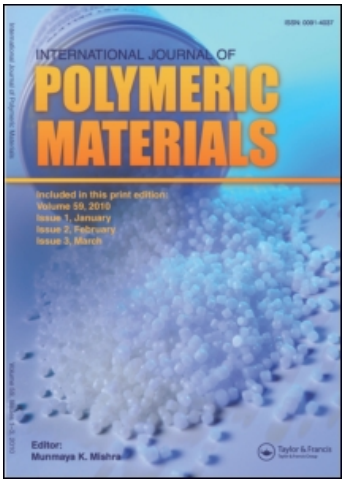

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Preparation and Swelling Study of a pH-Dependent Interpolymeric Hydrogel Based on Chitosan for Controlled Drug Release

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Novel pH-dependent, biodegradable interpolymeric network (IPN) hydrogels were prepared for controlled drug release investigations. The IPN hydrogels were prepared by irradiation of solutions of N-acryloyglycine (NAGly), polyethylene glycol diacrylate (PEGDA) mixed with chitosan, in the presence of a lower amount of glutaraldehyde as the crosslinker and using 2,2-dimethoxy-2-phenyl acetophenone as the photo-initiator. The equilibrium swelling studies were carried out for the gels at 37°C in buffer solutions of pH 2.1 and 7.4 (simulated gastric and intestinal fluids, respectively). 5-Fluorouracil (5-FU) was entrapped, as a model therapeutic agent, in the hydrogels and equilibrium-swelling studies were carried out for the drug-entrapped gels at 37°C. The in-vitro release profiles of the drug were established at 37°C in pH 2.1 and 7.4.

Keywords: biodegradable, chitosan, crosslinking, hydrogel, IPN, swelling

INTRODUCTION

A significant body of research has focused on the preparation and characterization of hydrogels for their use in controlled release of drugs, biotechnology, and many other fields of life [1–8]. However, the number of polymers suitable for the controlled release of viable

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therapeutics is quite limited compared to the total available synthetic polymers because of inherent toxicity or lack of certain properties such as biodegradability and swellability in specific environments.

Chitosan is a cationic biopolymer obtained through alkaline *N*-deacetylation of natural chitin. Chitosan has been considered as a biodegradable, non-toxic, and biocompatible polymer with many superior properties [9–12]. These interesting properties make chitosan an ideal candidate in the preparation of hydrogels for controlled drug release. However, chitosan also exhibits some shortcomings such as hydrophobicity and a high pH-dependence of its physical properties. Therefore, it is very difficult to control drug release with chitosan itself because of the various pHs of the internal organs of the human body. This may negatively reflect on the human body because of the drug's over-release [13].

To improve the hydrophilic nature of chitosan, some trials have been done by blending chitosan with hydrophilic polymers such as poly(vinyl alcohol), PVA [14], poly(ethylene glycol), PEG [13,15] or with poly(N-acryloyl glycine), P(NAGly) [16].

Poly(ethylene glycol) (PEG) is a highly water-soluble polymer widely used as a pharmacological product due to its non-toxicity, high hydrophilicity, and biocompatibility. The chemical modification of chitosan with PEG is considered to be a convenient route to synthesize drug carriers [15].

Glutaraldehyde is a common crosslinker used in the crosslinking of polypeptide, protein, and chitosan polymers due to the high reactivity of its aldehyde groups, which can easily from Schiff's base with the amino groups of the polymer [17]. However, from the health point of view, use of a high percent of glutaraldehyde as a crosslinker in a matrix for a drug delivery is not recommended. This study has developed, characterized, and studied a pH-sensitive interpolymeric (IPN) hydrogel for the controlled drug-release investigation. This IPN attempted to improve the chitosan hydrophilicity through the in situ copolymerization of two hydrophilic monomers, NAGly and PEG-diacrylate (PEGDA) in the presence of chitosan and a lower amount of glutaraldehyde (2–4 w% based on total matrix wt.). The diacrylate derivative of PEG was used to also act as a co-crosslinker with glutaraldehyde in the matrix.

EXPERIMENTAL

Materials

Chitosan, 5-fluorouracil (5-FU), and 2,2 dimethoxy-2-phenyl acetophenone were obtained from Acros Organics (New Jersey, USA).

Poly(ethylene glycol) diacrylate (PEGDA) of average M_n ca. 575 was supplied by Aldrich (Milwaukee, USA). Acryloyl chloride was purchased from Merck (Schuchardt OHG, Hohenbrunn, Germany). Glutaraldehyde (25% aqueous solution), glycine, and all other reagents and solvents were of analytical grade and used as received. The degree of *N*-deacetylation of chitosan was found to be 67% as determined by FTIR using the following relationship [18];

$$\%\,N\text{-deacetylation} = (1 - A_{1655}/A_{3340} \times 1/1.33) \times 100$$

where A is the absorbance value at the given wave number. Approximately the same value of *N*-deacetylation was estimated from the measured elemental analysis data for chitosan.

The molecular weight (M_w) of chitosan was determined to be 4.92×10^5 D, using the Mark-Houwink viscometry method [19], in a solvent of 0.1 M acetic acid/0.2 M NaCl maintained at 25°C. The solvent was prepared by addition of 571 µl of glacial acetic acid (17.5 M) to a solution of 1.17 g of NaCl in 100 ml distilled water. The efflux times of both the solvent and the chitosan solutions were measured with the aid of Cannon-Fenske Routine Viscometer (Cannon Instrument Co, State College, PA. 16801, USA). Each sample was measured three times.

Methods

Synthesis of (NAGly) Monomer

The modified method for the synthesis of NAGly has been described in detail in the authors' previous work [16]. Briefly, a solution of 2.00 g of glycine (26.7 mmol) in 20 mL of NaOH solution of (3 M) was cooled in an ice bath for 10 min with stirring. Then, a solution of acryloyl chloride (2.20 ml, 27.2 mmol) dissolved in 1,4-dioxan (10 ml) was added dropwise with vigorous stirring, and the final solution was stirred at 0°C for 1.5 h. The reaction mixture was washed with ether (2×25 ml), then the aqueous layer was acidified to pH 2 (4 M HCL), saturated with NaCl, and extracted with ethyl acetate (5×20 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, concentrated under reduced pressure, and the residue freeze-dried. The weight of crude product was 2.70 g (78%). A sample was recrystallized (1:1 ethyl acetate:diethyl ether) for analysis; m.p. 132–132.5°C. The FT-IR, MS, and ^{1}H-NMR spectroscopy of the product are given elsewhere [16].

Preparation of the Interpolymeric Network (IPN) Hydrogel

A pre-determined quantity of NAGly (see Table 1) dissolved in 30 ml of 1,4-dioxan was added to a solution of chitosan (3% w/v) in dilute

TABLE 1 Composition of the Prepared Poly[NAGly-PEGDA]-Chitosan Hydrogels

				Glutaraldehyde solution (25% aq.)	
Sample code	Chitosan (g)	NAGly (g)	PEGDA (g)	(μl)	(w% based on chitosan wt.)
GEC-1	2.50	—	—	500	5
GEC-2	2.50	—	—	1000	10
GEC-3	1.00	0.75	0.75	200	5
GEC-4	1.00	0.75	0.75	400	10
GEC-5	0.625	0.875	1.00	250	10

acetic acid (2% w/v). The appropriate weight of PEGDA dissolved in 10 ml of 1,4-dioxan was added to this mixture, then a solution of 2,2-dimethoxy-2-phenyl acetophenone (2 w% based on the total weight of NAGly and PEGDA) in THF (5 mL) was added dropwise. The calculated volume of glutaraldehyde (25% aqueous solution) was added with agitation. The reaction mixture was then poured into a glass Petri dish and the polymerization was initiated by irradiation with an incandescent broad-spectrum lamp (Philips Comptalux, 150 W), positioned 25 cm above the Petri dish. Irradiation was continued for 2 h until gelation occurred. The hydrogels produced were extensively washed with distilled water to remove any residual monomers, freeze-dried, and stored until further use. The compositions of the prepared IPNs are listed in Table 1.

Equipment

Characterization of NAGly was carried out by ^{1}H-NMR (JEOL-GX 270 FT-NMR spectrometer, D_2O solvent) spectroscopy, FT-IR (Perkin Elmer Paragon 1000 FT-IR spectrometer) and MS (Micro Mass ZMD ES-MS spectrometer). NAGly was also characterized qualitatively by using picrylsulphonic acid and ninhydrin reagents. The IR of the dried hydrogels was recorded with a Perkin Elmer Paragon 1000 FT-IR spectrometer in the range 4000–400 cm^{-1}. The elemental analysis was performed with Carlo Erba Elemental Analyser EA 1108 using a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand).

Entrapment of a Model Drug

The poly[NAGly-PEGDA]-chitosan hydrogels loaded with 5-FU as a model therapeutic agent were prepared in the same manner mentioned in a previous section. A predetermined amount of the drug was added to the reaction mixture, stirred vigorously, and then the

polymerization reaction was carried out. The obtained gels were washed with distilled water and freeze-dried.

Determination of the Amount of 5-FU Entrapped

The amount of 5-FU entrapped in the hydrogels was evaluated by an indirect method [16]. After the gel preparation, the washings were collected, filtered with a 0.45 mm Millipore filter and the amount of drug present estimated from the absorption at λ max of 268 nm. The difference between the amount of drug initially added to the gel and that estimated in the washings was taken as a measure of the amount of drug entrapped.

Equilibrium Swelling Measurements

The swelling behavior of the prepared interpolymeric hydrogels was studied at 37°C in pH 2.1 and 7.4 (simulating gastric and intestinal fluids, respectively). The buffer solutions were prepared from a mixture of phosphoric acid (54.0 mmol), boric acid (40.0 mmol), and acetic acid (42.0 mmol) then adjusting the pH to the required value by the dropwise addition of 0.2 N NaOH solution. The pH values were precisely checked by a pH-meter (PHM82/STANDARD, accuracy $\pm$ 0.1). The weights of the swollen gels were measured at intervals after removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by the following equation:

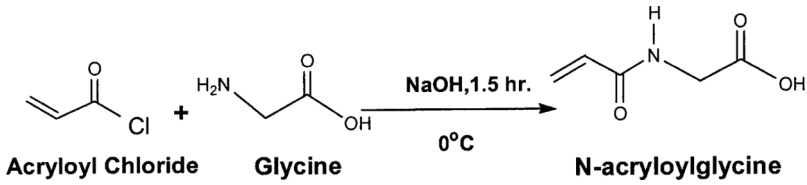
$$\%Swelling = [(W_t - W_0)/W_0] \times 100$$

where W_0 is the initial weight and W_t, the final weight of the swelled gel at time t. The data represents mean $\pm$ SDv. from three independent experiments.

For studying the effect of 5-FU entrapped (up to 5%) in the hydrogel on the gel swelling behavior, a cyclic swelling procedure was carried out. Known weights of the gel and the drug-loaded gel were left in the swelling medium until maximum equilibrium swelling was attained. The swelled sample was weighed after removal of surface liquid, completely freeze-dried and reweighed. This swelling-deswelling process was repeated three times for the same sample.

In-Vitro Cumulative Release Studies

The in-vitro release profiles of entrapped 5-FU were determined by placing the pre-weighed hydrogel loaded with drug in a buffer solution (pH 2.1 or 7.4, similar to that of gastric and intestinal fluids, respectively) at 37°C. At periodic intervals a 3 ml aliquot was withdrawn and its absorbance at 268 nm was measured. The withdrawn sample was



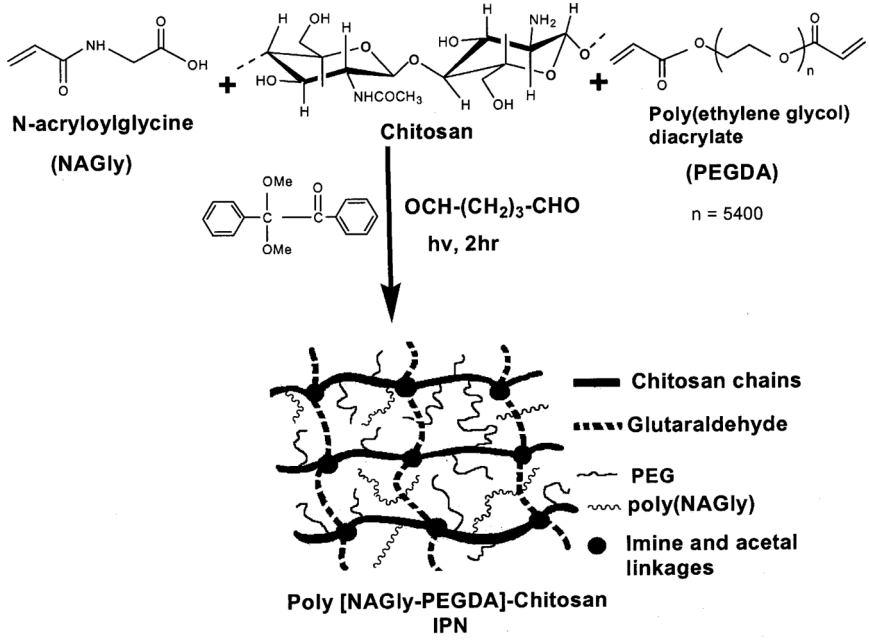
SCHEME 1 Preparation of NAGly.

replaced periodically with an equal volume of fresh buffer, to keep the volume of release media constant. The data represents the mean ± SDv. SDv. from three independent experiments.

RESULTS AND DISCUSSION

Synthesis and Characterization of NAGly

The preparation of NAGly in good yield (78%) by a modified method and its complete characterization were reported in an earlier study [16] (Scheme 1).



SCHEME 2 Preparation of poly[NAGly-PEGDA]-chitosan hydrogels.

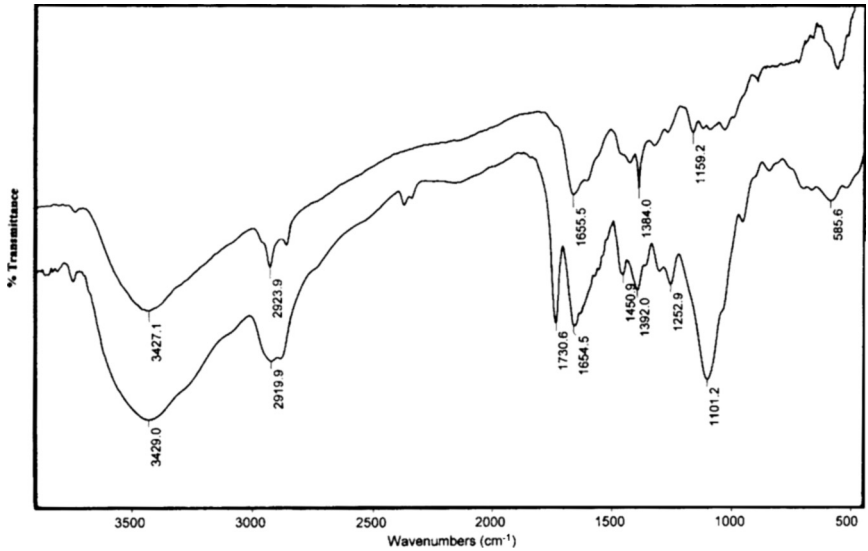


FIGURE 1 FT-IR spectra for chitosan (upper trace) and IPN hydrogel (lower trace).

Preparation and Characterization of the IPN Hydrogel

Scheme 2 shows the preparation of poly[NAGly-PEGDA]-chitosan hydrogels. Inside these crosslinked chitosan matrices, a type of intermixing in the form of graft copolymerized and entrapped poly(NAGly) and polyethylene glycol chains might occur. Also, the diacrylate groups of PEGDA can participate during the irradiation in the crosslinking process of chitosan. Figure 1 shows the FT-IR spectra for the poly[NAGly-PEGDA]-chitosan hydrogel as compared to that of chitosan. The signal appeared at 1730 cm^{-1} was assigned to the C=O stretching of both carboxylate (NAGly) and ester (PEGDA), whereas the absorption peak at 1654 cm^{-1} was attributed to both amide C=O and imine C=N stretching. No clear peaks appeared in the range of 1408–1425 cm^{-1}, which tends to indicate the absence of the vinylic double bond of both NAGly and PEGDA monomers.

Equilibrium Swelling Measurements

A preliminary study was carried out to detect the effect of the IPN composition on the swelling characteristics of chitosan. As shown in Table 2, chitosan itself has a high degree of swelling depending on

TABLE 2 Swelling Values of Poly[NAGly-PEGDA]-Chitosan Hydrogels (GEC 3–4) in pH 7.4 at 37°C as Compared to Crosslinked Chitosan (GEC 1–2)

	Swelling %							
Time (h)	GEC-1	±SDv	GEC-2	±SDv	GEC-3	±SDv	GEC-4	±SDv
0.25	45.8	2.1	40.1	4.9	18.7	5.8	10.16	4.5
0.5	59.2	3.8	55.3	5.1	22	7.5	9.7	3.7
1	110	4.5	100	2.1	21.8	3.7	10.9	5.9
2	185	7.1	150	6.8	36	5.9	16.3	2.1
3	250	5.1	215	9.5	42	2.1	17.9	2.1
4	246	4.8	204	6.1	56.1	6.1	20.9	4.1
5	245	10.5	210	8.8	58	9.8	19.3	3.7
6	241	6.1	205	4.5	60.1	2.5	19.9	6.8
24	240	3.9	200	3.7	60	6.1	21.5	3.4

±SDv: Standard deviation values.

the concentration of the crosslinking agent. For instance, the maximum equilibrium swelling attained about 240% (GEC-1) and 200% (GEC-2) for chitosan crosslinked with 5% and 10% of glutaraldehyde, respectively. In contrast, GEC-3 and GEC-4 attained a maximum equilibrium swelling of 60% and 21%, respectively. This indicates that the swelling of crosslinked chitosan alone is very high, which could be negatively reflected in the controlled drug release pattern. The decrease in the maximum equilibrium swelling for the IPN system is expected and can be controlled by changing the composition of the components as seen in Table 1 and discussed in this work.

The equilibrium swelling behavior of the hydrogels GEC 3-5, measured at 37°C in pH 2.1 and 7.4 are shown in Figures 2 and 3, respectively. The GEC-3 hydrogels attained 68% and 60% swelling at equilibrium in pH 2.1 and 7.4, respectively. The % equilibrium swelling of GEC-4 gels were 38% and 21% whereas GEC-5 gels were swollen to 72% and 62% at pH 2.1 and 7.4, respectively. These swelling values demonstrate the difference in swelling at equilibrium for gels prepared with varying amounts of PEGDA, NAGly, and glutaraldehyde.

Comparing the swelling values of GEC-3 and GEC-4 at the same pH implies that, as the percent of glutaraldehyde increases, the extent of crosslinking increases leading to a decrease of the equilibrium swelling. The % equilibrium swelling values, attained at the same pH for GEC-4 and GEC-5, showed that increasing the amounts of both NAGly and PEGDA increases the hydrophilicity of the gel and consequently the % equilibrium swelling increases. During the hydrogel preparation, some PEGDA may act as a co-crosslinker for chitosan with

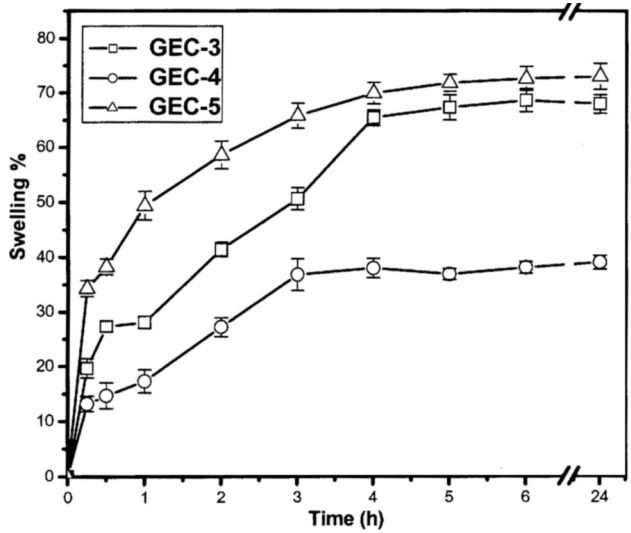


FIGURE 2 Swelling behavior of poly[NAGly-PEGDA]-chitosan hydrogels at pH 2.1 and 37°C.

glutaraldehyde and so it may work to decrease the swelling extent of the gel but from the swelling data obtained this factor seems to be less effective than the increasing of the gel hydrophilicity. Also, decreasing

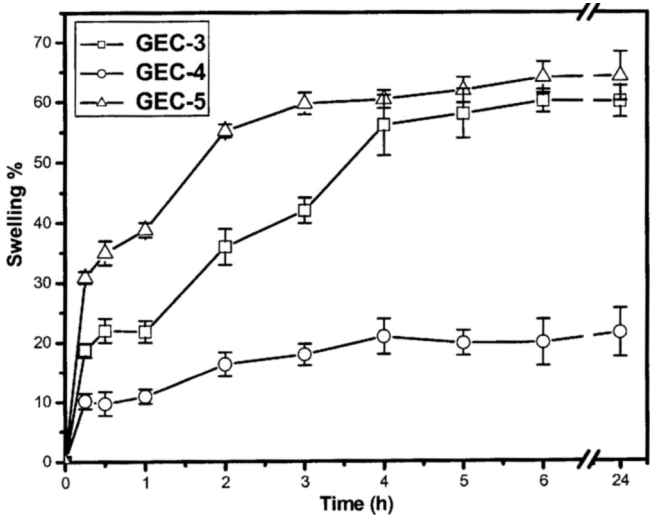


FIGURE 3 Swelling behavior of poly[NAGly-PEGDA]-chitosan hydrogels at pH 7.4 and 37°C.

the chitosan content in GEC-5 may have led to the decrease of the total crosslinking extent of the matrix.

The swelling values attained at equilibrium were found to be higher at pH 2.1 than at pH 7.4. This pH-responsive character of the hydrogels can be attributed to the chemical structure of chitosan where, at the acidic pH, protonation can occur at both of unreacted NH_2 groups of chitosan and the imine (C=N) groups leading to dissociation of the hydrogen bonding involving amino/imine groups. Moreover, the acidic medium can hydrolyze the imine bond leading to facilitation of the entrance of solvent into the gel to attain higher values of swelling. The swelling process of hydrogels involves the ionization of the unreacted amino groups in the acidic buffer solution, then the acid would be attracted to the gels by ionic bonds leading to increasing the weight of the gels in the acidic buffer [16,20]. In addition, the equilibrium swelling values at pH 7.4 will be lower than the values in pH 2.1 due to the increased hydrophobicity of the chitosan-based hydrogels dominating at higher pH values, thus preventing faster swelling in neutral and alkaline media [21].

Figure 4 shows the % equilibrium swelling of GEC-4 and GEC-4 loaded with 5-FU (GEC-4FU, 5 w% uploading) at pH 2.1 and 7.4. At pH 2.1, GEC-4 reached equilibrium at about 38% swelling after 3 h while under the same conditions GEC-4FU attained equilibrium at 53% after approximately 4.5 h. Similar behavior was observed at pH

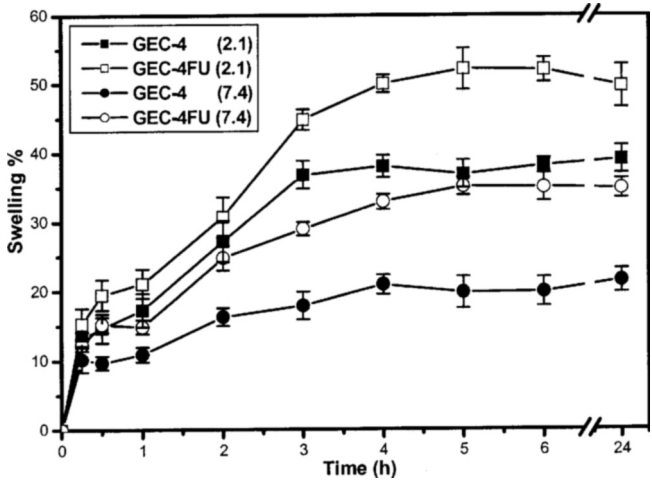


FIGURE 4 Swelling behavior of GEC-4 and GEC-4FU in pH 2.1 and 7.4 at 37°C.

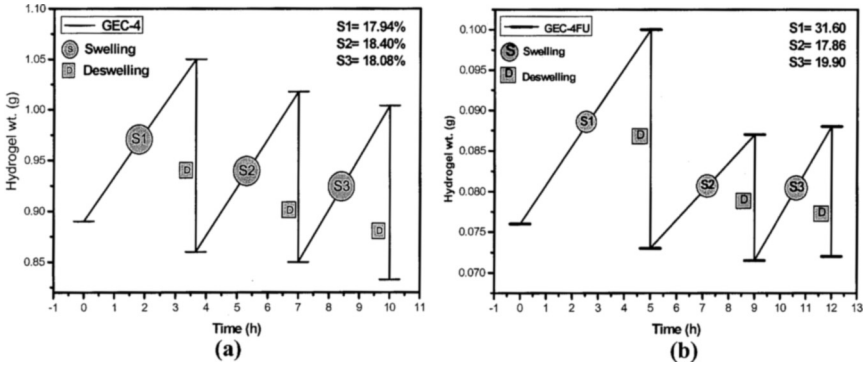


FIGURE 5 The cyclic swelling for (a) GEC-4 and (b) GEC-4FU in pH 7.4 at 37°C.

7.4. This increase in % swelling upon loading 5-FU can be attributed to the hydrophilic nature of the 5-FU molecules, which facilitates the diffusion of the swelling fluids into the partially swollen hydrogels.

The change in the swelling behavior of the gel upon loading the drug was also confirmed by carrying out cyclic swelling for both the drug-loaded (GEC-4FU) and the drug-free (GEC-4) samples as shown in Figure 5. From this figure, GEC-4 gel (Figure 5a) attained about 18% equilibrium swelling in the first cycle. Approximately no change was noticed in the other two cycles except a slight decrease in the weight of the dried gel. In the case of the drug-loaded gel (Figure 5b), the equilibrium swelling for the first cycle attained about 31%. Deswelling of the sample and repeating the experiment shows that the equilibrium swelling decreases and achieved its maximum value after a shorter time. The third cycle shows approximately no change in the maximum equilibrium swelling with a slight decrease in the time of achieving this equilibrium. These results confirm that 5-FU is responsible for the increase of equilibrium swelling value due to its hydrophilic nature. Figure 5b shows also that all the accessible 5-FU is released from the matrix in the first cycle. The lack of change seen in the other two cycles implies the role played by 5-FU and tends to indicate that little or no soluble fraction remains in the matrix after 5 h of initial swelling.

In-Vitro Cumulative Release Studies

The in-vitro cumulative release profiles of 5-FU from the hydrogels in pH 2.1 at 37°C are shown in Figure 6. Comparing the extent of release

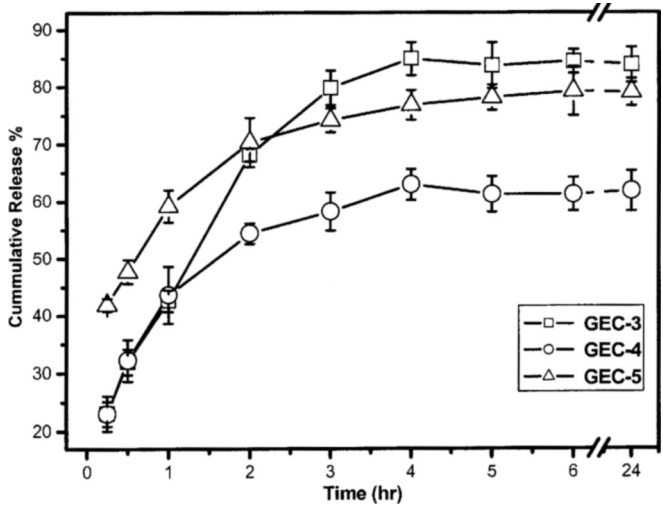


FIGURE 6 In-vitro cumulative release measurements of 5-FU loaded poly[NAGly-PEGDA]-chitosan hydrogels at pH 2.1 and 37°C.

from GEC-3 and GEC-4 clearly confirms that the amount of drug released at equilibrium is inversely related to the degree of crosslinking. GEC-5 (higher NAGly and PEGDA contents) exhibits a particularly high extent of release as compared to GEC-4. The same behavior of drug release from the hydrogels was noted in pH 7.4 (Table 3).

TABLE 3 In-vitro Cumulative Release Measurements of 5-FU Loaded poly[NAGly-PEGDA]-Chitosan Hydrogels at pH 7.4 at 37°C

	Average cumulative release % (pH 7.4)					
Time (h)	GEC-3	±SDv	GEC-4	±SDv	GEC-5	±SDv
0.25	23.4	3.2	21.0	1.1	31.7	2.1
0.5	30.3	2.8	25.2	2.6	40.6	1.0
1	40.1	1.3	36.6	2.0	53.2	1.8
2	65.8	1.2	48.0	2.8	65.3	2.8
3	76.3	3.5	50.4	1.3	68.4	2.1
4	78.1	1.1	52.9	2.4	71.8	2.2
5	80.3	2.4	54.2	3.1	74.3	2.7
6	79.4	2.0	53.9	2.7	79.2	4.1
24	80.0	4.1	54.3	2.5	79.0	2.5

±SDv: Standard deviation values.

The gels exhibited higher percent of drug released at pH 2.1 than at pH 7.4. This difference in the release rate can be attributed to the difference in swelling behavior of the gels where the drug release may attribute to the diffusion-dissolution mechanism through the swollen gels. As discussed earlier, the swelling of the prepared gels in the acidic medium is higher than in weakly basic medium. In the case of GEC-5, the extent of drug release shows little pH sensitivity due to the presence of smaller amount of chitosan.

As shown in Figure 6 and Table 3, the gels attained equilibrium after about 4–6 h and 5–6 h in pH 2.1 and 7.4, respectively. Varying the crosslinking density and/or the percent of the IPN components can modulate this release time. Also, repeating this preliminary study using other model drugs may change both the time and the rate of release.

CONCLUSIONS

Novel biodegradable pH-dependent IPN hydrogels of poly[NAGly-PEGDA]-chitosan were prepared by irradiation for controlled drug release studies. The pH-responsive behavior of these gels was observed through studying their equilibrium swelling at 37°C in simulated body fluids (pH 2.1 and 7.4). The in-vitro release profiles of 5-FU, as a model therapeutic agent, from the gels were also estimated at the same pH values. The results obtained showed that the inclusion of PEGDA in the matrix assembly was beneficial. In the presence of PEGDA, longer release times were achieved than the previous study [16] with reduced use of the relatively toxic crosslinker, glutaraldehyde. This preliminary study points to these chitosan-based interpolymeric hydrogels being good candidate matrices that may be tailored for utilization in drug delivery applications.

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