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Hydrolyzed Collagen-Based Hydrogel System Design, Characterization and Application in Drug Delivery

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Abstract

This work was intended to develop a new hydrogel of collagen-g-Poly (acrylamide-co-itaconic acid) through chemical cross-linking by graft copolymerization of acrylamide (AM) and itaconic acid (IA) on to collagen (CGN) via redox initiator system of ammonium persulfate (APS) and *N, N, N', N'*-tetramethylethylenediamine (TMED), in presence of *N, N'*-methylene bis acrylamide (MBA) as crosslinking agent. Characterization of the hydrogel was done by FT-IR, TGA, SEM, LCMS/MS and HPLC. Valsartan (VAL) was successfully loaded into the prepared hydrogel. CGN-g-P(AM-co-IA) (H_{10}) formulation showed highest swelling capacity as well as VAL release in the biological media and release was controlled up to 24h. The release data of various formulations were fitted to Zero order, First order and Higuchi's kinetic models. It was observed that the release of drug from all the formulations followed Higuchi's kinetic model as its value of coefficient of determination is greater than that of others.

Keywords: Hydrogel, Collagen, Acrylamide, Itaconic acid, Residual monomers, Valsartan delivery.

1. Introduction

Hydrogels are lightly crosslinked networks of hydrophilic polymer chains, which can retain large amount of water within its structure but do not dissolve into it. Polymers from natural, synthetic or semi-synthetic sources can be used for preparing hydrogels. It involves crosslinking of either linear polymers or simultaneous polymerization and crosslinking of monomers with polyfunctional monomers [1, 2]. The most

effective hydrogels are obtained from polymers having dissociative ionic functional groups. The presence of ionic groups in the hydrogel structure changes its charge state against changes in pH and ionic strength of the medium. The absorbent properties of polymeric hydrogels have been commercially exploited in hygiene products, agricultural and site specific controlled drug delivery applications. At present, stimuli sensitive drug delivery systems (DDSs) have been an attractive theme for controlled release [3, 4]. The release behaviors of drugs can be easily controlled by surrounding properties, such as pH, temperature, ionic strength and electric field. As compared with conventional administration, drugs can prolong their duration time by hydrogel DDSs.

Collagen (CGN) is the most abundant protein in animal kingdom as it represents close to 30 % of mammalian protein matter. Because of its spatial structure and high molecular weight, native collagen is naturally insoluble in water. In order to be separated from the other constituents of animal tissues, it is made soluble through an extraction process, which includes first a partial and controlled hydrolysis of the protein chain and then a warm water extraction. This yields hydrolyzed collagen. In nutraceuticals, hydrolyzed collagen could be incorporated in various "dosage forms". Numerous hydrogels composed of synthetic acrylic derivatives like hydroxyethyl methacrylate, acrylic acid, methacrylic acid, N-isopropylacrylamide, and natural polysaccharides like chitosan exhibit pH-sensitive behavior and subsequently used for colonic drug delivery [5-9]. There are various copolymers of itaconic acid which also shows the pH-sensitive swelling and drug release behavior. Therefore, the diseases of the colon are treated through direct delivery of the drugs to the site of action. Itaconic acid is one of the monomers, which is readily available at low cost. It is obtained from renewable resources by fermentation with *Aspergillus terrus* using carbohydrate materials as molasses and hydrolyzed starch [10]. Itaconic acid easily copolymerizes and provides polymer chains with carboxylic side groups, which are highly hydrophilic and able to form hydrogen bonds with corresponding groups. Unlike monocarboxylic methacrylic and acrylic acid, itaconic acid has two -COOH groups with different pKa (pKa_1=3.85 and 5.45) values, so that very small amounts of IA render good pH sensitivity and increased degree of hydrogel swelling. In addition, incorporation of co-monomers which can contribute to H-bonding can increase the mechanical strength of the hydrogel. Furthermore, IA is very hydrophilic and is expected to show high biocompatibility because of its natural source.

The purpose of this study was to synthesize intelligent CGN-based hydrogels to be used as pH sensitive carriers for the controlled delivery of valsartan (VAL), a long-acting angiotensin receptor blocker (ARB). VAL was used as model drug because it is absorbed at different sites in the human gastrointestinal tract [11].

2. Experimental

2.1. Materials Materials

Hydrolyzed collagen (CGN) (Himedia Laboratories Pvt. Ltd., Mumbai) is of industrial grade which is available in market and has nearly 25% insoluble phosphate salt. Acrylamide (AM), Ammonium persulfate (APS), *N, N, N'N'*-tetramethylethylenediamine (TMED), *N,N*'methylene-bisacrylamide (MBA) (Merk) were used without further purification. Itaconic acid was purchased from fluka and used as received. Orthophosphoric acid from E-MERK, Germany. Acrylamide was supplied by Sigma Chemical Company (St. Louis, MO, USA). Acrylamide-d3 was purchased from Polymer Source Inc. (Dorval, Quebec, Canada). Individual stock standard solutions of 100 mg/l in water were prepared and stored at 4^0C for 6 months. Analytically pure valsartan was purchased from Fluka AG, Chemische Febrik, Switzerland. All other chemicals used in this study were of analytical grade quality, with deionized water (milli Q, Millipore (India) Pvt Ltd, Bangalore) being used for preparing the solutions.

2.2 Synthesis

Synthesis of Collagen-g-Poly (acrylamide-co-itaconic acid), CGN-g-Poly (AM-co-IA)

A pre-weighed amount of hydrolyzed CGN (1.0 g) was added to 50 ml deionized water and filtered to remove its insoluble phosphate salt.

Then the solution was added to a three-necked 500 ml reactor equipped with a mechanical stirrer (RZR 2021), a three-blade propeller (Heidolph, Schwabach, Germany) and stirred at 250 rpm for 10 min. The reactor was placed in a thermostated water bath to control the reaction temperature at 80 ºC. After dissolving and homogenizing the mixture, the monomers AM, IA and the crosslinker, MBA[12] were simultaneously added and the reaction mixture was stirred for 15 min. Then the initiator APS (oxidant) and TMED (reductant) were added (Table I). The solution was stirred at 400–500 rpm while maintaining the temperature and inert atmosphere. The temperature was maintained at 80ºC and the reaction mixture was stirred continuously for 24 h. The low molecular weight substances remaining in the samples after polymerization were extracted with boiling ethanol for 24 h. The product was collected by centrifugation and dried in the oven under vacuum at 60 ºC for 24 h. The dried graft polymer was added to 300 ml deionized water. It was allowed to swell during agitation in a water bath at the constant temperature of 60 ºC for 24 h. Then it was extracted with ethanol in a soxhlet for 6 h followed by water at 100 ºC for 72 h. The Hydrogel was filtered and dried under vacuum at 60ºC. After grinding, the resulting powder was stored away from moisture, heat and light. Homopolymer, PAM (poly acrylamide) and copolymer and copolymer Poly (AM-co-IA) were also synthesized using the same method and by FT-IR studies (Fig. 1).

Table I Composition of the feed mixture

Polymer code	IA (mg)	MBA 1% (w/v) ml	TMED 1% (w/v) ml	APS 1% (w/v) ml	% Yield
H1	0	0.4	0.3	0.2	51
H2	20	0.4	0.3	0.2	54
H3	40	0.4	0.3	0.2	61
H4	60	0.4	0.3	0.2	78
H5	80	0.4	0.3	0.2	58
H6	60	0.2	0.3	0.2	62
H7	60	0.6	0.3	0.2	59
H8	60	0.8	0.3	0.2	54
H9	60	0.4	0.1	0.2	63
H10	60	0.4	0.2	0.2	92
H11	60	0.4	0.4	0.2	79
H12	60	0.4	0.2	0.1	70
H13	60	0.4	0.2	0.3	72
H14	60	0.4	0.2	0.4	73

2.3 Characterization

FTIR spectra of individual and crosslinked polymers were recorded in the range 400-4000 cm^{-1} on a Perkin Elmer Paragon 500 FTIR spectrophotometer using KBr pellets. The thermogravimetric analysis data were recorded with a Shimadzu DTG-50 thermal analyzer. The samples were heated from room temperature to 600°C at a heating rate of 10°C per min. The SEM of gold-coated samples were obtained using JSM - 6390LV scanning electron microscope (Jeol Ltd, Japan) at a magnification of x 5 to 300,000 (Resolution-HV 3.0 nm). Residual AM was detected and quantified by Liquid Chromatography Mass Spectrometry (LCMS/MS). Analysis was performed on a Perkin-Elmer 200 Micro pump series system (perkin-Elmer, Uberlingen, Germany) coupled to an Applied Biosystem API 2000 triple quadrupole mass spectrometer equipped with a Turboionspary ionization source (Applied Biosystem, Foster City, CA). MS detection was performed in the positive

ion mode using multiple reactions monitoring (MRM). Data was acquired and processed by Analyst software (version 1.4.1). Residual IA was detected and quantified by HPLC (Prominence, Shimadzu Corporation, Japan). The chromatographic system consisted of a computer-controlled pump (model LC 20AT), autosampler (model SIL-10AF) equipped with a 200µl sample loop, photodiode array (PDA) detector (model SPD-M20A). Shimadzu LC Solution software was used for the system and data management. The separation was performed in isocratic mode at a flow rate of 1.0 ml /min and a temperature of 40 °C on an analytical column Gemini 5µ C18, 150 x 4.6 mm (Phenomenex, USA). An RP C18 Security guard (4 x 3mm, Phenomenex) was employed to protect the analytical column. The mobile phase was aqueous 0.05% orthophosphoric acid and the injection volume was 50µl. The observed backpressure values were in the range from 1400 to 1450 psi. Data was acquired and processed by LC solution software (Shimadzu, Japan).

2.4 Swelling behavior of CGN-g-P (AM-co-IA)

Swelling behavior of CGN-g-Poly (AM-co-IA) was performed by tea bag [13] method. About 0.100 g of sample was added to a small bag made of nylon (50 mm x 90 mm; 200 mesh). Then the bag was completely immersed in the swelling medium (200 ml) at room temperature for 24 h to reach the swelling equilibrium. The pH-dependent equilibrium swelling of the hydrogel was studied both in the simulated gastric fluid (SGF, 3.2 mg/ml pepsin in 0.05 M hydrochloric acid, pH 1.2) and simulated intestinal fluid (SIF, 10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4). Adhered liquid droplets on the surface of the particles were removed by blotting with tissue papers. The swollen hydrogels were weighed and dried in an oven at 60 °C for 6 h until there was no change in the dry mass of the samples.

The % equilibrium swelling (ES) was defined as follows:

$$ES\ (\%) = \frac{(W_S - W_d)}{W_d} \times 100$$

Where W_S and W_d are the weights of the swollen sample and the weight of dried gel, respectively.

2.5 Drug loading

CGN-g-Poly(AM-co-IA) was loaded with VAL (as model drug) by soaking in an aqueous solution containing 10 % (w/v) of VAL. Soaking was done for nearly 2 days in order to achieve complete equilibrium. The formulations were filtered and the surface-adhered drug solution was removed by washing and blotting with soft filter paper and dried in air before storing in a desiccator.

2.6 Drug entrapment efficiency (%)

VAL loaded CGN-g-Poly (AM-co-IA) (50 mg) from each batch were dispersed separately in water and kept for 24 h, filtered through 0.22 µm microfilter and absorbance was measured using UV/VIS spectrophotometer (Varian, Cary 50 Bio,USA) at 250 nm. The obtained absorbance was plotted on the standard curve to get the exact concentration of the entrapped drug. VAL content were determined and expressed in terms of weight of VAL per weight of hydrogel, thus determining the actual entrapment ratio (AER).

% of Entrapment efficiency = AER / TER x 100

where AER = Measured drug wt / formulation wt and

TER = Drug wt. /drug wt. and polymer wt.

2.7 *In vitro* drug release study in pH progressive media

The *in vitro* drug release was carried out by filling the calculated amount of VAL loaded CGN-g-Poly (AM-co-IA) in capsule shell (size 2), analyzed using USP-I basket dissolution apparatus and proper simulation of gastro intestinal (GIT) condition was maintained by altering the pH of dissolution medium at different time intervals following two step-dissolution conditions. To simulate the physiological conditions of GIT, first 2 h of dissolution were carried out in 900 ml of simulated gastric fluid (SGF, 3.2 mg/ml pepsin in 0.05M hydrochloric acid, pH 1.2) and the rest of the time in 900 ml of simulated intestinal fluid (SIF, 10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4). The media was stirred at 100 rpm at 37 ± 0.5 °C. At predetermined time intervals, specified amount of dissolution medium was removed;

filtered through 0.22 μm microfilter and analyzed in UV spectrophotometer at 250 nm. After each sampling, an equal volume of fresh dissolution media was added to the dissolution medium. All the dissolution studies were repeated six times.

2.8 Kinetics of drug release

Different mathematical models may be applied for describing the kinetics of the drug release process from the CGN-g-Poly (AM-co-IA) hydrogel matrix; the most suited being the one which best fits the experimental results. The kinetics of VAL release from hydrogel was determined by finding the best fit of the dissolution data (drug release *vs.* time) to distinct models: Zero order [eq.1], first-order [eq.2] and Higuchi [eq. 3].

$Q_t = k_0 t$ [1]

$Q_t = Q_\infty (1\text{-}e^{-k_1 t})$ [2]

$Q_t = k_H t^{1/2}$ [3]

where Q_∞ being the total amount of drug in the matrix, k_0 the zero order kinetic constant, k_1 the first order kinetic constant and k_H representing the Higuchi rate constant.

2.9 Stability studies

To assess the physical stability, selected VAL delivery systems (H4, H10 and H11) were stored in the stability chamber at 40 °C /75% relative humidity condition as per I.C.H. guidelines [14]. Samples of definite amount from each batch were withdrawn after three months to see the effect of VAL release from the gel on storage.

3. Results and Discussions

3.1 FT-IR spectra

Fig. 1 shows the FTIR spectra of PAM, Poly (AM-co-IA), CGN-g-Poly (AM-co-IA), VAL, VAL incorporated into graft copolymer. For CGN-g-Poly (AM-co-IA) (c), the peaks found at 3401, 1649, and 1600 cm^{-1} indicate the N–H stretching, the C=O stretching and N–H bending of the amide bands, respectively, which are characteristics of the —$CONH_2$ group containing in the acrylamide. In addition, the peak at 1390 cm^{-1} is for the –C–N stretching and 599 cm^{-1} for the weak band N–H out of plane bending. These are the typical absorption bands of the amide. The broad band at 3200-3600 $cm^{-1}$is due to the overlapping of N–H stretching band of amide with –OH stretching band of the collagen portion of the graft copolymer. Moreover, band at 1711 cm^{-1} was assigned to carboxylic carbonyl group of itaconic acid. This is in good agreement with the results reported in the literature [15]. VAL alone showed two carbonyl absorption bands at 1729 and 1609 cm^{-1}, assigned to the carboxyl carbonyl and amide carbonyl stretching, respectively (d). These bands are of indicative value to elucidate drug–polymer interactions. The two absorption bands of the pure drug appeared unchanged in the graft copolymer (e).

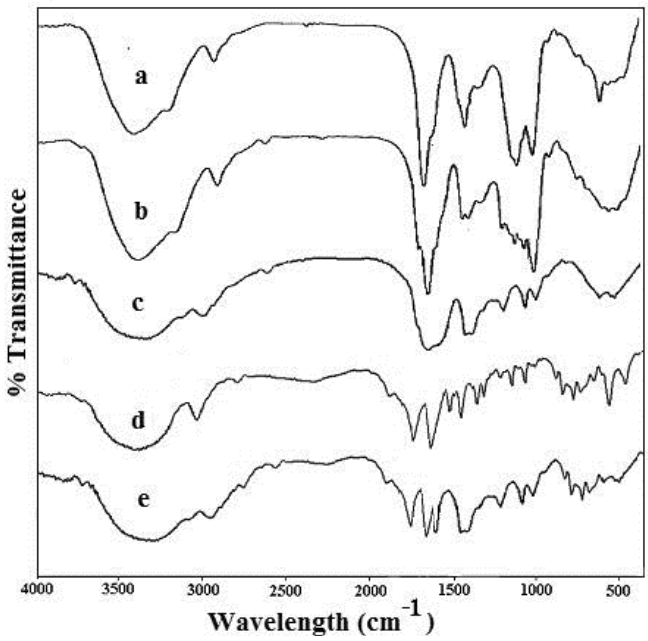

Figure 1. FTIR spectra of PAM (a), Poly (AM-co-IA) (b), CGN-g-Poly (AM-co-IA) (c), VAL (d) VAL incorporated into graft copolymer (e).

3.2 Thermal analysis

The results of thermogravimetric analysis (TGA) technique employed to characterize the thermal properties of the obtained graft copolymers as shown in Fig. 2. CGN (a) shows a two-step characteristic thermogram, wherein the major weight loss (73 %) takes place in the second step within the temperature range of 176–334 ºC, the temperature for a maximum decomposition was 318 ºC. The thermogram of Poly (AM-co-IA) (b) showed three decomposition stages. The first decomposition stage in the range 38-110 ºC was

attributed to the loss of bound water. The second one in the interval of 181.1-292 ºC had been described to the decarboxylation of IA coupled with the chain scission. The Weight loss in the third or main stage of decomposition (295-387 ºC) can be assigned to the degradation of acrylamide portion. In case of CGN grafted with Poly (AM-co-IA) (c), four stages of decomposition were observed. It is suggested that in an initial stage of the thermal diagram, when the temperature in a range from ambient temperature to about 150 ºC, the weight loss is a result of the dehydration process of the water contained in such a hydrophilic polymer. At the second stage from 151 to 269 ºC, there is a decomposition peak in the side groups and branches of the graft copolymer (carboxyl group in itaconic acid proportion). At the third stage from 270 to 337 ºC, there is a degradation of CGN in the graft copolymer. However, at the fourth stage about 338 ºC, the weight loss was found as a result of the degradation of the polymer chain and matrices (degradation of polysaccharide and acrylamide portion). From the TGA curves, it can be concluded that the thermal stability of the polysaccharide decreases with the grafting of Poly (AM-co-IA) chains onto the polysaccharide backbone. This may be attributed to the low thermal stability of CGN and itaconic acid. Similar phenomenon has also been reported by N. Isıklan [14]. He has indicated that thermal stability of the polymer reduced with the grafting of IA onto sodium alginate.

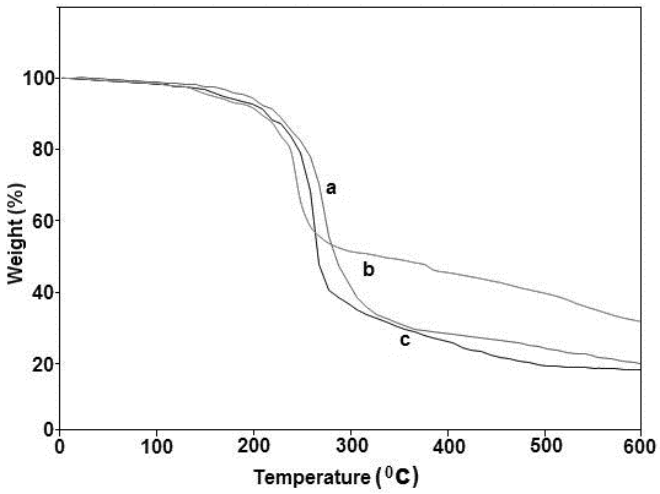

Figure 2. TGA of (a) CGN (b) Poly (AM-co-IA) (c) CGN-g-Poly (AM-co-IA) [H10].

3.3 Scanning Electron Microscopy

Scanning electron microscopy (Fig. 3) allows much high magnification of the hydrogel structure in which we can see the surface. This picture verifies that the graft copolymers prepared in this work have a porous structure. The surface of the hydrogel is rougher and the approximate diameter of the pores was found to be in between 50 to 150 µm. The uneven surface may be due to a quite high viscosity of the gel and solvent evaporation process. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the incorporated drug or hydrophilic.

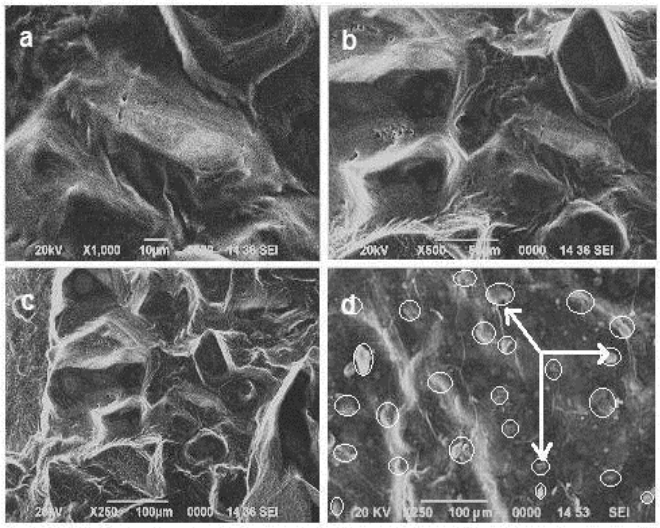

Figure 3. SEM image of the CGN-g-Poly (AM-co-IA) [H10] at different magnifications (a, b and c), VAL loaded H10 (d).

groups of the graft copolymers. Therefore, the porous structure is the predominant reason for the higher swelling rate. The results show that the drug is uniformly distributed in the hydrogel (Fig. 3 d).

3.4 Residual AM determination

Dried xerogel (0.100 g) was accurately weighed and added to 4.5 ml water in an amber vial (Supleco, USA). 500 µl of the internal standard (100 ng/ml) solution, d3-acrylamide, was added. After vortex (Cyclo mixer, CM 101, Remi Instruments, India) for 10min the sample hydrogels were placed in an orbital shaker (Labline instruments, India) at 37 ºC with constant agitation (200 rpm) for 24 h. Then it was

centrifuged (model 510R, Eppendorf, Germany) for 10 min at 4000 rpm at 5 ºC. The samples are respectively shaked during 1 min on a Vortex and 10 min by orbital rotation. The extract is centrifuged at 5 ºC with a speed of 4000 rpm; the supernatant was collected filtered on a nylon membrane. The supernatant was passed through Oasis® HLB SPE cartridges previously conditioned with 5ml of methanol and 5ml of water. Elution occurs with 5ml of water. A second SPE purification involves the Bond Elut-Accucat® cartridges conditioned with 5ml of methanol and 5ml of water before loading with the totality of the extract. Eluent was directly collected and filtered. 20 µl was injected into LCMS/MS.

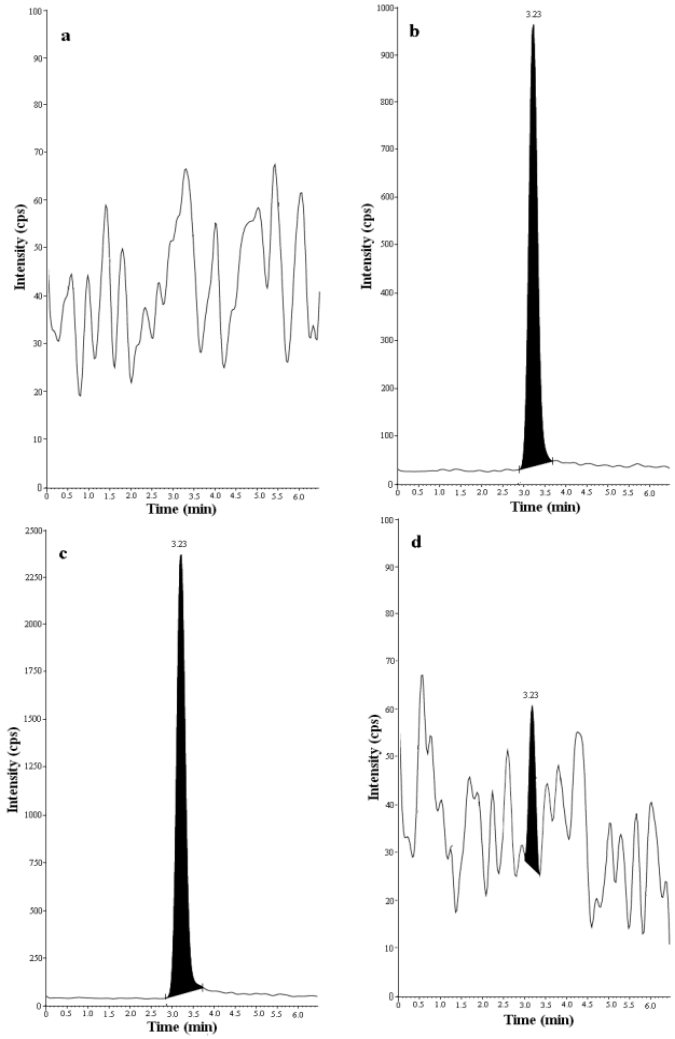

Figure 4. LCMS/MS Chromatograms. (a) Blank (Methanol) , (b) AM standard 0.250 µg/ml, (c) AM in sample (H10) (before extraction),(d) AM in sample (H10) (after soxhlet extraction).

For the detection by LC–MS/MS, identification occurs with the relative retention time (RRT) and diagnostic ions consisting mainly of the precursor ion at *m*/*z* 72.04 and one daughter ion (quantifier) resulting from a loss of HCN at *m*/*z* 55.10 [16]. For confirmatory purpose, a comparison with quality control samples were made using acceptable deviations of ± 2.5% for relative retention time and ± 20% for the ionic relative abundance as described in the European

Commission Decision 2002/657/EC. It can be seen from the Fig. 4 that the amount of the residual AM was decreased markedly after soxhlet extraction (the amount was decreased from 28.41 µg/g to 0.503 µg/g). The decrease in residual AM content was due to its diffusion from gel network to the water, as it was allowed to swell in the excess of water, but a little amount was still detected as few monomers were trapped in the polymer chain.

3.5 Residual IA determination

Powdered hydrogel sample (0.300 g) was accurately weighed and added to 10 ml methanolic orthophosphoric acid (10:90, pH=2.3) in a polypropylene tube. After vortex (Cyclo mixer, CM 101, Remi Instruments, India) for 10 minutes the sample hydrogels were placed in an ultrasonicator bath (Toshcon, SW-7, India) for half an hour followed by placing in an orbital shaker (Labline instruments, India) at 37 ºC with constant agitation (200 rpm) for 12 h. Then it was centrifuged (Eppendorf, 510R, Germany) for 10 minutes at 3500 rpm at 4 ºC. The supernatant was taken by means of a syringe, then filtered through a 0.45 µm syringe filter (Millipore millex-HV, Hydrophillic PVDF) and finally put in a sample vial (Waters,USA). The PDA absorbance over the 205-500 nm range was recorded and the wavelength used for quantification was 210 nm.

Standard solutions of IA (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml), each in five replicates were injected into the system. Linearity of the analytical procedure was evaluated by plotting detector response (peak area) against analyte concentration. The regression equation was Y= a X + b, where Y denotes peak area and X is the concentration of IA (µg/ml). The r^2 values for IA was found to be >0.9990, confirming the linear relationship

between the concentration of the IA and area under the curve. Methanolic orthophosphoric acid (10:90) was used as extraction solvent. The coiled and packed chains of hydrogel matrix unfold and make rooms or voids for solvent molecules as it was allowed to swell and the total residual monomer in form of either acid or its salt diffuses from gel network to the extracting solution. Representative HPLC chromatograms of itaconic acid in different hydrogel matrix are shown in Fig. 5. In this case also residual IA graft co-polymer decreased considerably after soxhlet extraction i.e. from 24.01 µg/g to 7.33 µg/g.

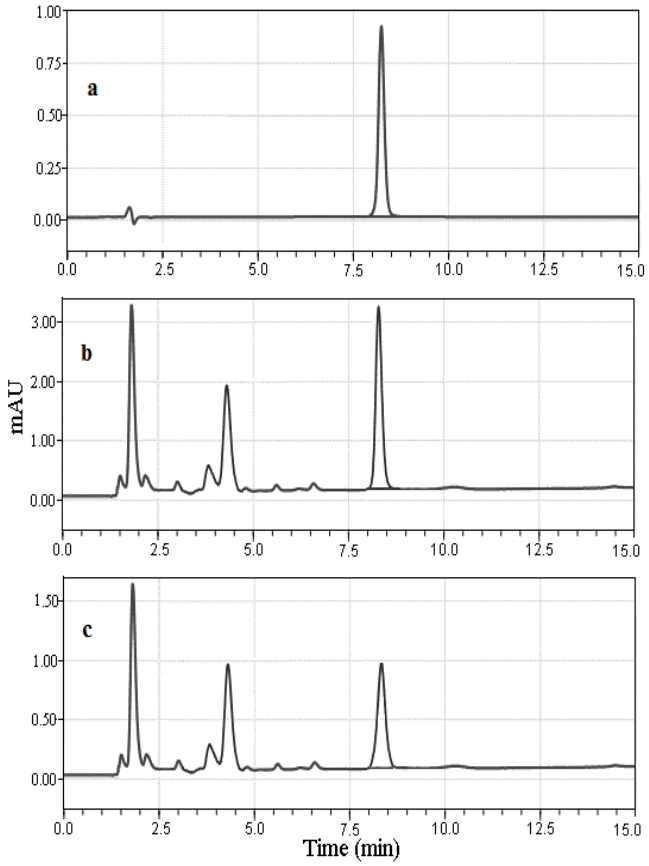

Figure 5. Representative HPLC chromatograms of standard itaconic acid (0.2µg/ml) (a), residual itaconic acid detected in CGN-g-Poly (AM-co-IA) (before soxhlet extraction) (b), after soxhlet extraction (c).

3.6 Swelling behavior

Swelling studies were performed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) as shown in Fig. 6. From the study, swelling of hydrogels in SIF media was found to be much higher than that in SGF. Under acidic pH values (SGF), most of the carboxylate anions were protonated. So the main anion–anion repulsive forces were eliminated and consequently swelling values were decreased. At higher pH values (SIF), some of the carboxylate groups were ionized and the electrostatic repulsion between COO^- groups caused an enhancement of the swelling capacity. CGN-g-P (AM-co-IA) (H10) showed slightly highest swelling capacity in both the media compared to other hydrogels.

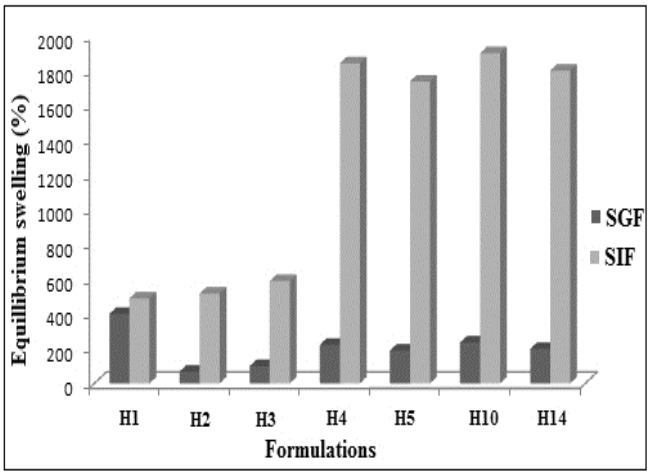

Figure 6. Equilibrium swelling (%) of selected formulations in biological media. SGF: Simulated Gastric fluid; SIF: Simulated Intestinal Fluid

3.7 Drug entrapment efficiency (%)

Entrapment efficiency is the amount of added drug (in percent) that is encapsulated in the formulation (Table II). Among the selected formulations, formulation H10 found to have higher VAL loading and entrapment efficiency. This might be due to comparatively higher swelling capacity of the formulation H10. Low coefficient of variance (< 2.5%) in % VAL entrapment indicates uniformity of drug entrapment in different batches.

Table II VAL loading into CGN-g-Poly (AM-co-IA) formulations, Hydrolyzed collagen: (1.0 g); acrylamide: 1.0 g, H2O: 30 ml, temperature: 80 ◦C

Polymer Code	%VAL loading	%VAL entrapment
H4	96±2.0	79±2.1
H10	**98±2.3**	**83±2.5**
H11	95±1.9	76± 1.7

3.8 *In vitro* drug release study

In vitro VAL release from CGN-g-Poly (AM-co-IA) hydrogel systems were evaluated in pH progressive media *i.e.* SGF as well as in SIF as the dissolution medium to see the release behavior in different pH conditions. The applied formulative variable *i.e.* change in monomer ratio, concentration of the crosslinker in the crosslinked polymer was compared for their influence on drug release rate. Formulation H11 showed slowest drug release profile in both the medium than other formulations and formulation H10 showed relatively more release in both the media (Fig. 7). The crosslinking between the polymer networks found to play major role in the release of VAL. The release of VAL from the hydrogel system was also found to be dependent on swelling properties of the hydrogel in particular medium. Comparatively much higher VAL release was observed in SIF than that in SGF. This may be due to the higher swelling capacity of the system in SIF. VAL release from hydrogel was found to be controlled up to 24h.

3.9 *In vitro* drug release kinetic mechanism

To determine the mechanism of drug release from CGN-g-P (AM-co-IA) matrices, different kinetic models like zero order kinetic, first order kinetic, Higuchi model were used. Regression coefficient (R^2) values of each kinetic model were compared to find out the best fit model. By comparing the R^2 values of different models, Higuchi model was found to be best fit (Table III). So it could be predicted that release of VAL from the hydrogel formulations were of diffusion type. After storing the formulations for three months at accelerated stability condition *i.e.* 40°C/75% RH as per I.C.H. guidelines, the VAL loaded hydrogel were found to retain the same drug content with minor deviations

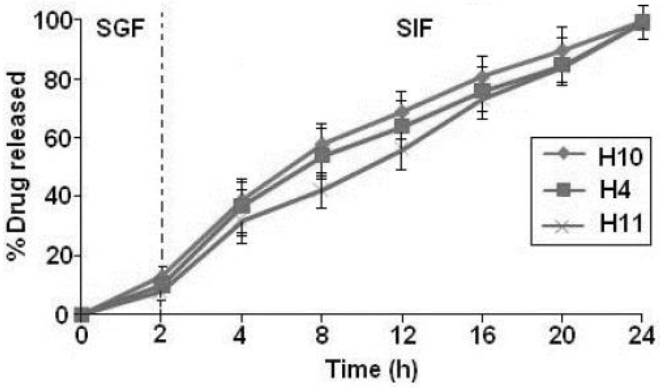

Figure 7 Comparison of % VAL release from VAL loaded formulations (H4, H10 and H11) in pH progressive biological media

Table III. Fitting results of experimental VAL release data of CGN-g-P (AM-co-IA) hydrogel formulations H4, H10 and H11 to different kinetic equations.

Formulation	Zero order (k_0)	R^2	First order (k_1)	R^2	Higuchi (k_H)	R^2
H4	9.521 (1.650)	0.9302 (0.090)	0.714 (0.109)	0.9854 (0.015)	59.475 (1.371)	**0.9868 (0.001)**
H10	7.965 (1.446)	0.9604 (0.061)	0.049 (0.124)	0.9410 (0.011)	21.341 (1.423)	**0.9884 (0.006)**
H11	8.634 (1.244)	0.9831 (0.018)	0.078 (0.191)	0.9280 (0.024)	24.142 (1.482)	**0.9792 (0.009)**

4. Conclusion

In this study, a series of hydrogels based on CGN, AM and IA was prepared through free radical polymerization. These pH sensitive gels respond to small change of pH to much sharper extent than other pH-sensitive gels, which may be due to the presence of two carboxylic groups of IA in the hydrogels. Hydrogels with high content of itaconic acid showed more drug release than those gels with low content of IA. A negligible amount of VAL is released in SGF while in SIF more than 80% of the total drug is released. From the *in vitro* drug release study in pH progressive media, formulation H_{10} showed comparatively higher release extending up to 24 h. From the drug release kinetic study, Higuchi model was found to be best fit among all the models. The mechanism of VAL release from the hydrogel matrix was found to be of diffusion type. To be used for biomedical applications, the amount of the residual monomers in the prepared hydrogel must be kept as low as possible. So we used LCMS/MS and HPLC technique respectively to detect and quantify the residual AM and IA in the synthesized hydrogel. In addition, it is expected that the resulted porous polymers show more compatibility with body when they use as drug delivery systems, because of use of protein as a natural backbone. These results indicate that CGN-g-P- (AM-co-IA) can be used as the sustained-release carrier of VAL in oral administration.

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