Turk J Pharm Sci 2018;15(1):63-76
DOI: 10.4274/tjps.64326

ORIGINAL ARTICLE

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

Setirizin HCl'nin Kontrollü Salımı için pH Duyarlı İnterpenetrasyon Jelatin/Sodyum Aljinat-esaslı Polimerik Kompozitlerin Yapı, Kristalinite ve Termal Özellikleri

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ABSTRACT

Objectives: The present work 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 through free radical polymerization using glutaraldehyde as a crosslinker. These polymeric composites were used as model systems to envisage various important characterizations. The *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 such as 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 were confirmed using 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 polymers, and porosity decreased when the concentration of the crosslinker was increased. 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, *in vitro* release, controlled delivery

ÖZ

Amaç: Bu çalışmada, her bir polimerin farklı oranları kullanılarak pH duyarlı çapraz bağlı jelatin/sodyum aljinat hidrojellerinin tasarlanması ve sentezlemesi ve her bir polimerin dinamik, kararlı şişme ve model etken madde olarak seçilen setirizin hidroklorürün *in vitro* salım profiline etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu jelatin ve sodyum aljinat hidrojelleri, oda sıcaklığında, çapraz bağlayıcı olarak glutaraldehitin kullanımıyla serbest radikal polimerizasyonu ile hazırlandı. Bu polimerik kompozitler, çeşitli önemli karakterizasyonları öngörmek için model sistemler olarak kullanıldı. Etken maddelerin *in vitro* salım modeli üç farklı ortamda (pH 1.2, 5.5, 7.5 iyonik kuvveti sabit tutulan fosfat tampon çözeltisi) araştırıldı. Solunum analizi, gözeneklilik, sol-jel analizi, çapraz bağlar arasındaki ortalama molekül ağırlığı (Mc), çözücü etkileşim parametresi (χ), polimerin hacim fraksiyonu ($V_{2,s}$) ve difüzyon gibi salım davranışını etkileyen çeşitli yapı özellik ilişkileri belirlendi. FTIR, XRD ve DSC analizi kullanılarak yapı, kristalinite ve termal stabilite doğrulandı.

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Received: 02.04.2017, Accepted: 20.04.2017

Bulgular: Bu hidrojeller pH 1.2'de maksimum şişme gösterdi. Etken madde salım modelini göstermek için sıfır derece, birinci derece, Higuchi ve Peppas modelleri uygulandı. Etken madde salımı, non-Fick difüzyon veya anomolus mekanizmasıyla gerçekleşti. Her iki polimer konsantrasyonundaki artışla porozitenin arttığı ve çapraz bağlayıcı konsantrasyonu arttıkça gözenekliliğin azaldığı bulundu. Sodyum aljinat, jelatin ve glutaraldehit konsantrasyonunda artış ile jel fraksiyonu artmıştır.

Sonuç: Hazırlanan pH'ya duyarlı hidrojeller, setirizin hidroklorür sürekli salımı için potansiyel bir taşıyıcı olarak kullanılabilir.

Anahtar kelimeler: pH'ya duyarlı, dinamik şişme, setirizin HCl, *in vitro* salım, kontrollü salım

INTRODUCTION

Modern research is oriented towards site-targeted and controlled release of drug. Various peptides and proteins have 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 three-dimensional networks that have the ability to absorb a considerable amount of water.[2] The water-absorbing ability depends upon the nature of the aqueous environment and polymer composition.[3] Hydrogels have found extensive applications as drug delivery systems, contact lenses, wound dressings, and artificial lung and joint biomaterials. They are involved in catheterization and endoscopy to reduce surface friction for the comfort of patients.[4,5] Hydrogels have the ability to protect drugs from aggressive environments e.g., the presence of certain enzymes and low pH of the stomach.

Natural polysaccharides play a vital role in developing solid dose forms for drug delivery.[6] Natural polymers are generally cheap and have attracted the attention of researchers to prepare natural polymer-based hydrogels.[7] In the present study, gelatin and sodium alginate (SA) were used to prepare hydrogels. Both of these natural polymers are biodegradable and are employed for sustained release of drug because 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 amounts in gelatin and others to a 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 makes it the best option to develop hydrogels for sustained drug delivery. The 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 increased concentration of pyrrolidines. In order to form a 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.

SA belongs to a group of agents that were studied extensively and these agents were made of stiff linear polysaccharides. SA belongs to the most commonly used gel-forming agent obtained from seaweed. They contain 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 a form gel when in contact with moisture due to the formation of a strong hydrophilic gel.[11] Hence, it is nontoxic, biocompatible, and non-carcinogenic.[12,13] These are the polysaccharides that can be used 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 (GA) 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 the concentration of the crosslinker. Hydrogels with a higher degree of crosslinking swell less than those with a 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 antagonists, cetirizine is less able to cross the blood–brain barrier and induce drowsiness. However, some serious adverse effects such as somnolence, fatigue, dry mouth, and insomnia have been reported with CTZ HCl. Therfore, in order to control the toxicity associated with this drug, the delivery system needs to be modified. Figure 1 indicates the chemical structure of CTZ HCl.

The main objective of the current study was to prepare pH-sensitive Ge/SA hydrogels for sustained delivery of an antihistaminic drug (CTZ HCl) to the gastrointestinal tract. By developing these stimuli-responsive polymeric hydrogel systems, the main idea was to provide controlled delivery and metabolism of the drug, and in turn, to reduce the adverse effects associated with this drug. The hydrogel samples were developed to achieve 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 (CTZ HCl); 4) To evaluate sol-gel fraction analysis, porosity measurement, networking parameters, and the diffusion coefficient; 5) To evaluate the best release mechanism by applying various mathematical release models; 6) To confirm the

network structure of the hydrogels by various characterization tools such as Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and differential scanning calorimetric (DSC) were used to investigate the stability of the hydrogel samples.

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. GA was obtained from Merck-Schuchardt. Acetic acid (AA), which was 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, and sodium chloride were used as received. All chemicals used were of analytical grade. Double-distilled water was used for the preparation of the hydrogels and buffer solutions.

Synthesis of pH-sensitive gelatin/sodium alginate (Ge/SA) hydrogels

Ge/SA hydrogels crosslinked with GA was prepared at room temperature using free radical polymerization as reported earlier with minor modifications.[18] Briefly, both polymers were taken in different concentrations. SA solution was made by adding the desired quantity of SA in bidistilled water at 60˚C for 1 hr. The SA solution was then cooled down. An aqueous solution of gelatin was made with the addition of a weighed quantity of gelatin in a 3% solution of AA at a temperature of 40˚C. The gelatin solution was placed at room temperature and then added to the SA solution and stirred for 45 min. Varying quantities of GA were added to this homogeneous mixture. Double-distilled water was added to make the final weight up to 50 grams. The final homogeneous mixture was poured into 16-mm internal diameter test tubes (Pyrex) with 150 mm length. The oxygen was removed from the glass tubes by nitrogen bubbling for 15 to 20 min. 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 5-mm length discs. These discs were dried at room temperature. After drying these discs, they were extensively washed 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 became equal to the pH of the water/ethanol mixture. Finally, the synthesized Ge/SA discs were placed first at room temperature and afterwards in an oven under vacuum at 45˚C until solid, reaching a stable mass. These gelatin and SA hydrogels were stored in a vacuum desiccator for future use.[18] The various formulations of SA/Ge hydrogels are given in Table 1. Figure 2 indicates the presumptive structure of Ge/SA hydrogels.

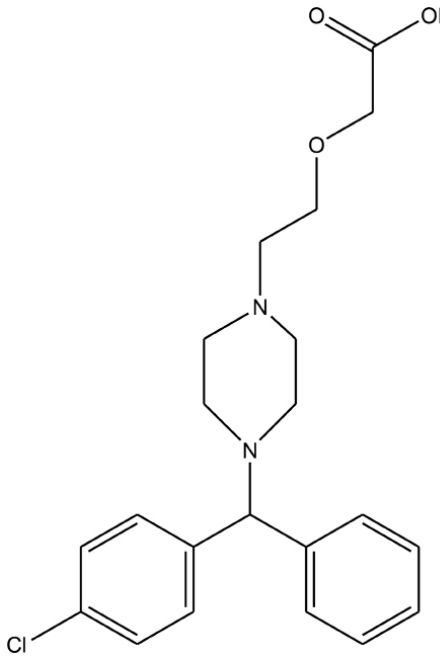

Figure 1. Structure of cetirizine hydrochloride

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). The buffering agent concentration was 0.05 M. A 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-weighed dried hydrogel discs were allowed to swell in different pH solutions (1.2, 5.5, 6.5, and 7.5) at room temperature i.e. 25-30˚C. The swollen discs were removed from the desired pH solutions at predetermined regular time intervals. The discs were first blotted with filter paper to

Table 1. Feed composition of various formulations of Ge/SA hydrogels

Sample codes	Gelatin (g)/100 g solution	SA (g)/100 g solution	Ge: SA	GA (g)/100 g 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

Ge/SA: Gelatin/sodium alginate

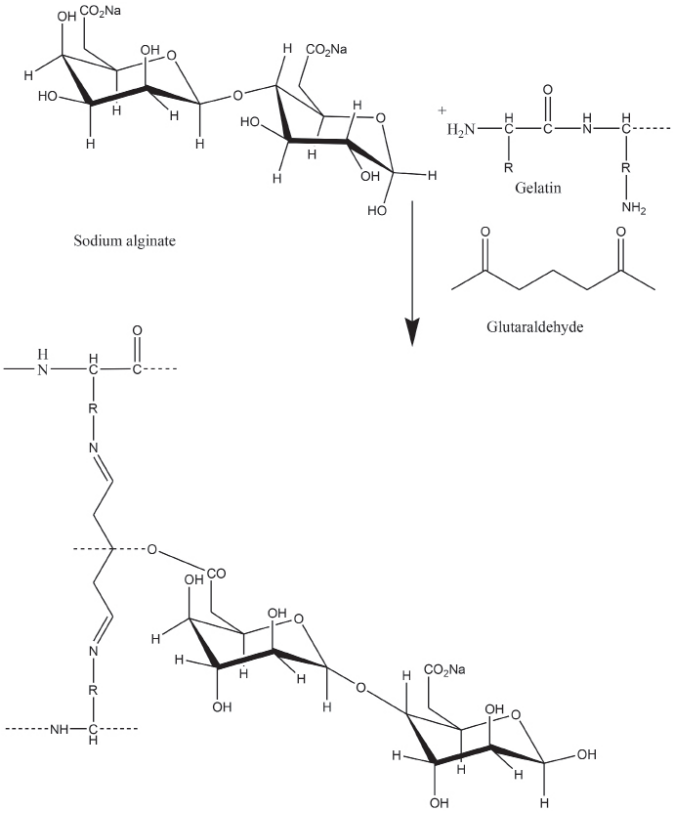

Figure 2. Proposed structure of Ge/SA hydrogel cross-linked with glutaraldehyde

remove excess solution and then weighed and positioned in the same bath solution. These studies were performed 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 were conducted by allowing the hydrogel discs to swell till they attained a constant weight and reached a state of equilibrium. The discs at higher pH absorbed much more water and became fragile and had to be handled carefully to avoid breakage.

The following equation provided the 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

Diffusion coefficient (DC) represents the quantity of substance diffusing across a unit area through a concentration gradient in unit time. DC is dependent on the amount and nature of chemicals in the polymer. The following equation was used for the 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 the hydrogel, we performed the following 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] The 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)

The solvent interaction parameter χ was calculated by using the Flory–Huggins theory.[23,24] These parameters were used to determine whether the polymers were compatible with the molecules of the surrounding fluid. According to this theory, the following 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

The 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. The 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)

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

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

M_r=Molecular weight of the repeating unit and was determined using the following 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 SA, gelatin, and GA, respectively. M_{SA}, M_{Ge}, and M_{GA} are the molar masses of SA, gelatin and GA.

Sol-gel analysis

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

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

Gel fraction (%) = (100 – Sol fraction) (10)

Measurement of porosity

This 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%. The dried gelatin/SA hydrogels were submerged in absolute ethanol for one night and excess ethanol was blotted using filter paper. The blotted hydrogels were then weighed and porosity was determined using the following equation:

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

Here, M_1=weight of the hydrogel before placing in 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 performed on Ge/SA hydrogel samples that had maximum swelling. The discs were loaded with drug by dipping them in a 1% w/v aqueous solution of CTZ HCl. The desired solution of CTZ HCl was made by dissolving the drug in water. The discs were allowed to remain in the CTZ HCl solution till equilibrium swelling was achieved. The swollen hydrogels were removed and first dried by placing them at room temperature, followed by oven drying at 46°C to a consistent weight.[18]

Measuring cetirizine hydrochloride loading

Three methods were used to measure the amount of CTZ HCl loaded in the Ge/SA hydrogels.[20] The equation used to determine the amount of cetirizine loaded by weight method is shown belowr:

Amount of drug = W_D- W_d (12)

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

In this equation, 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, the weighed hydrogel disc was placed in CTZ HCl solution until reaching equilibrium swelling. The hydrogels loaded with drug were removed and weighed once more after removing excess fluid with blotting paper to determine the amount of absorbed drug solution. The difference in weight of the gels gave the volume of cetirizine hydrochloride loaded or entrapped in the Ge/SA hydrogels. The amount of CTZ HCl was calculated from the volume.

In the extraction method, the drug was determined by repeatedly extracting the weighed amount of loaded gels in the presence of distilled water. In each turn, 25 mL of new distilled water was added until there was no drug left in the solution. The quantity of CTZ HCl was measured using a spectrophotometer. Quantities of drug present in all parts of the extract were added and this provided the means to measure the total drug loaded.

Release studies of Cetirizine hydrochloride

An *in vitro* dissolution test was used to determine the release of CTZ HCl 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 the dissolution medium, which comprised various pH values (1.2, 5.5, and 7.5). The weighed Ge/SA gel discs were placed in 900 mL dissolution medium at 37±2°C. The prepared medium was kept stirring at 100 rpm to evenly distribute the released drug in the medium. The CTZ HCl release study was conducted at 229 nm for up to 12 hours. Each time, 5 mL of dissolution medium was taken for UV analysis to verify the concentration of drug. The withdrawn solution was replaced with the 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, the Higuchi and Korsmeyer–Peppas models were employed. To achieve controlled release of drug, it is necessary that the drug diffuses faster than the swelling of the gel. The 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] $In\ (1-F)=-K_{1t}$ (15)

F represents 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

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

X-ray diffraction analysis

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 1°, 1°, 1°, and 0.15°, respectively, was used. The percentage crystallinity was determined using the equation below:[20]

% Crystallinity = Crystalline area / Total area × 100 (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 KBr (Merck IR spectroscopy grade) in 1:100 proportions and dried at 40°C. The mixture was compressed to a 12-mm semi-transparent disk by applying a pressure of 65 kN (pressure gauge, Shimadzu) for 2 min. The FTIR spectra over the wavelength range 4500-400 cm^{-1} were recorded using an FTIR spectrometer (FT-IR 8400 S, Shimadzu).[18]

Statistical analysis

For the statistical analysis of data, Student's t-test was used to compare the results and to determine the statistical significant/non-significant interpretation at 95% confidence interval; p values less than 0.05 were considered as significantly different. Data are presented as mean ± standard deviation (SD).

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 solutions of various pH values. The hydrogels were sensitive to pH; their swelling was dependent on pK_a and pH of the swelling medium. The prepared hydrogels contained both NH_2 (amine) and -COOH (carboxylic group), known as polyampholytic gels. These hydrogels showed the maximum swelling in pH 1.2 solution and the second highest swelling in pH buffer 7.5. Swelling of hydrogels was maximal at pH 1.2 because of protonation of $–NH_2$ groups and then these amine groups were ionized. Electrostatic repulsion of similar charges was the main cause of swelling in the hydrogels.[30] At pH 7.5, (–COOH) groups present in both SA and Ge were changed to (COO^-) groups, resulting in anion-anion repulsion that eventually increased the swelling of the hydrogels. The results of swelling studies (dynamic and equilibrium swelling) are shown in Table 2, which showed that the swelling of the hydrogels were decreased

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, Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

when the pK_a value of the swelling medium was less than that of the polymer. These synthesized SA/Ge hydrogels can swell at both acidic and basic pHs. Therefore, they can be used for the sustained delivery of drug.

For loading of CTZ HCl, hydrogel samples were selected that showed maximum swelling. CTZ HCl 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 quantities of gelatin (S_4-S_7) were selected for the loading of drug. Table 3 shows the amount of loaded drug in the selected samples.

In order to determine the effect of pH on CTZ HCl release, the loaded hydrogel samples were immersed in solutions of pH 1.2, 5.5, and 7.5. A dissolution apparatus was used to determine the release of drug. The maximum amount of drug was released when the hydrogels were immersed in a pH 1.2 solution and the second highest release of drug was observed at pH 7.5; the minimum amount of drug was released in a pH 5.5 solution. Table 4 refers to the effect of pH of the dissolution medium on percentage drug release of Ge/SA hydrogels.

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/100 g in Ge/SA hydrogels with GA 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 CTZ HCl from the hydrogels. It was observed that with an increase in gelatin content, an increase in drug release and swelling occurred. 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 the quantity of gelatin was increased, an increase in drug release was observed. The drug release increased from 78.15% to 81.28% in pH 1.2, 49.86% to 52.31% in pH 5.5, and 66.72% to 70.21% in pH 7.5. Figure 4-6 indicates the effect of different concentrations of Ge on the *in vitro* release of CTZ HCl as a 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 CTZ HCl 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

Ge/SA: Gelatin/sodium alginate, CTZ HCl: Cetirizine dihydrochloride

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

The concentrations of SA used in the preparation of hydrogels were 1, 1.5, and 2 g/100 g of the sample solution (S_1 to S_3) with a constant amount of gelatin and GA. 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 the SA, which hindered the diffusion of water into SA/Ge hydrogels. Bajpai et al.[32], also experienced a similar pattern for hydrogels prepared with SA. Figure 7 refers to the impact of SA on the dynamic swelling coefficient of Ge/SA hydrogels in PBS of variable pH values.

Effect of crosslinker quantity on swelling behavior and drug release from Ge/SA hydrogels

The extent of crosslinking was a major factor that affected the swelling and CTZ HCl release properties of hydrogels. To highlight this factor, hydrogels prepared with different

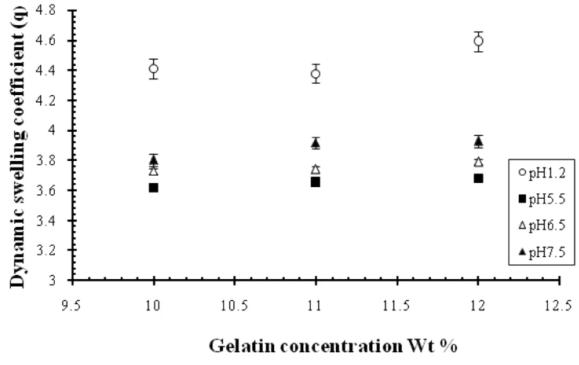

Figure 3. Dynamic swelling behavior of SA/Ge hydrogels with different concentrations of Ge (S_4-S_6), keeping the concentration of SA and GA constant in solutions of various pHs (1.2, 5.5, 6.5, and 7.5)

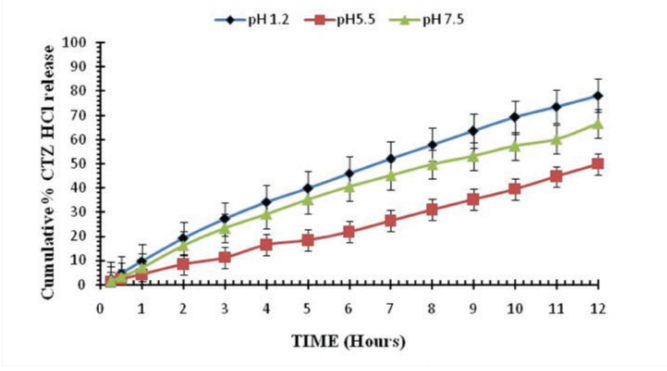

Figure 4. Pulsatile cumulative percentage drug release of CTZ HCl from Ge/SA hydrogels (10/2 g) in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride

concentrations (S_7 to S_9) of GA (3.5%, 3.75%, and 4%) while keeping the quantity of both the polymers constant (SA/Ge = 2/11g). When increased quantities of crosslinker was used, dense networks were produced with shrunken mesh size and a tighter structure. This resulted in minimal spaces for the entrance and accommodation of water and swelling decreased.[33] Figure 8 refers to the effect of GA on the dynamic swelling coefficient of Ge/SA hydrogels in PBS of variable pH values. Drug release from hydrogels is dependent on swelling and samples with the 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 1.2, 51.02% to 48.96% in 5.5, and 72.86% to 69.01% in pH 7.5, respectively. Figures 9-11 indicate the effect of different concentrations of GA on the *in vitro* release of CTZ HCl as a function of time from Ge/SA hydrogels in PBS of various pH values.

Molecular weight between crosslinks (M_c) and solvent interaction parameters

The concentration of gelatin has a direct relation with M_c values. An increase in gelatin concentration enhanced the M_c 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 the concentration of gelatin. The M_c value has a 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.

Diffusion coefficient of polymers (D)

The 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. The diffusion coefficient was found to be decreased when increased concentrations of SA and GA were used. The diffusion coefficient increased with increasing concentrations of gelatin because swelling increases with increased concentrations of gelatin. Table 5 indicates the values of D.

Sol-gel analysis

Sol-gel analysis was performed to determine the uncrosslinked polymer concentration in the hydrogel. Different compositions of SA/Ge were used to determine the effect of polymers and degree of crosslinking on the gel fraction of the hydrogels. The gel fraction increased with an increase in concentrations of SA, Ge, and GA, and the sol fraction was decreased. This mechanism was observed due to enhanced grafting, and

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

Ge/SA: Gelatin/sodium alginate

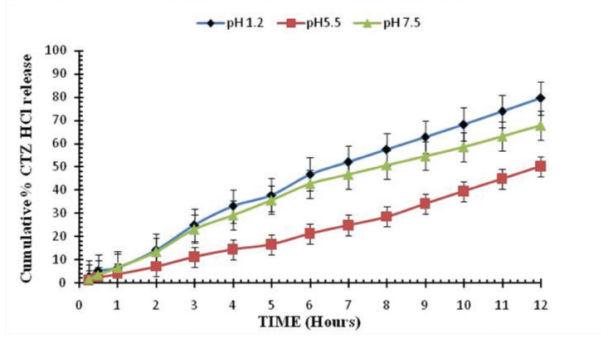

Figure 5. Pulsatile cumulative drug release of CTZ HCl from Ge/SA hydrogels (11/2 g) in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride, Ge/SA: Gelatin/sodium alginate

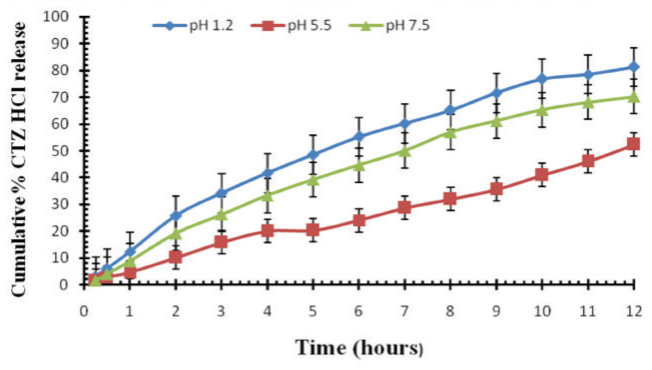

Figure 6. Pulsatile cumulative percentage drug release of CTZ HCl from Ge/SA hydrogels (12/2 g) in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride, Ge/SA: Gelatin/sodium alginate

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

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

Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

increased concentrations of polymers (SA and Ge) and crosslinker resulted in extensive crosslinking; this mechanism was not observed with lower concentrations of these agents. Similar findings were reported by Ranjha and Mudassir[27], who prepared hydrogels composed of chitosan and acrylic acid. Natural polymers showed an increase in gel fraction at higher concentrations. Table 6 indicates the gel fraction (%) of Ge/SA hydrogels.

Porosity

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

By increasing the concentration of GA, porosity deceases. Increased crosslinker concentration causes the shrinkage in mesh size of the resulting gels, lesser pores are formed, and eventually porosity decreases. Figures 12-14 indicate the effect of variables on porosity percentage of Ge/SA hydrogels. Ranjha and Mudassir[27] used chitosan to prepare hydrogels and their results showed that chitosan formed a viscous solution that entrapped the bubbles, which lead to voids in the hydrogel matrix. Table 6 indicates the 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 was CTZ HCl. The most appropriate method to determine best model for drug is based on values of the regression coefficient denoted by r. The model should have an r value close to one.

Regression coefficient values (r) with different concentrations of Ge and GA are given in Tables 7 and 8. The r values of the Higuchi model at various Ge and GA concentrations showed greatest linearity and the CTZ HCl release mechanism was found to be diffusion controlled.

The effect of varying amounts of Ge and GA on the release exponent (n) at different pH solutions is shown in Tables 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 CTZ HCl release from

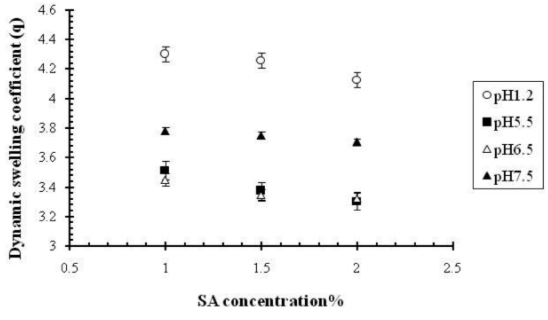

Figure 7. Dynamic swelling behavior of SA/Ge hydrogels with different concentrations of SA (S_1-S_3), keeping concentrations of Ge and GA constant in various pH solutions (1.2, 5.5, 6.5, and 7.5)

SA: Sodium alginate

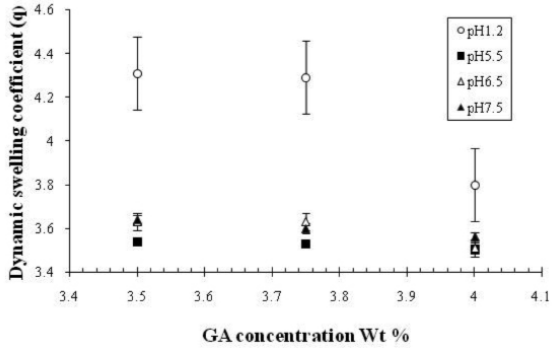

Figure 8. Dynamic selling behavior of SA/Ge hydrogels with different concentrations of GA (S_7-S_9), keeping SA and Ge constant at solutions of various pH (1.2, 5.5, 6.5, and 7.5)

GA: Glutaraldehyde

Table 6. Gel fraction and porosity percentage of various formulations of Ge/SA hydrogels

Sample codes	$V_{2,s}$	χ	M_c	M_r	q	$D \times 10^{-5}$ (cm² sec^{-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

Ge/SA: Gelatin/sodium alginate

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

Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

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

Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

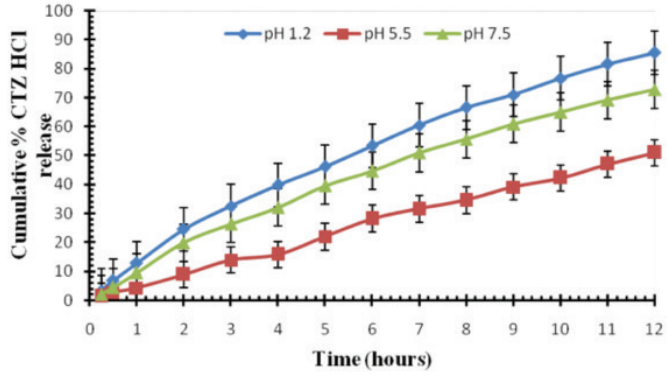

Figure 9. Pulsatile cumulative percentage drug release of CTZ HCl from Ge/SA hydrogels (11/2 g) using 3.5% GA in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride

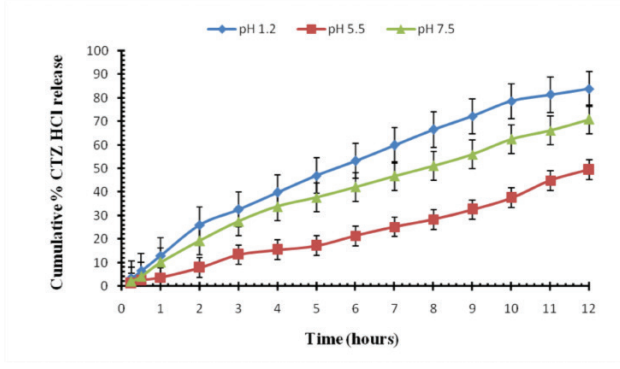

Figure 10. Pulsatile cumulative percentage drug release of CTZ HCl from Ge/SA hydrogels (11/2 g) using 3.75% GA in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride

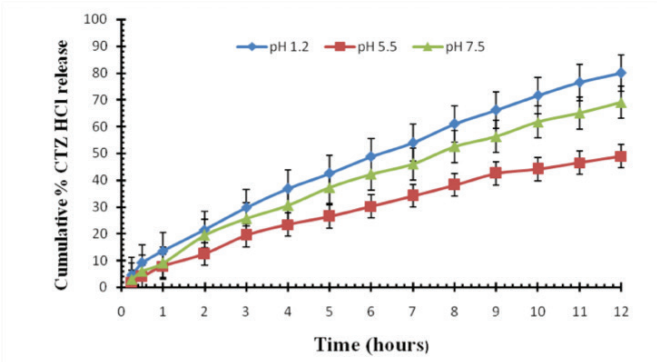

Figure 11. Pulsatile cumulative percentage drug release of CTZ HCl from Ge/SA hydrogels (11/2 g) using 4% GA in phosphate buffer solutions of various pH values. Each point represents the mean ± standard deviation of n=3 experiments

CTZ HCl: Cetirizine dihydrochloride

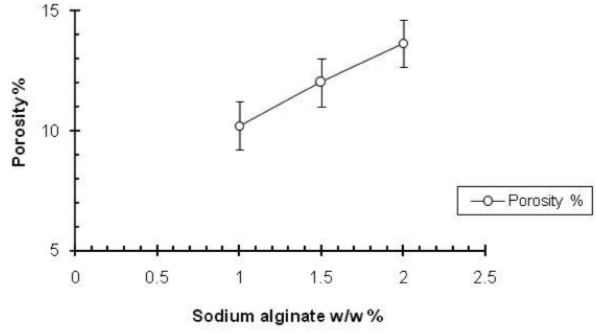

Figure 12. Effect of different concentrations of SA (1, 1.5, and 2 g) on the porosity percentage of Ge/SA hydrogels

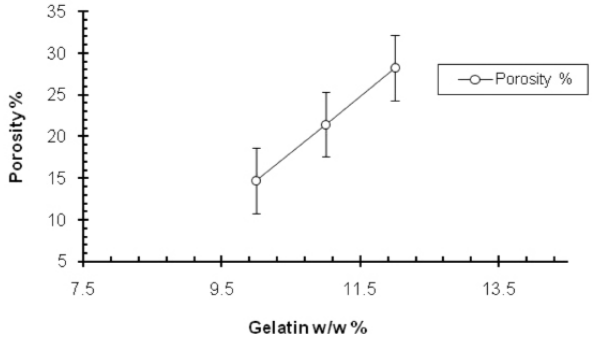

Figure 13. Effect of different concentrations of Ge (10, 11, and 12 g) on the porosity percentage of Ge/SA hydrogels

hydrogels is due to the swelling and relaxation of polymers, which, in this case, were Ge and SA.

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 indicate a sharp melting peak of CTZ HCl at about 201.3°C, followed by a decomposition peak at about 260°C. The drug-

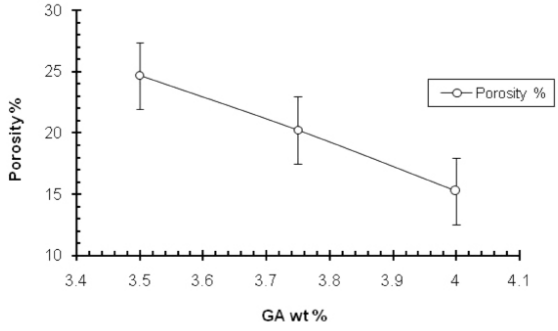

Figure 14. Effect of different concentrations of GA (3.5, 3.75, and 4%) on the porosity percentage of Ge/SA hydrogels

GA: Glutaraldehyde

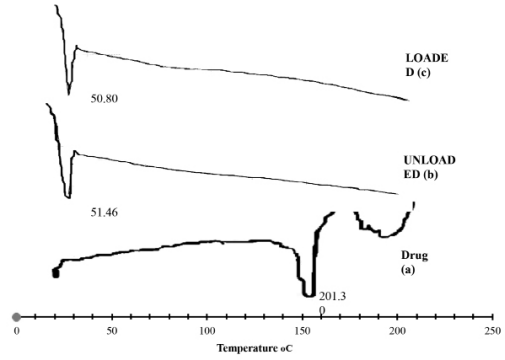

Figure 15. DSC thermogram of a) Pure drug b) Unloaded and c) Drug-loaded hydrogel

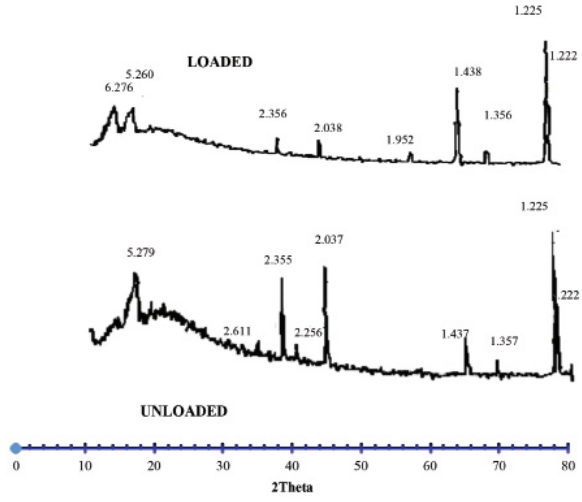

Figure 16. XRD patterns of loaded and unloaded Ge/SA hydrogels

loaded hydrogel showed an absence of drug melting peak, which indicates molecular dispersion of drug in the prepared hydrogels. The unloaded sample showed no endothermic transitions due to the rigid polymer network structure because of chain entanglement. The prepared hydrogels were found to be stable.

X-ray diffraction (XRD) analysis

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

FTIR spectroscopic analysis

The FTIR spectra of the Ge/SA hydrogels are shown in Figure 17. The FTIR spectra 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^{-1}, 1080 cm^{-1}, and the peak at 1248 cm^{-1} corresponds to the anti-symmetric stretching of C-O-C.

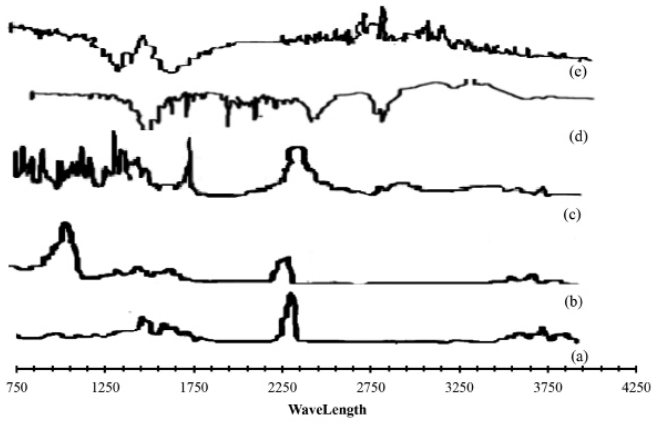

Figure 17. FTIR of a) Gelatin b) Sodium alginate c) Cetirizine hydrochloride d) Unloaded and e) Loaded samples

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

Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

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

Ge/SA: Gelatin/sodium alginate, GA: Glutaraldehyde

CONCLUSION

pH-sensitive hydrogels composed of SA and Ge were prepared in the presence of GA as a crosslinker at room temperature. The swelling ratio of cross-linked hydrogels was more in acidic media than in basic media. The 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 the highest swelling and drug release. In contrast, a decreasing trend in drug release was observed with an 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 pattern 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.

ACKNOWLEDGEMENTS

The authors highly acknowledge Department of Pharmacy, Bahauddin Zakariya University Multan for providing us the laboratory facilities.

Conflict of Interest: No conflict of interest was declared by the authors.

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