Synthesis and Characterization of Biodegradable Hydrogels for Oral Delivery of 5-Fluorouracil Targeted to Colon: Screening with Preliminary *In-vivo* Studies

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Abstract

In this communication, we developed a thermally stable, biocompatible and colonically-degradable hydrogel-based device [Pectin-co-poly(MAA)] for oral delivery of 5-Flurouracil (5-FU) to treat colon cancer with minimal upper gastrointestinal invasion. Towards this end, ethylene glycol dimethacrylate (EGDMA) crosslinked hydrogels of pectin were synthesized. Methacrylic acid (MAA) was grafted to impart pH-responsive-character while benzoyl peroxide (BPO) was applied for simultaneous grafting and crosslinking polymerization. The hydrogels were characterized by FTIR, TGA, DSC and XRD. SEM micrographs were taken to analyze the surface morphology. Swelling behaviour was analyzed to assess better performance of biodegradable hydrogels for optimized loading and release of the drug targeted to colon. Gel fraction, swelling ratio, diffusion coefficient, drug loading and cumulative release increased with increase of pectin ratio and decreased on increase of MAA and EGDMA ratio. Strategically, hydrogels with higher amounts of pectin were prepared for complete degradation in colon. Our investigations indicate that Pectin-co-poly(MAA) hydrogel is a suitable delivery system developed for oral delivery of the drug targeted to colon.

Keywords Biodegradable, Colon targeting, 5-Fluorouracil, Croslinking, Hydrogels, Copolymerization

Introduction

Hydrogels are high molecular weight, three-dimensional cross-linked polymeric networks composed of a wide variety of hydrophilic polymers. Cross-linked polymers are being investigated at tremendous rate for numerous applications in controlled release, sustained release, targeted drug-delivery, protective drug-release and tissue engineering.[1-3] The hydrogel networks can be fabricated from a wide range of monomers, polymers (natural or synthetic origin), biodegradable, non-biodegradable or combinations of both. The resulting cross-linked polymeric systems impart extensive diversity in terms of bulk physical properties. However, crosslinked natural polymeric matrices are desirable being environment friendly.[4]

Pectin, a naturally occurring carbohydrate polymer derived from citrus sources. Pectin is chemically comprised of poly α 1–4-galacturonic acids having varying degree of methylation of carboxylic acid residues.[5-7] Pectin shows excellent characteristics for the effective delivery of various agents. The promising properties of pectin include good gelling properties, stability in acidic conditions as well as at higher temperature, no toxicity, low production cost and easy availability.[8-12]

A particular issue with 5-FU is the need for site specific delivery to the colon with little or no release of the drug in the upper gastro-intestinal tract (GIT). Pectin appears to be ideal in resolving this issue as it is stable at low pH and resistant to proteases and amylases that are active in upper GIT. Therefore, hydrogels based on pectin would retain their integrity in the upper GIT. Further, pectin is completely degraded by colonic microorganisms; thereby it can provide the required specific delivery in the colon. Keeping in view of these suitable properties of pectin, we selected the polymer for hydrogel synthesis for colonic delivery of 5-FU.[13-17]

Natural polymers have good biocompatibility, biodegradability and have evident advantages over the synthetic polymers.[18] However, natural polymers impart less mechanical strength to polymeric formulations. Pure pectin has poor capacity to hold the drug that causes premature release of drug.[19] Higher amounts of pectin cannot be incorporated easily in dosage forms. Poor entrapment efficiency and low mechanical strength of pectin based formulations necessitate its chemical modifications.[17]

Colon cancer is the fourth most commonly occurring among cancers.[13] The 5-FU is commonly administered in colorectal cancer by intravenous bolus injection in standard regimen.[20] Oral conventional delivery of 5-FU cannot be adopted due to its erratic absorption through GIT. Gastrointestinal absorption of 5-FU is rapid, yielding peak levels in the blood between 15 and 60 minutes after ingestion. However, there are high variations in plasma concentrations due to pre-systemic elimination. While intravenous delivery distributes 5-FU equally in all compartments of body. The plasma half-life is extremely short being in the range 8 to 20 minutes.[21] 5-FU has low lipid solubility but can cross the blood brain barrier.[22] From a patient compliance point of view, oral therapy, were it effective, is preferred.[17, 23] 5-FU is a cytotoxic drug and intravenous therapy of this anticancer agent may cause serious side effects in various parts of body (where exposure to this drug would be inappropriate) other than the colon. Oral site specific delivery of 5-FU in colon cancer has the potential to minimize these complications.[20, 24, 25]

Various approaches have been adopted to prevent unwanted effects by developing delivery systems and formulations that offer site specific delivery.[13] Recently, stimuli sensitive (pH-sensitive) hydrogels prepared by synthetic polymers have been studied for colonic delivery of 5-FU.[19, 26] Various other techniques (e.g. pH sensitive nanoparticles) have also been investigated for 5-FU targeting.[27] But most of these studies have been conducted

without considering the GI factors (especially inter-individual variations) that might cause burst release of 5-FU in the upper GIT.[17]

We present the development of stable hydrogels for site specific oral delivery for 5-FU using a more efficient methodology and a novel combination of natural biodegradable polymer, pectin. The current method is an enhancement of the previously reported methods of Bettini et al., (1995) and Sutar et al., (2008). [28, 29] The study presented here reports an efficient methodology for synthesis of colon specific biodegradable hydrogels consisting of a combination of pectin, MAA and EGDMA. Chemically crosslinked matrices show high and uniform swelling than radiation-induced crosslinking. [30] EGDMA showed excellent crosslinking agent between pectin and MAA. Based on physical characterization and drug release kinetics, the newly developed hydrogel system appeared effective for targeted oral delivery of 5-FU.

Materials and Methods

Chemicals

5-Fluorouracil was obtained from Pharmedic Laboratories (Pvt.) Ltd. Lahore, Pakistan. Pectin (MW≈ 30000-100000), methacrylic acid, ethylene glycol dimethacrylate and benzoyl peroxide were purchased from Sigma Aldrich, UK., and sodium dihydrogen phosphate from Merck, Germany.

Synthesis of Pectin-co-poly(MAA) hydrogels

The solution polymerization process was adopted and crosslinked hydrogels were formulated by varying pectin, MAA and EGDMA contents as shown in Table 1. A specific amount of pectin was weighed and dissolved in a small portion in water with continuous stirring. The temperature of reaction mixture was maintained at 40^{o}C. Purging of pectin solution was

carried out for 30 minutes to remove dissolved oxygen. The benzoyl peroxide employed as a reaction initiator (1% of MAA) was dissolved in a weighed amount of MAA. This benzoyl peroxide-MAA mixture was slowly mixed into the pectin solution at 40^{o}C. Finally, the crosslinking agent EGDMA was added with continuous stirring and the final volume made up to 100 g with water (Table 1). The reaction mixture was carefully transferred to glass tubes and placed in a water bath at 65^{o}C for 12 hours. After this treatment, all tubes were cooled to room temperature and cylindrical firm hydrogels were drawn. These were cut into small disks 6 mm in thickness having same diameter and washed with ethanol-water (70:30) to remove unreacted monomers and catalyst. Fresh solvent was used until there was no difference in the pH of the washings and fresh solvent. Initially the disks were dried in laminar flow air for 24 hours and then in vacuum oven at 40^{o}C for one week.

FTIR

FTIR spectra of the raw materials pectin, benzoyl peroxide and methacrylic acid were recorded. Samples were thoroughly ground and analyzed by attenuated total reflectance ATR-FTIR (Schimadzu, Germany) in the range of 4000-650 cm^{-1}. All the hydrogel formulations were also characterized by FTIR.

TGA

Thermal analysis was performed using a thermo-gravimetric analyzer (TGA) and differential scanning calorimetry (DSC). TGA analysis was performed on the TGA module of TA instruments Q5000 series Thermal Analysis System (TA instruments, West Sussex, UK). A quantity between 0.5-5mg was placed in an open pan (platinum 100 µl) attached to a microbalance. The samples were heated at 20^{o}C/min from 25-500^{o}C under dry nitrogen with a flow rate of 10ml/min in standard mode with ramp test type. All the measurements were made in triplicate.

DSC

DSC analysis was carried out using the DSC module of TA instruments Q2000 Series Thermal Analysis system (TA Instruments, West Sussex, UK). Indium (99%, melting point 156.6) was used to calibrate the DSC and validated using a zinc standard with a melting point of 419.5^{o}C. Samples of pure pectin and the various formulations (0.5-3mg) were precisely weighed into an aluminum pan onto which aluminum lid with a centrally pierced hole was crimped. The samples were then scanned under a stream of nitrogen gas from 25 – 200^{o}C using a heating rate of 20^{o}C/min. All samples were analyzed in triplicate.

PXRD

Powdered X-ray diffraction data were obtained using a Bruker D-8 powder diffractometer (Bruker, Kahlsruhl, Germany) at room temperature. Sample preparation involved filling a plastic sample holder with the powder of pure pectin and smoothing the surface with a glass slide. Samples were scanned over the range 5-50° 2θ at a rate of 1° 2θ/min using a copper Kα radiation source with a wavelength of 1.542Å and 1mm slits. All formulations were analyzed in triplicate.

SEM

The shape and surface morphology of hydrogels was investigated using scanning electron microscopy (SEM) by a Quanta 400 SEM (FEI Company, Cambridge, UK). Completely dried discs of hydrogels were cut to optimum sizes to fix on a double-adhesive tape stuck to an aluminum stub. The stubs were coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were randomly scanned and photomicrographs were recorded to reveal surface morphology.

Sol-gel ratio

The extent of reactants consumed in the synthesis of Pectin-co-poly(MAA) hydrogels was calculated by measuring sol and gel contents. [31] The sol content represents the soluble unreacted content of the polymerization reaction. To measure the sol content, the formed hydrogel were cut into 2 mm size slabs. The slabs were dried at 55^{o}C to constant weight. The dried gel pieces were placed in a Soxhelt apparatus and extracted for twelve hours in boiling deionized water. The extracted gels were removed and dried to constant weight at 55°C. The sol and gel fraction was measured by Equation (1) and (2) respectively:

$$Sol\ fraction = \frac{M_i - M_s}{M_s} \times 100 \quad (1)$$

Where M_i denotes the initial weight of dry gel before extraction and M_s indicates the weight of dry gel after extraction.

$$Gel\ fraction = 100 - Sol\ fraction \quad (2)$$

Swellability of hydrogels

The swelling properties of hydrogels were determined at 37 ˚C by immersing samples of known weight in 100 ml 0.5 M buffer solutions of pH 1.2 and 7.4. The samples were removed at specific intervals to determine the dynamic swelling, weighed after removing excess of water by blotting with tissue paper. The swelling studies were continued up to equilibrium weight. The degree of swelling and equilibrium water content were determined using Equation (3) and (4) respectively: [32]

$$Q = \frac{M_s}{M_d} \quad (3)$$

$$EWC\% = \frac{M_{sq} - M_d}{M_d} \times 100 \quad (4)$$

Where M_s indicates mass of swelling at predetermined time interval and M_d represents the weight of dry gel before initiation of swelling experiments.

Diffusion coefficient

Diffusivity across crosslinked hydrogels is an important physicochemical property and has applications in absorbance and controlled release of drugs at target area. Diffusivity represents the amount of mass transfer across unit area of matrix in unit time. [33] Swelling data was used to calculate diffusion coefficient (D_c) by following Equation: [34]

$$D_c = \pi\left(\frac{l\theta}{4Q_{eq}}\right)^2 \qquad (5)$$

where, l indicates the thickness of dry hydrogel slab before start of swelling experiment, θ is the slope obtained by plotting initial 60% swelling ratio against time and Q_{eq} is the equilibrium swelling ratio of hydrogels.

Drug loading

5-FU was loaded in all hydrogel formulations by diffusion. The hydrogel slabs were allowed to swell completely until equilibrium in 100 ml 5-FU solution (1.0%) in phosphate buffer of pH 7.4. The pH 7.4 was selected in order to increase mesh size of crosslinked network and which ultimately enhances the entrapment of drug entrapment. [28] All disks were completely washed with deionized distilled water to remove any remaining drug on the surface.[35]

Drug entrapment efficiency and release study

Some Pectin-co-poly(MAA) hydrogel disks loaded with drug were ground in a clean and dried pestle and mortar. Weighed quantity of this powder was placed in a phosphate buffer solution pH 7.4 for 30 minutes with periodic shaking and centrifuged at 3000 rpm. The supernatant layer was separated, filtered and assayed for 5-FU using UV-spectrophotometer at λ_{max}266 nm.

In-vitro drug release study was performed to determine the pH dependant delivery of 5-FU from hydrogel network. Drug loaded disks were placed in 900 ml phosphate buffer solutions of pH 1.2 and 7.4 in a USP dissolution apparatus-II at 37 ± 0.5^{o}C. A 10 ml volume of dissolution medium was periodically withdrawn and replaced with fresh buffer solution to maintain the sink conditions. The collected samples were then analysed at 266 nm using UV-Vis-spectrophotometer. 5-FU release has been presented in Table 2.

Biological Evaluation of 5-FU Loaded Hydrogels

In-vivo evaluation was performed on rabbits to assess targeting properties of the developed formulations. Study was started after the approval of Pharmacy Research Ethics Committee (PREC). The selected animal models were kept in separation, properly tagged and grouped into two parts. 5-FU standard solution was administered to Group-A and Group-B received Pectin hydrogel samples with 50mg/kg dosing. Analyses were carried out to investigate the absorption level (through plasma drug concentrations) and drug amount in recovered hydrogels from rabbit intestine (assay of remaining drug amount in hydrogels). Samples were assayed through a newly developed and validated HPLC method for 5-FU, we have reported this method in our recent publication.[36]

Results and discussions

FTIR

FTIR spectra of pure pectin, MAA and all cross-linked Pectin-co-poly(MAA) hydrogels taken to confirm the cross-linking between polymer (pectin) and monomers (MAA) are shown in Figure 1. The spectrum of pure pectin shows a peak at 3437 cm^{-1}due to –OH stretching. The spectral peak at 2931 cm^{-1}indicates –C–H stretching vibrations, while the peak at 1749 cm^{-1} suggests the presence of >C=O, those at 1628 cm^{-1} and 1444 cm^{-1}correspond to C=C and $–CH_2$ stretching vibrations.

The IR spectrum of methacrylic acid reveals various characteristic peaks, with the peak at 2929 cm^{-1} being assigned to methyl C–H asymmetric stretching. Importantly the peak range 1725-1700 cm^{-1} is assigned for carboxylic acid (1699 cm^{-1} indicate carboxylic acid) and the peak at 1633 cm^{-1} shows the C=C stretching vibrations.

The FTIR spectrum of cross-linked Pectin-co-poly(MAA) hydrogel shows a different peak intensity pattern from the spectra of pure pectin, MAA and EGDMA indicating development of new polymer. The peak range 3570-3200 cm^{-1} is assigned to –OH stretching, peaks at 3457 cm^{-1} and 3245 cm^{-1} indicate the hydrogen bonded –OH cm^{-1} stretching vibrations.

TGA and DSC

TGA analysis was performed on raw material and all formulations. MAA showed complete decomposition at 110^{o}C and pure pectin at 240^{o}C. However, the decomposition of the crosslinked polymer was observed above 400^{o}C. The DSC thermograms of pectin, MAA and crosslinked Pectin-co-poly(MAA) hydrogels are presented in Figures 2 and 3, respectively. DSC of pure monomer MAA showed an endothermic peak below 25^{o}C for MAA and at 130^{o}C for pectin. The DSC thermogram of Pectin-co-poly(MAA) hydrogel shows a small

peak at 100°C and then a large endothermic-peak at about 240°C. TGA and DSC characterization confirmed the formation of a new co-graft polymer. Crosslinked matrices of Pectin-co-poly(MAA) revealed high thermal stability compared with their individual components. The DSC peaks at 100°C indicate the water loss from the matrices which is followed by decomposition at approximately 240°C. Clearly our method of hydrogel fabrication yields thermally stable crosslinked matrices of pectin.

PXRD

Powder x-ray diffraction was employed to study the crystallinity of pectin and the crosslinked pectin hydrogels. The diffraction patterns are presented in Figure 4. Pure pectin gives a different PXRD pattern compared to the crosslinked matrices confirming the formation of a new material. The PXRD pattern of Pectin-co-poly(MAA) hydrogel indicates less crystalline structural characteristics than pure pectin. The decrease in crystallinity might occur due to incorporation of new bulkier groups within the pectin polymer by copolymerization. Addition of the groups decreases the intermolecular hydrogen bonding.[29, 37]

Surface morphology of Pectin-co-poly(MAA) hydrogels

Surface morphology of crosslinked Pectin-co-poly(MAA) hydrogels was studied by scanning electron microscopy. Dried hydrogel discs were observed for intact surface and some discs were cut to take images of cross-sectional morphology.

SEM micrographs revealed different surface morphology for the hydrogels with higher concentration of MAA from that observed for hydrogels having high amount of pectin. The intact surface of dried discs containing high MAA contents showed a smooth surface and apparently less porous while hydrogels with high content of pectin showed horizontal grooves like longitudinal channels. High porosity was observed by cross-sectional view of

discs for hydrogels with high content of pectin. Hydrogels produce smooth and dense surface with MAA at higher ratio and higher amounts of pectin make highly porous surfaces. Some SEM micrographs have been shown in Figure 5 a, b, c, d.

Gel fraction

The gel fraction of the pectin-co-MAA polymer increased with increase of pectin, crosslinker and monomeric ratio. This trend is attributed to the fact that an increase in ratio of any of reactant in the feed mixture provides more active sites for free radical polymerization reaction. The velocity and extent of the reaction is based on the feed composition of hydrogel mixture.

Swelling studies

The results of swelling capabilities of the new gels are presented in Figure 6. All formulations of new copolymer showed pH-dependent swelling. The swelling index of hydrogels was very low at pH 1.2 than swelling at pH 7.4. The low swelling of gels is attributed to the presence of grafted carboxylic groups of MAA. At low pH, the carboxylic groups remain protonated and the network structure of the crosslinked polymer remains intact.[29] The segmental chain mobility of gels is minimal at low pH. However, there was a high swelling index of hydrogels at pH 7.4. This is thought to be associated with high segmental mobility of the polymer chains and resulting increase in network mesh size. The pKa value of MAA is 4.5. On increase of pH above the pKa value of MAA, the grafted carboxylic groups deprotonate.[38] The deprotonated grafted carboxylic anions push each other resulting in extension of the crosslinked network. The pore size of the extended network is substantially increased due to which water enters rapidly into the gel leading to a high swelling index.[39] Swelling kinetics showed a low swelling index with the increase of MAA ratio. The fact of low swelling with increase of MAA could be explained that higher cross linking between monomers and or

monomer with polymer (pectin) restricts swelling ability. High crosslinking between components increases the crosslinked density. High crosslinking density reduces the exposure and restricts swelling. However, rapid swelling of the new polymer at high pH is a desirable property which can be tuned to control drug release. The drug release from hydrogels is driven by the swelling capacity of hydrogels. At pH 1.2, the swelling index of gels is very low therefore; negligible amount of drug will be released at this pH. However high swelling index at pH 7.4 make the new polymeric gels suitable carriers for site specific release of 5-FU in colon.

With increase in the pectin ratio, the swelling index was increased. This can be attributed to the hydrophilic nature of the polymer which attracts and retains more water. The unreacted carboxylic and methoxyl groups of pectin may contribute to low swelling at pH 1.2 and high swelling index at pH 7.4.

The swelling index was reduced with increase of EGDMA ratio. The decrease in swelling index with increase degree of crosslinking is associated with formation of more tight junctions or high crosslinking density. The segmental mobility of highly crosslinked polymeric chains is restricted which results in a low degree of swelling.[40] The analysis of the effect of feed composition on swelling index provides a way to develop formulations with more suitable drug release properties.

Diffusion coefficient

The diffusion of water was dependent on polymeric, monomeric and crosslinking ratio of the hydrogels. The values of the diffusion coefficient also correlate with the swellability of gels. On increase in the polymeric ratio, there was an increase in diffusion coefficient. However, the diffusion coefficient decreased with an increased degree of crosslinking and monomeric

ratio. Similarly, there was a low swelling ratio and equilibrium swelling with an increase in the crosslinking ratio in feed mixture.

Drug entrapment and release profile of 5-FU

Drug entrapment of all prepared pectin-co-poly(MAA) hydrogels revealed high efficiency with higher swellability. Drug entrapment in hydrogels increased with increase of pectin but decreased on increase of MAA and EGDMA ratio. Pectin-co-poly(MAA) hydrogels showed 58-60% drug release at pH 1.2 and more than 90% release at pH 7.4 after 12 hours dissolution experiments. Recent studies on targeted delivery of 5-FU reported the site-specific drug carriers, through cross-linked and enteric coated microspheres, [41] controlled and pH sensitive microsphere blends. [42-43] The release profile of 5-FU is shown in Figure 7. The release profile was associated with swelling behaviour of hydrogels. Due to rapid and massive uptake of water, the rate and extent of drug release was high at pH 7.4. There was an increase in drug loading and release on increase of pectin ratio. However, 5-FU loading and release decreased on increase of MAA and EGDMA contents. The gel formulations which showed a low swelling ratio and correspondingly low diffusion coefficient values also showed low drug entrapment and slow release of 5-FU. Influence of gel composition on the diffusion coefficient, gel content and drug loading is presented in Table 2.

In-Vivo Studies

A previously reported work has confirmed that a colon targeting of 5-FU prolongs the cytotoxic effect as compared to conventional immediate release systems. [44] 5-FU-loaded hydrogels administered to rabbits and samples were collected up to 24 hours because limited number of samples can be collected from rabbits. Pectin hydrogels showed gradual increase in plasma concentration, T_{last} 24.0 hours is time at which last sample was collected and C_{max}

(210.219 ± 2.112 μg/ml) is maximum drug concentration that was found in 24 hour samples. C_{max} of hydrogel was less than C_{max} of oral solution, it can be expected that drug release could remain continue to last part of GIT. After 24 hours, rabbits were slaughtered and intestinal contents were removed and 5-FU concentration was assessed by HPLC method.[36] The results showed that approximately 10-15 mg remaining amount of drug was found in total intestinal contents of studied rabbits. The estimation of remaining drug contents confirmed the slow release of drug from prepared hydrogels. The developed pectin based systems could effectively deliver the optimum concentration of 5-FU locally in colon part of the GIT. In-vivo profiles of oral solution and hydrogels of 5-FU are presented in Figure 8 and 9.

Conclusion

Hydrogels offer the possibility of pH-selective release kinetics of loaded material which can be exploited for site specific drug delivery. We developed and characterised pectin-based hydrogels for the delivery of 5-FU to the colon. The material was designed with the aim to minimize exposure of anti-cancer drug in the upper GIT and maximize its concentration in cancerous colon. FTIR spectra and SEM micrographs confirmed the formation of highly crosslinked porous network structures. All fabricated hydrogels expressed good pH-sensitivity required for site specific delivery of the drug. Swelling index and drug release were found to be dependent on the pH of external media. The diffusion coefficient, gel fraction, drug loading and cumulative release were dependent on hydrogel composition. DSC and XRD analysis revealed the development of a stable and less crystalline polymer. The drug release data shows that Pectin-co-poly(MAA) hydrogels have a high potential for site specific delivery of 5-FU targeted to colon with minimum exposure of the upper gastrointestinal tract.

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Table 1 Formulation contents of Pectin-co-poly(MAA) hydrogels

Formulation	Pectin (g/100 g)	MAA (g/100 g)	Pectin/MAA (Wt %)	EGDMA (g/100 g)
FM-1	0.60	30.0	1.96/98.04	0.2
FM-2	0.60	40.0	1.48/98.52	0.2
FM-3	0.60	50.0	1.19/98.81	0.2
FP-1	0.20	35.0	0.57/99.43	0.2
FP-2	0.80	35.0	2.23/97.77	0.2
FP-3	1.00	35.0	2.78/97.22	0.2
FE-1	0.60	35.0	1.68/98.32	0.1
FE-2	0.60	35.0	1.68/98.32	0.2
FE-3	0.60	35.0	1.68/98.32	0.3

Table 2 Effect of reaction variables on diffusion coefficient (D_c), gel fraction and drug loading

Formulation	D_c $10^7 cm^2 sec^{-1}$	Gel fraction	5FU loading mg/g of dry gel
FM-1	8.32	93.34	127.7
FM-2	6.24	93.78	84.05
FM-3	5.37	95.37	57.53
FP-1	6.35	91.21	68.99
FP-2	7.11	93.45	65.92
FP-3	7.78	94.10	64.22
FE-1	11.56	92.63	96.44
FE-2	9.74	94.58	89.23
FE-3	8.42	95.33	77.79

Figure captions

Fig. 1 FTIR spectra of pectin, MAA and Pectin-co-poly(MAA) hydrogel

Fig. 2 TGA curves of pectin, MAA and Pectin-co-poly(MAA) hydrogel

Fig. 3 DSC curves of pectin, MAA and Pectin-co-poly(MAA) hydrogel

Fig. 4 PXRD pattern of pectin and Pectin-co-poly(MAA) hydrogel

Fig. 5 a, b, c, d SEM micrographs of intact and cross-section surfaces

Fig. 6 Swelling ratio of Pectin-co-poly(MAA) hydrogels

Fig. 7 5-FU release profile of FP-3 Pectin-co-poly(MAA) hydrogels

Fig. 8 Plasma vs time plot of oral solution of 5-FU (Standard)

Fig. 9 Plasma vs time plot of hydrogel formulation of 5-FU

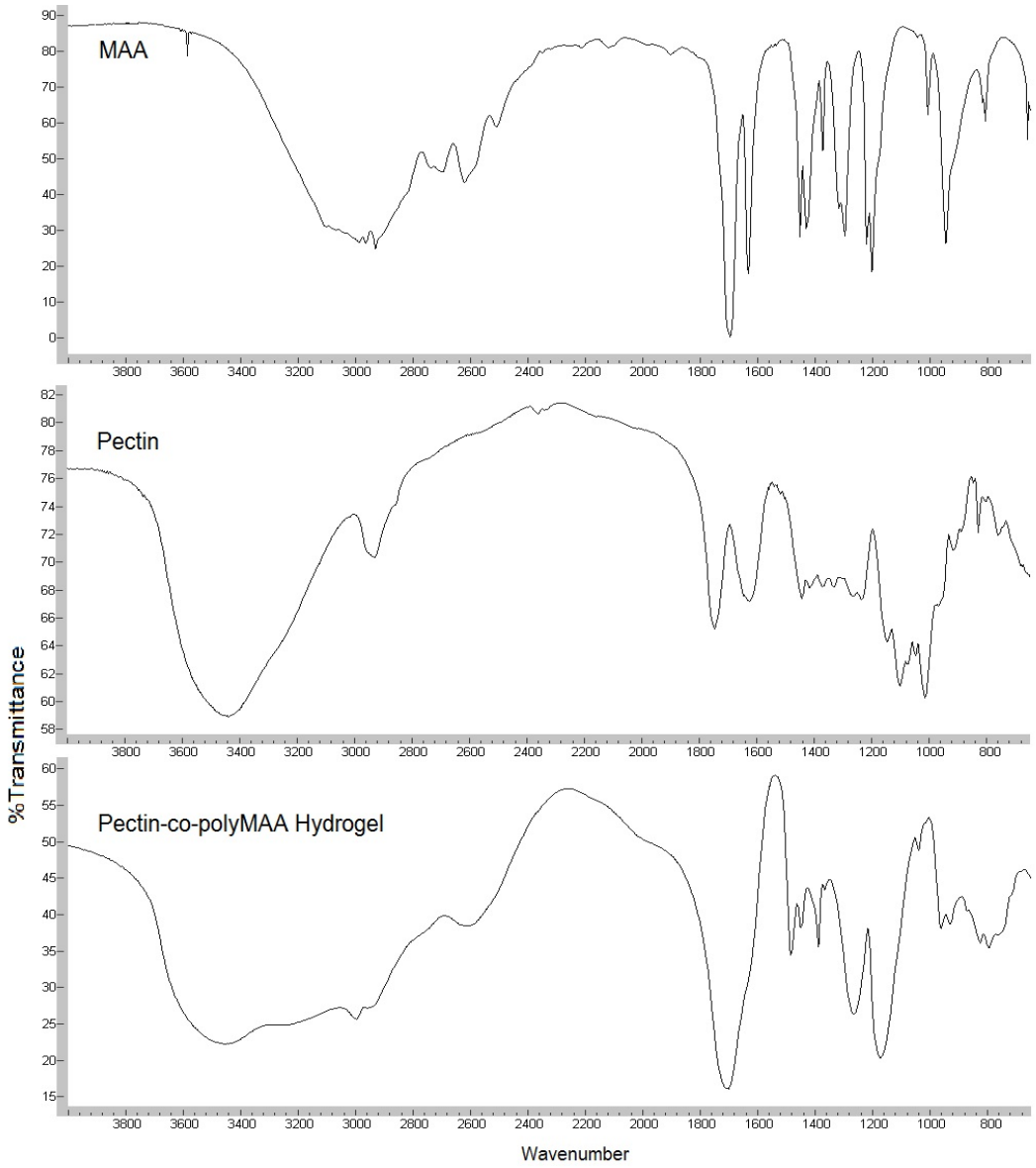
MAA
Pectin
Pectin-co-polyMAA Hydrogel
%Transmittance
Wavenumber

Fig. 1

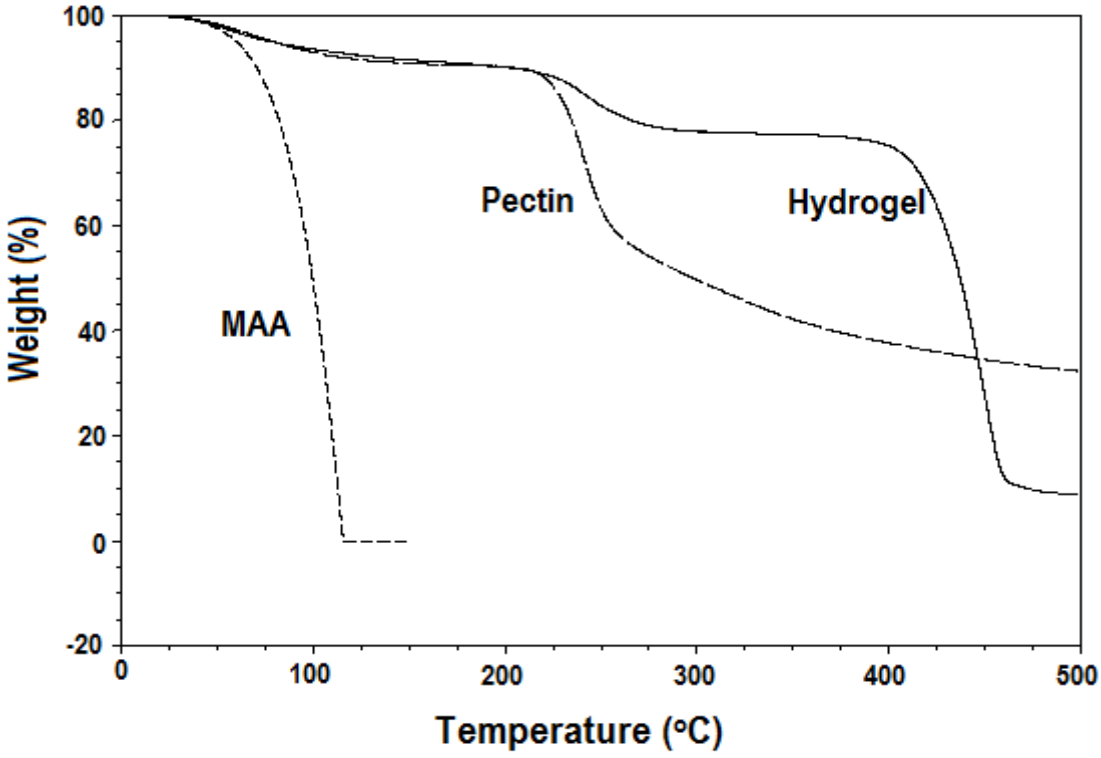
Weight (%)
Temperature (°C)
MAA
Pectin
Hydrogel
100
80
60
40
20
0
-20
0
100
200
300
400
500

Fig. 2

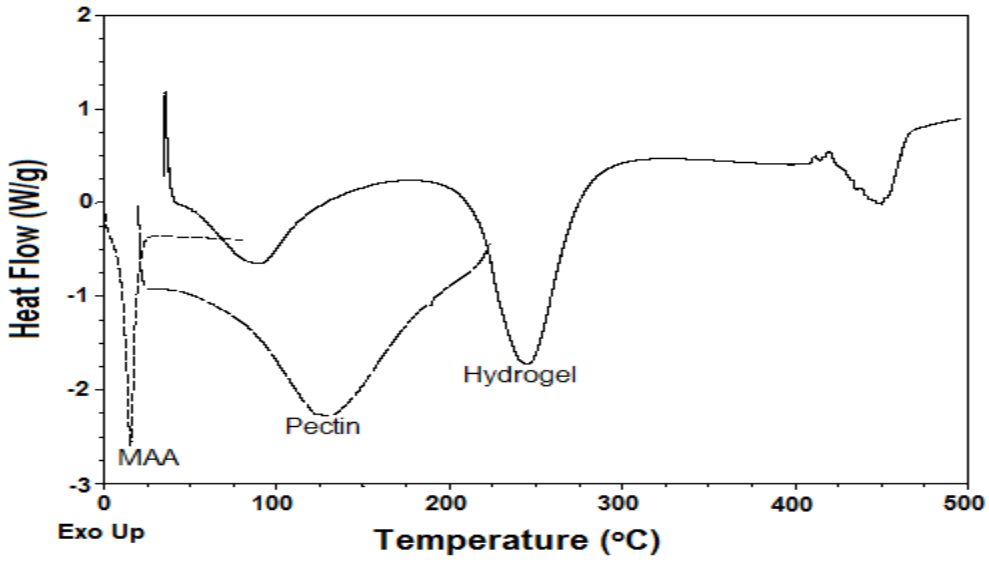
Heat Flow (W/g)
Temperature (°C)
Exo Up
MAA
Pectin
Hydrogel
2
1
0
-1
-2
-3
0
100
200
300
400
500

Fig. 3

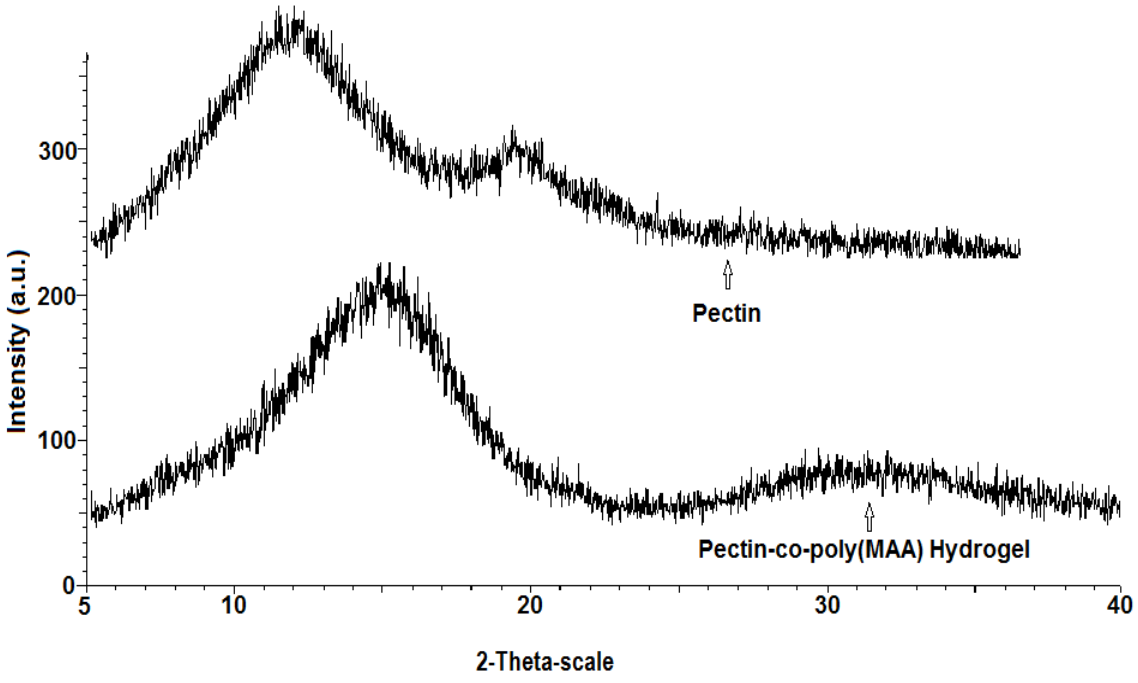

Fig. 4

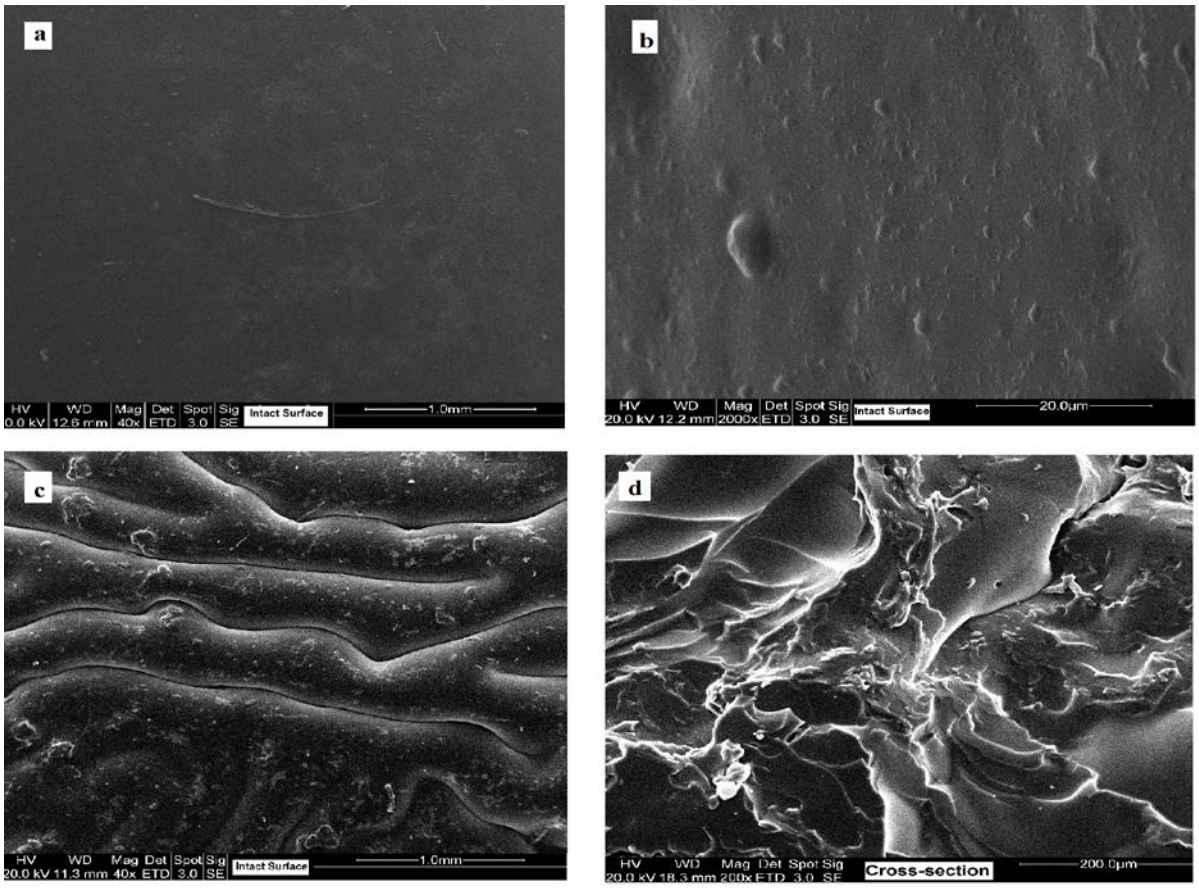

Fig. 5 a, b, c, d

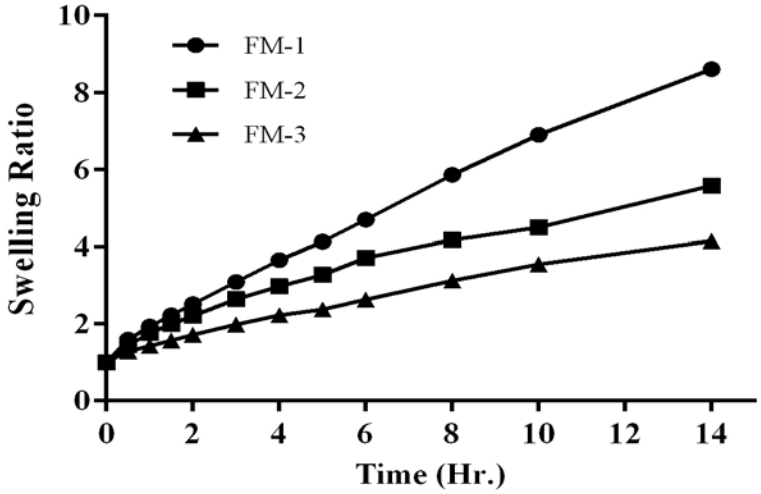
FM-1
FM-2
FM-3
Swelling Ratio
Time (Hr.)
0
2
4
6
8
10
12
14

Fig. 6

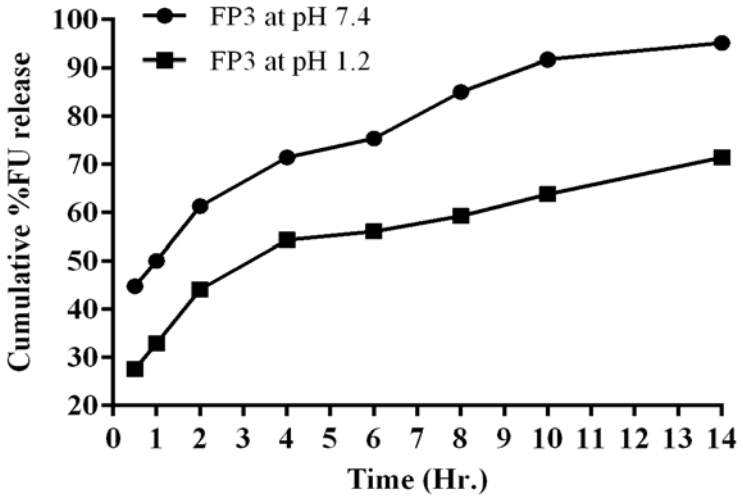
FP3 at pH 7.4
FP3 at pH 1.2
Cumulative %FU release
Time (Hr.)
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 7

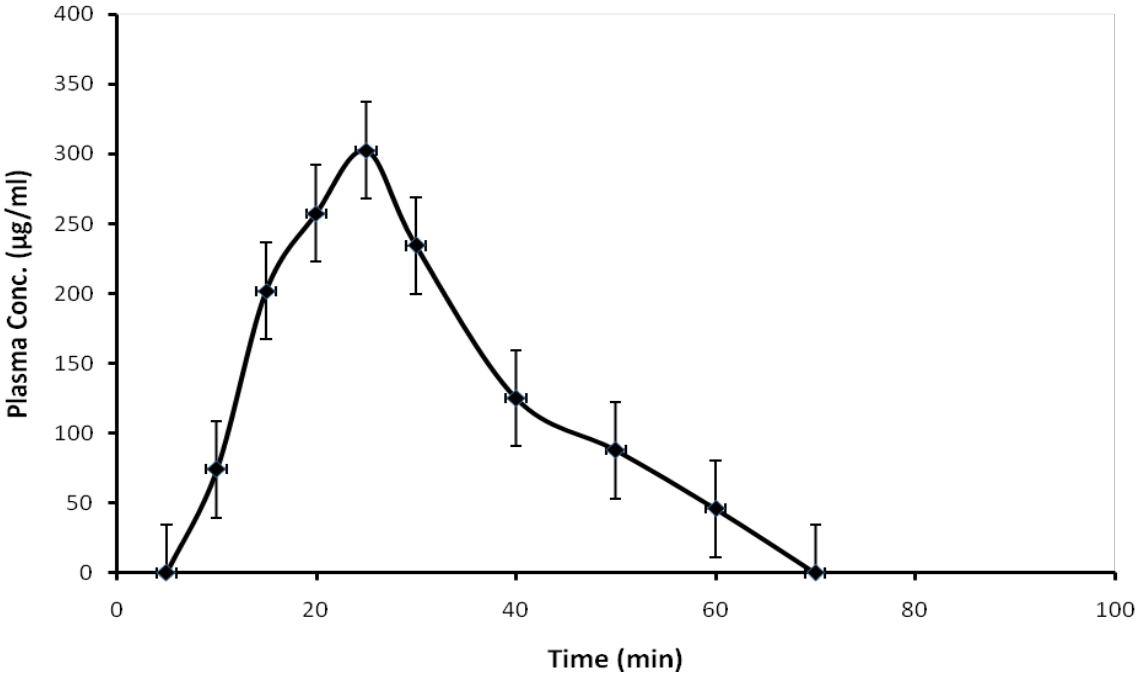
Plasma Conc. (µg/ml)
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
Time (min)

Fig. 8

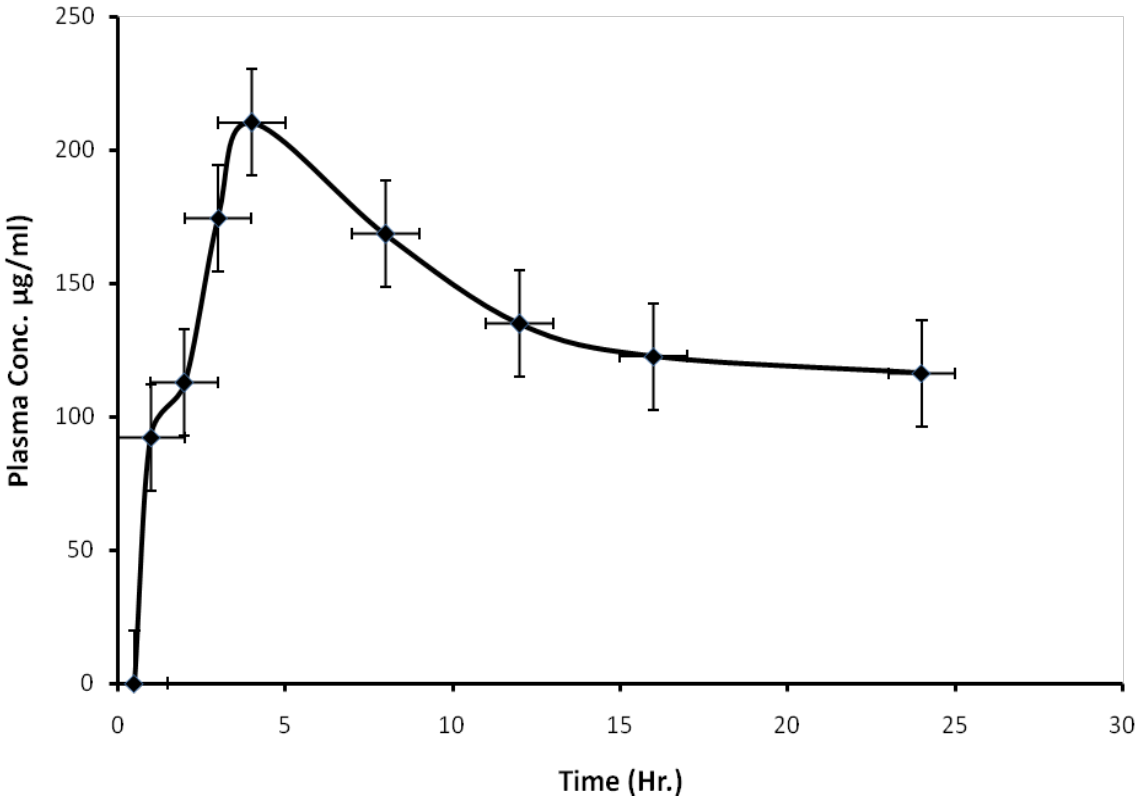
Plasma Conc. µg/ml)
250
200
150
100
50
0
0
5
10
15
20
25
30
Time (Hr.)

Fig. 9