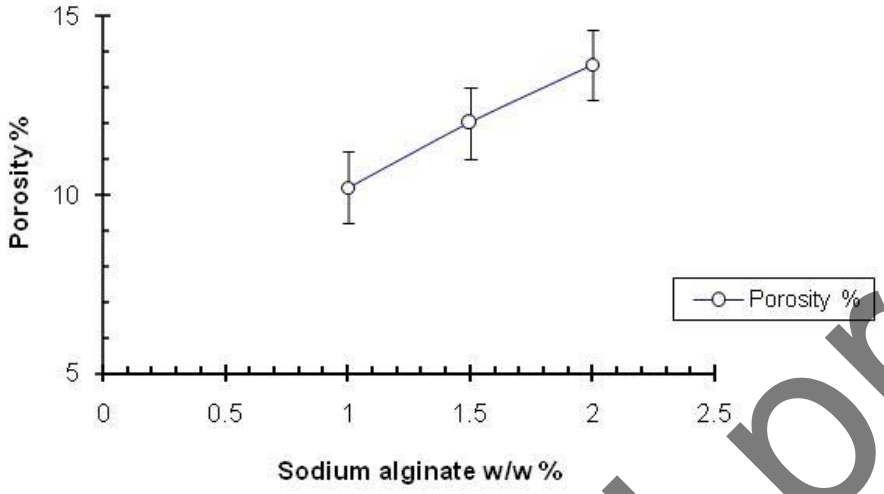

Figure 6. Effect of different concentrations of SA (1, 1.5 and 2g) on porosity % of Ge/SA hydrogels.

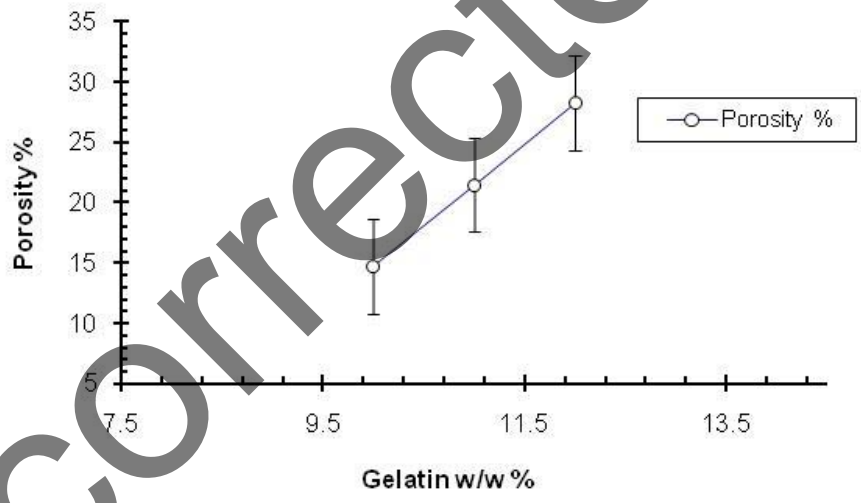

Figure 7. Effect of different concentrations of Ge (10, 11 and 12g) on porosity % of Ge/SA hydrogels.

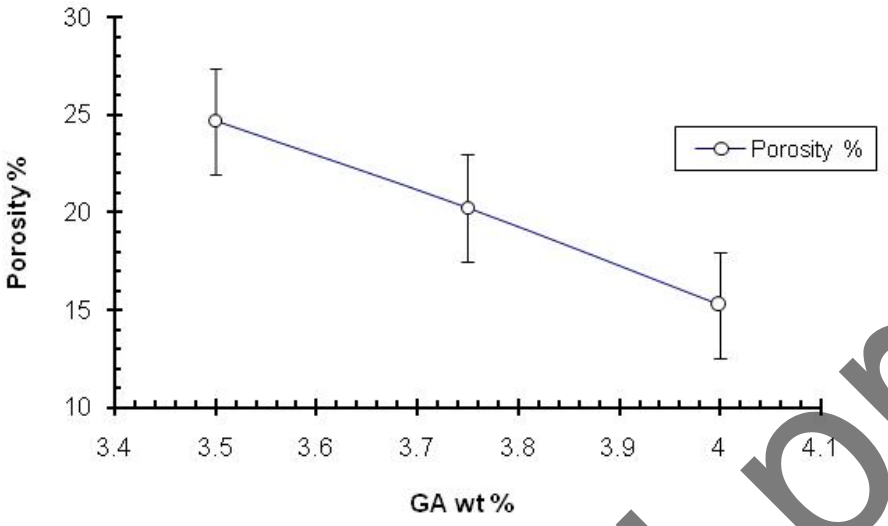

Figure 8. Effect of different concentrations of GA (3.5, 3.75 and 4%) on porosity % of Ge/SA hydrogels.

Cetirizine hydrochloride release mechanism

When hydrogels are immersed in water, they swell due to diffusion of water molecules in the polymeric network. This swelling of hydrogels leads to the release of drug which in this case is cetirizine hydrochloride. The most appropriate method to determine best model for drug is based on values of regression coefficient denoted by r. The model should have r value close to one.

Regression coefficient value (r) with different concentrations of Ge and GA are given in Table 7 and 8. The r values of Higuchi model at various Ge and GA concentrations showed greatest linearity and cetirizine hydrochloride release mechanism is found to be diffusion controlled.

Effect of varying amounts of Ge and GA on release exponent n at different pH solutions is shown in Table 9 and 10. All the values lie between 0.5-1.0 and no n value is above or below this range which shows non-fickian behavior at various pHs (1.2, 5.5 and 7.5). This means that cetirizine hydrochloride release from hydrogels is due to swelling and relaxation of polymers that in this case is Ge and SA.

Table 7. Effect of various concentrations of Ge on drug release kinetics of Ge/SA hydrogel in varying pH solutions using GA as a crosslinker.

Sample Codes	Ge contents	pH	Zero order kinetics		First order kinetics		Higuchi Model	
			K_o (h^{-1})	R^2	K_1 (h^{-1})	R^2	K_2 (h^{-1})	R^2
S_4	10	1.2	6.324	0.990	0.122	0.991	0.270	0.998
		5.5	4.024	0.997	0.054	0.981	0.167	0.954
		7.5	5.426	0.989	0.089	0.998	0.233	0.999
S_5	11	1.2	6.658	0.992	0.128	0.988	0.283	0.990
		5.5	4.014	0.993	0.054	0.969	0.165	0.930
		7.5	5.692	0.986	0.094	0.998	0.244	0.996
S_6	12	1.2	6.500	0.968	0.14	0.998	0.282	0.999
		5.5	4.053	0.991	0.056	0.974	0.170	0.959
		7.5	5.821	0.983	0.103	0.998	0.251	0.998

Table 8. Effect of various quantities of GA on drug release kinetics of Ge/SA hydrogel in solution of various pH values.

Samples No	GA Contents	pH	Zero order kinetics		First order kinetics		Higuchi Model	
			$K_o(h^{-1})$	R^2	$K_1(h^{-1})$	R^2	$K_2(h^{-1})$	R^2
S_7	3.5%	1.2	6.754	0.988	0.153	0.990	0.290	0.999
		5.5	4.262	0.999	0.058	0.994	0.179	0.972
		7.5	5.853	0.988	0.106	0.998	0.251	0.999
S_8	3.75%	1.2	6.695	0.981	0.150	0.992	0.288	0.998
		5.5	3.860	0.990	0.052	0.968	0.160	0.942
		7.5	5.502	0.984	0.097	0.995	0.236	0.997
S_9	4%	1.2	6.212	0.996	0.127	0.992	0.265	0.993
		5.5	3.877	0.989	0.054	0.999	0.166	0.997
		7.5	5.508	0.991	0.095	0.998	0.235	0.997

Table 9. Effect of Ge concentration on drug release mechanism of Ge/ SA hydrogels in buffer solutions of various pH.

Samples No	Ge Contents	pH	Release exponent (n)	R^2	Order of release
S_4	10	1.2	0.863	0.999	Non-fickian
		5.5	0.967	0.998	Non-fickian
		7.5	0.927	0.989	Non-fickian
S_5	11	1.2	0.933	0.990	Non-fickian
		5.5	0.944	0.994	Non-fickian
		7.5	0.953	0.995	Non-fickian
S_6	12	1.2	0.799	0.988	Non-fickian
		5.5	0.917	0.990	Non-fickian
		7.5	0.889	0.988	Non-fickian

Table 10. Effect of GA concentration on the release kinetics of drug from Ge/SA hydrogels when placed in various pH solutions.

Sample Codes	GA Contents	pH	(Release exponent) "n"	R^2	Release order
S_7	3.5%	1.2	0.781	0.998	Non-fickian
		5.5	0.965	0.997	Non-fickian
		7.5	0.854	0.990	Non-fickian
S_8	3.75%	1.2	0.784	0.992	Non-fickian
		5.5	0.952	0.990	Non-fickian
		7.5	0.830	0.980	Non-fickian
S_9	4%	1.2	0.702	0.999	Non-fickian
		5.5	0.777	0.996	Non-fickian
		7.5	0.784	0.999	Non-fickian

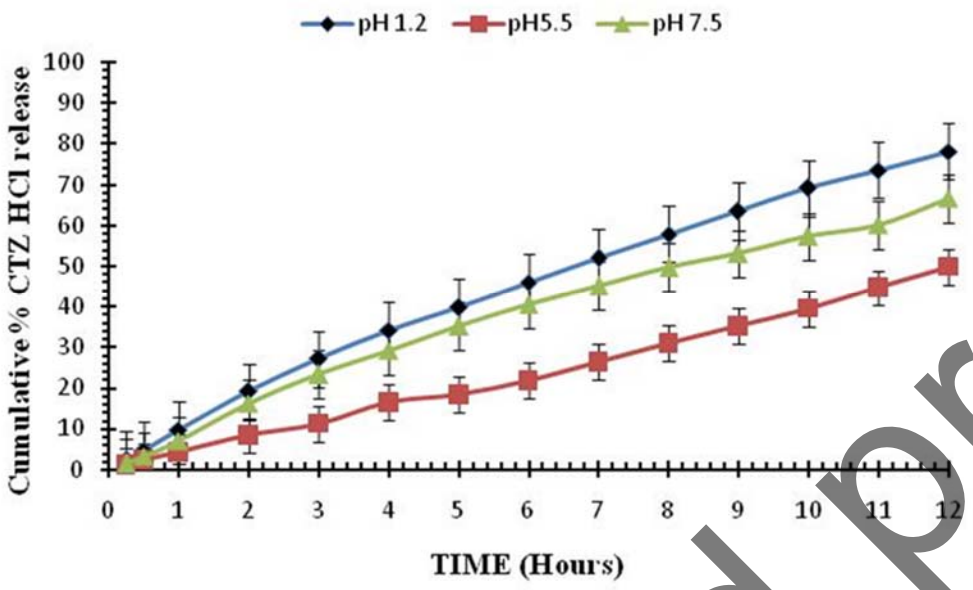

Figure 9. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (10/2 g) in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments.

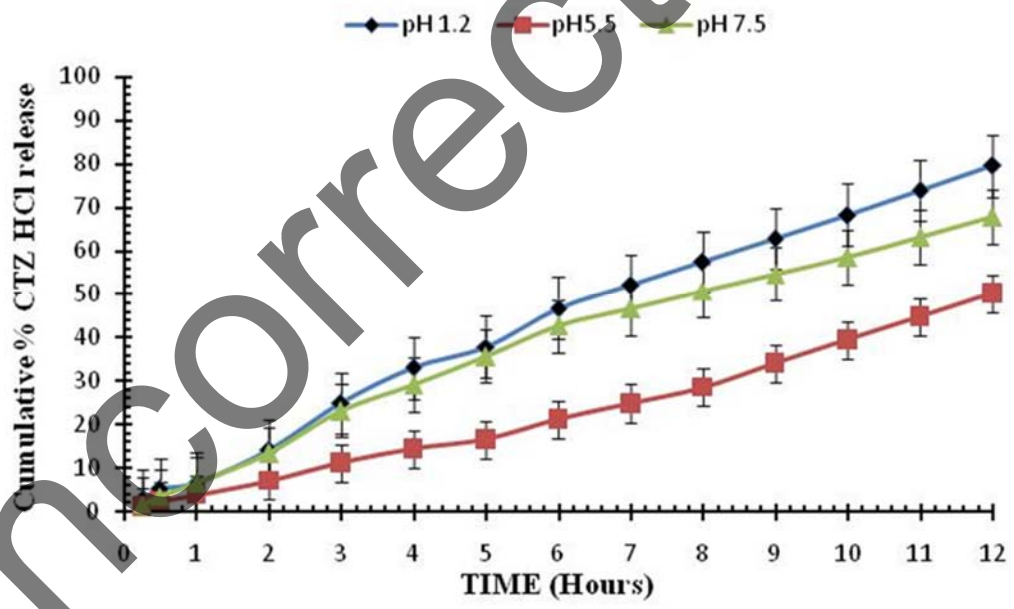

Figure 10. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (11/ 2 g) in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments.

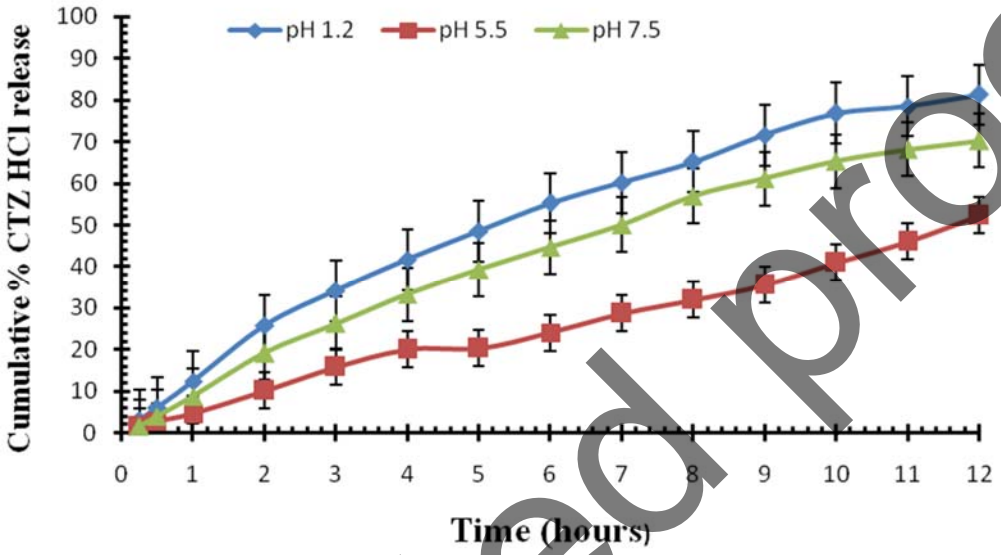

Figure 11. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (12/ 2 g) in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments

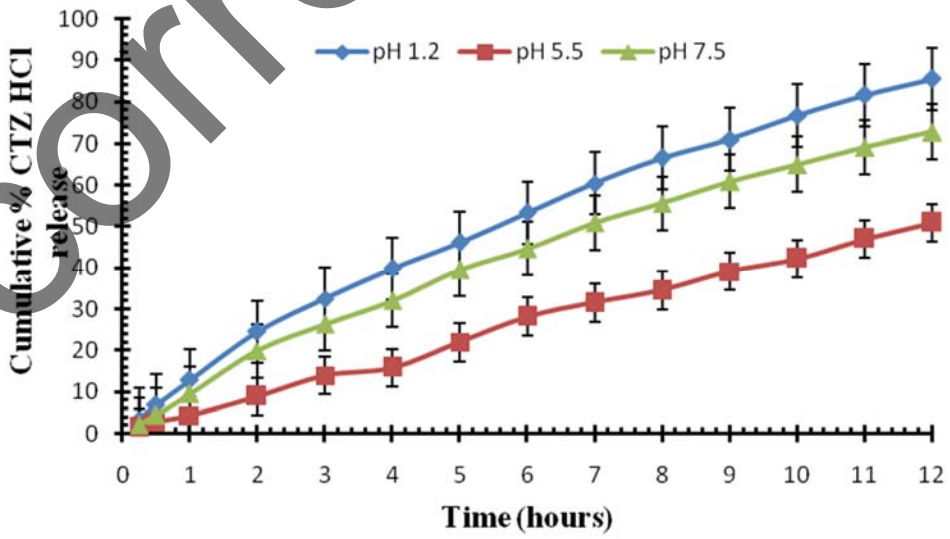

Figure 12. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (11/ 2 g) using 3.5 % GA in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments

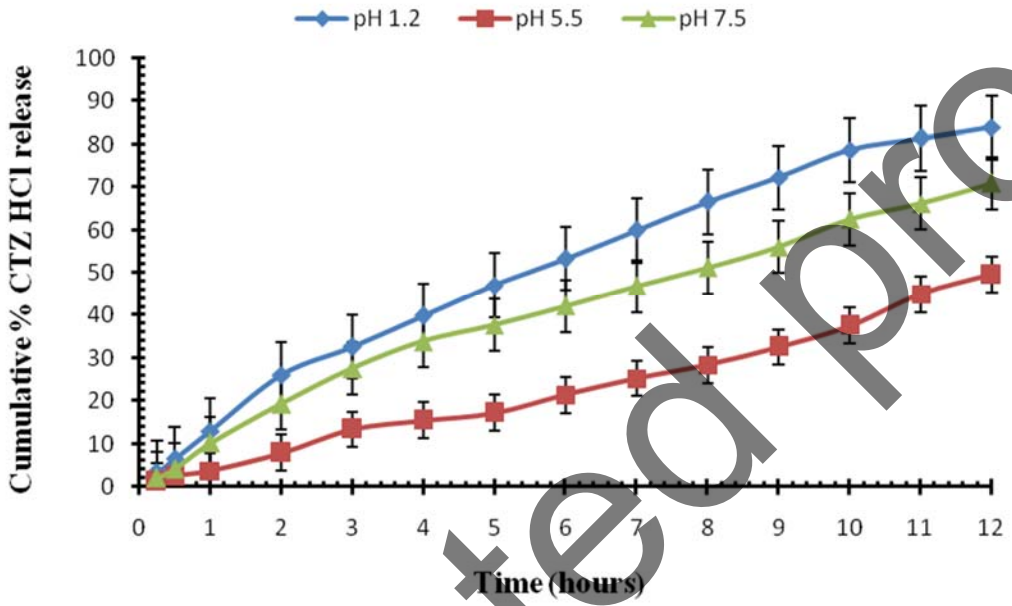

Figure 13. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (11/ 2 g) using 3.75 % GA in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments

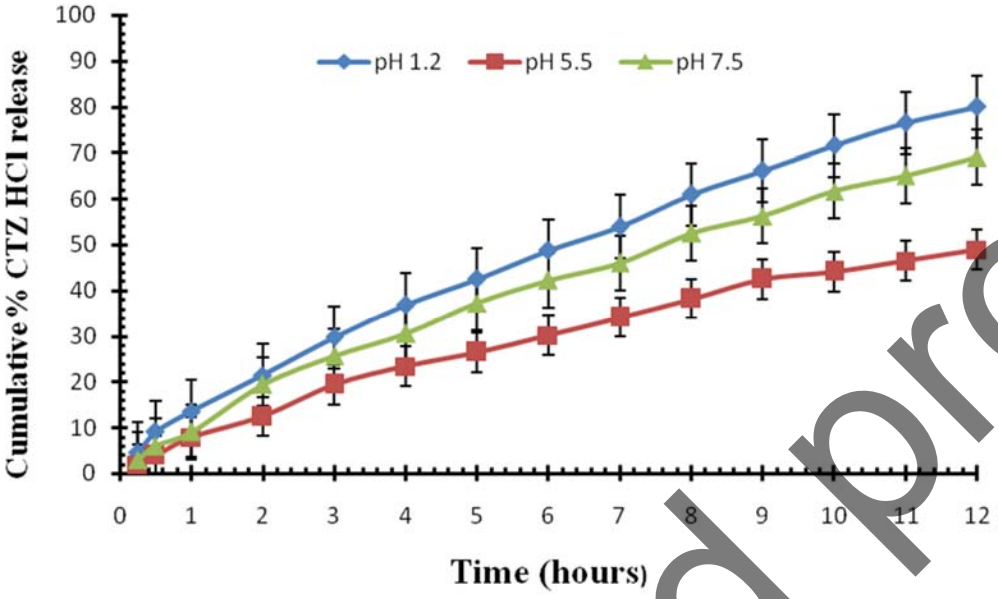

Figure 14. Pulsatile Cumulative % drug release of cetirizine hydrochloride from Ge/SA hydrogels (11/ 2 g) using 4 % GA in phosphate buffer solutions of various pH values. Each point represent the mean ± standard deviation of n=3 experiments

Characterization of Ge/SA Hydrogels

Differential scanning calorimetry

DSC thermograms of pure drug, unloaded, and drug-loaded hydrogels are presented in Figure 15. The thermograms of DSC clearly indicates a sharp melting peak of cetirizine hydrochloride at about 201.3°C followed by a decomposition peak at about 260°C. The drug-loaded hydrogel showed an absence of drug melting peak which indicates molecular dispersion of drug in the prepared hydrogels. The unloaded sample did not show any endothermic transitions due to rigid polymer network structure because of chain entanglement. The prepared hydrogels are found to be stable.

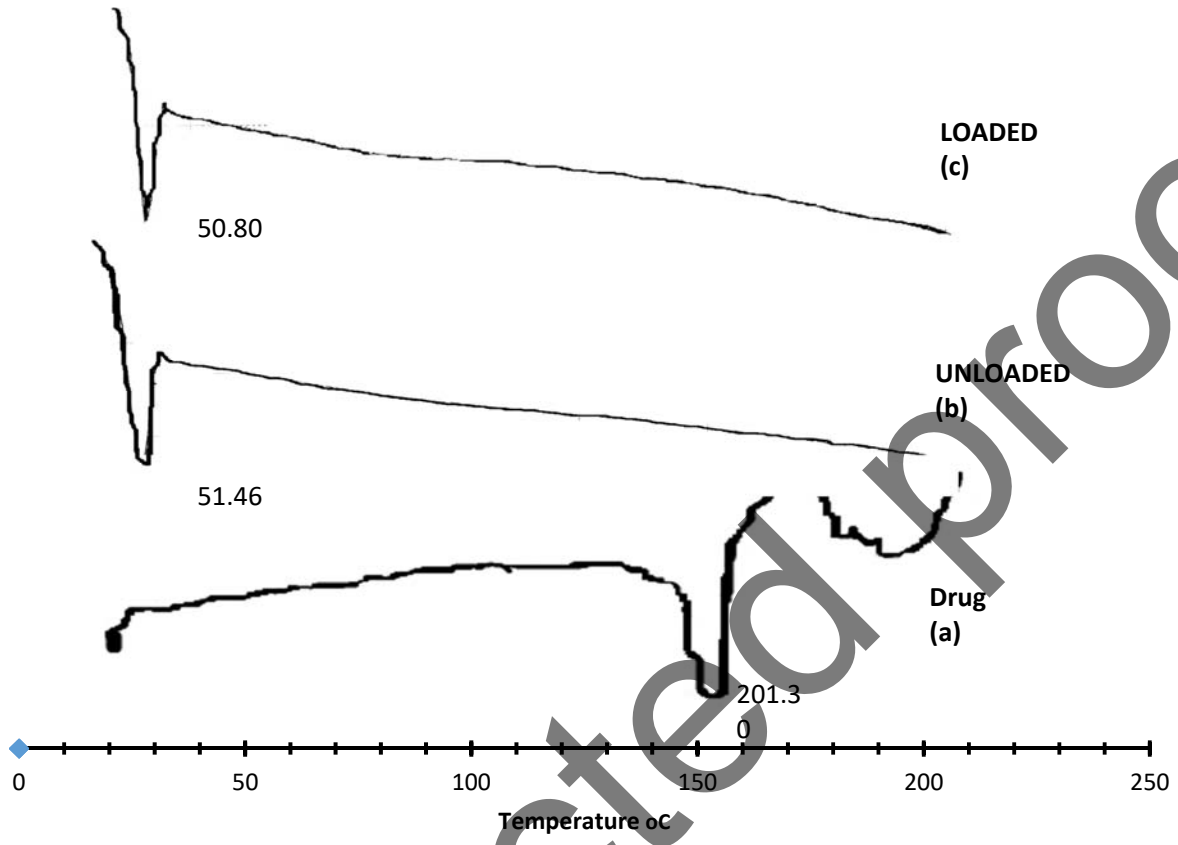

Figure 15. DSC thermogram of a) Pure drug b) Unloaded and c) drug loaded hydrogel.

X-RAY diffraction (XRD) analysis

The XRD pattern of Ge/SA hydrogel and drug loaded Ge/SA hydrogel has been depicted in Figure 16. The diffractogram of unloaded Ge/SA hydrogel indicated peak at ~16.780º, 38.180º, 44.440º, 64.820º, 69.200º, 77.940º, 78.180º (2θ). While the diffractogram of drug loaded Ge/SA hydrogel indicated peaks at ~ 16.840º, 38.160º, 44.420º, 64.800º, 69.220º, 77.940º and 78.180º (2θ). These values were nearly same as that of Ge/SA hydrogel sample without drug. It means that there was no apparent interaction reported between drug and hydrogel.

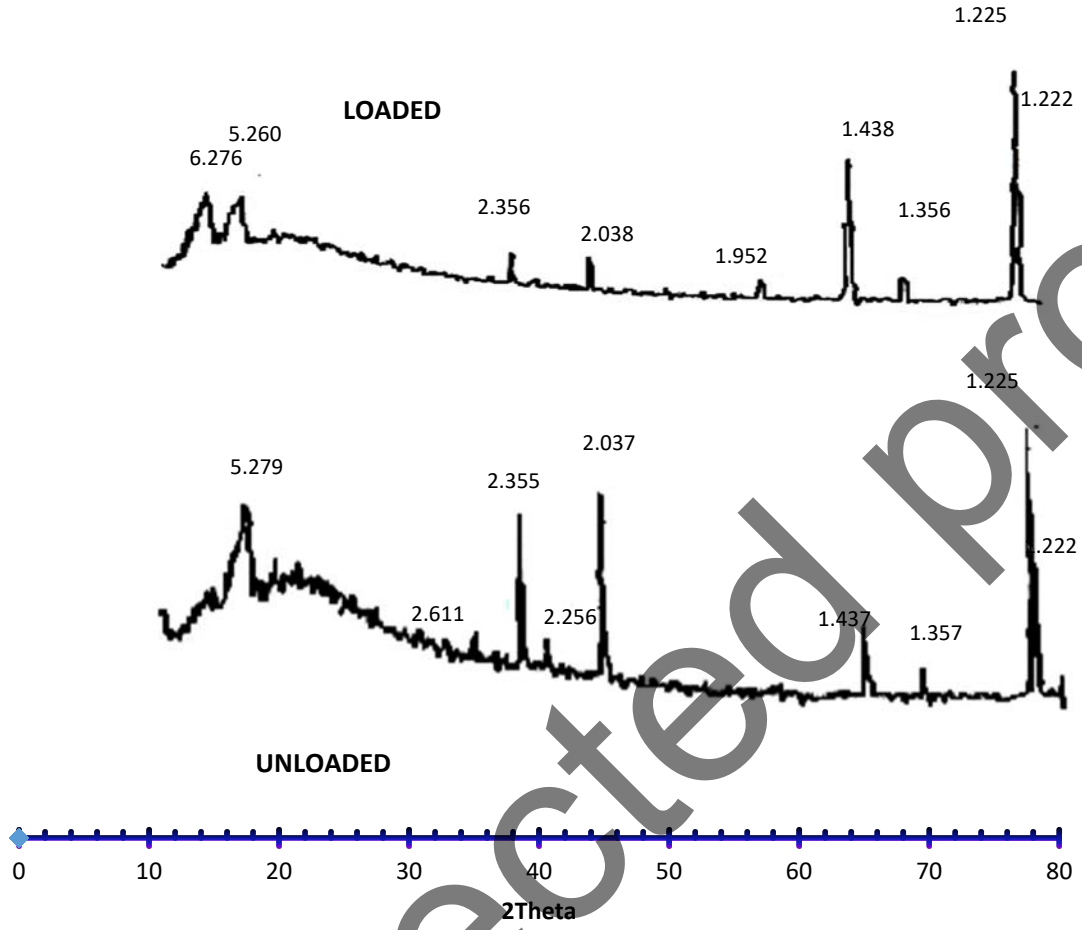

Figure 16. XRD Patterns of loaded and unloaded Ge/SA hydrogels.

FTIR spectroscopic analysis

The FTIR spectra of Ge/SA hydrogels are shown in Figure 17. The FTIR spectrum of SA/Ge indicates the characteristic absorption peaks observed at 3274 cm^{-1} typical for hydroxyl stretching and a peak at 1637 cm^{-1} which corresponds to a stretch of C=O. Peaks at 1521, 1458, 1408, and 1349 cm^{-1} in the SA spectrum indicate the anti-symmetric stretch and symmetric stretch of $–COO^-$ in associated carboxylic acid salt. Two other interactions in the C-O stretch of C-OH groups can be found at 1030, 1080 cm^{-1} and the peak at 1248 cm^{-1} corresponds to the anti-symmetric stretching of C-O-C.

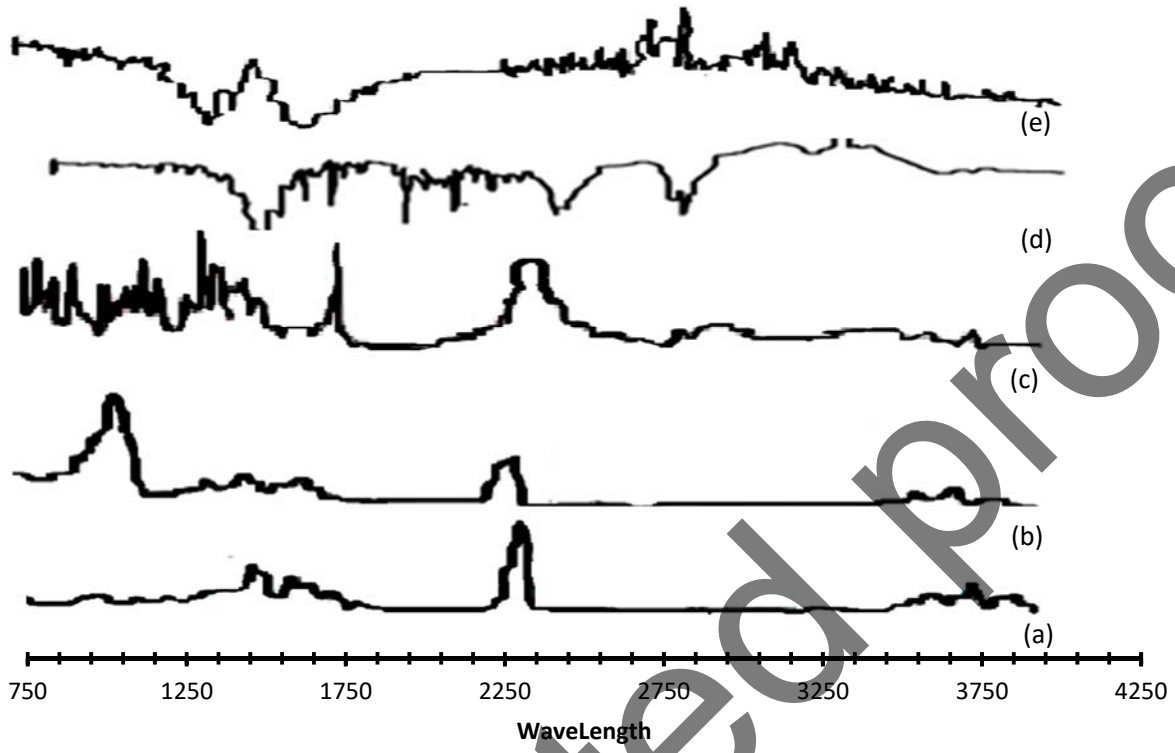

Figure 17. FTIR of a) Gelatin b) Sodium alginate c) Cetirizine hydrochloride d) Unloaded and e) Loaded samples.

CONCLUSION

pH sensitive hydrogels composed of SA and Ge were prepared in the presence of GA as a crosslinker at room temperature. Swelling ratio of cross-linked hydrogels was more in acidic media than in basic media. Gel fraction increased as the concentration of Ge, SA and GA increased. Cetirizine hydrochloride was loaded as a model drug. Hydrogels with higher content of Ge showed highest swelling and drug release. Whereas, decreasing trend in drug release was observed with increasing degree of crosslinking. The analysis of drug release showed that CTZ HCl was released from Ge/SA hydrogels by non-fickian diffusion. FTIR analysis showed the successful formation of cross-linked structure. XRD patterns analysis showed the crystalline nature of Ge/SA hydrogels. DSC analysis confirmed the thermal stability of the Ge/SA hydrogels over an extended temperature range. The results showed that the prepared hydrogels were a suitable candidate for sustained delivery of drug.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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