

SYNTHESIS AND CHARACTERIZATION OF β -CYCLODEXTRIN HYDROGELS: CROSSLINKED POLYMERIC NETWORK FOR TARGETED DELIVERY OF 5-FLUOROURACIL

MUHAMMAD USMAN MINHAS,* MAHMOOD AHMAD,* SHAHZEBA KHAN,**
LIAQAT ALI* and MUHAMMAD SOHAIL****

*Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, Islamia University of
Bahawalpur, Bahawalpur-63100, Punjab-Pakistan

**Department of Pharmacy, University of Malakand, KPK-Pakistan

***Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad-Pakistan

✉ Corresponding author: Mahmood Ahmad, ma786_786@yahoo.com

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The aim of the study was to synthesize and characterize a potential crosslinked polymeric system for oral targeted delivery of 5-Fluorouracil, a first-line anticancer agent in colorectal cancer. β -Cyclodextrin crosslinked with methacrylic acid (β -CD MAA) hydrogels were developed using free radical copolymerization in aqueous media. ATR-FTIR spectra of β -CD MAA hydrogels confirmed the formation of a new crosslinked polymeric structure and SEM images revealed a porous network structure. Thermogravimetric analysis and differential scanning calorimetry (TGA & DSC) analyses clearly indicated high thermal stability of the developed new crosslinked polymer hydrogel structure. Physicochemical properties such as swelling index, gel fraction and porosity of hydrogels were measured. 5-Fluorouracil (5-FU), an antineoplastic agent, was incorporated as a model drug. Rapid swelling and drug release were observed at pH 7.4. However, the swelling index and 5-FU release remained very low at pH 1.2. This investigation demonstrated the strong site specific release potential of β -CD MAA hydrogels for colonic delivery of antineoplastic agent 5-FU.

Keywords: targeted delivery, cyclodextrin, 5-Fluorouracil, crosslinking, copolymerization

INTRODUCTION

Targeted delivery to colon offers some promising therapeutic advantages in the treatment of non-cancerous bowel diseases and colon carcinomas. Colon targeted delivery ensures high drug concentration locally at the disease site, thereby helps to improve efficacy and reduce side effects. A drug delivery system having a triggering mechanism and responsive to physiological variations in gastrointestinal tract may concentrate the drug particularly into colon.¹

Hydrogels are versatile, reproducible, and finely tunable materials that have gained a huge interest as biomedical, and drug carrier devices in last few decades.²⁻⁴ Hydrogels are gaining a great interest in drug targeting due to their promising stimuli responsive and controlled release characteristics. Two main approaches used for site specific delivery by hydrogels are: first, diffusion, erosion or swelling in response to physiological stimuli (temperature, pH, electric or magnetic

field, light, and ionic potential), and second, breakage of labile covalent bonds on exposure to specific biological conditions.⁵⁻⁷ High hydrophilicity of non-crosslinked hydrogels limit their control over the release of hydrophilic agents and retard the loading of hydrophobic drugs. However, crosslinked hydrogels keep their original shape, remain intact and do not degrade under ordinary pressure of intestinal movements.⁸ Chemically crosslinked hydrogels are being studied extensively for various pharmaceutical and biopharmaceutical applications. As compared to conventional drug carriers, hydrogels are more appropriate polymeric matrices for site specific drug delivery.⁹⁻¹⁰

β -CD is a torus-shaped cyclic oligosaccharide linked by α -1,4 glycosidic bonds. Its internal hydrophobic cavity and external hydrophilic surface make it a unique polymer for a variety of applications in food, purification, analysis,

pharmaceuticals and other biomedical applications. β -CD forms inclusion complexes through host-guest interactions. Cyclodextrins have been used in pharmaceutical technology, cell biology and gene delivery. Powerful functionalization potential, unique inclusion capability, low immunogenicity, low toxicity, and excellent biocompatibility of cyclodextrins (CD_s) and their derivatives render them highly suitable materials to be engineered for biomedical applications.¹¹⁻¹³ CD_s can be modified to smart hydrogels using different polymerization techniques for various applications in controlled drug delivery and gene therapy.¹⁴⁻¹⁷ Yu-Yang and co-researchers synthesized the β -CD interpenetrating polymeric network (β -CD-IPN) microgels. They studied the pH dependent inclusion complexes of methyl orange with β -CD.¹⁸ Chen and coworkers synthesized crosslinked poly(ethylene glycol)- β -CD-copoly(acrylic acid) hydrogels by emulsion polymerization technique and electron beam irradiation.¹⁹ Jose-Fernando and coworkers developed CD based hydrogels as contact lenses with tunable mechanical, loading and drug release properties. These hydrogels showed high cytocompatibility and negligible macrophage response. Hydrocortisone and acetazolamide were successfully incorporated into hydrogels and their release was extended to several days.²⁰

5-FU is extensively applied for treatment of solid tumors of the rectum or colon. Some serious side effects of 5-FU chemotherapy include cell growth defects, liver disease, hair loss, fatigue, mouth sores, birth defects, ulcers and temporary suppression of bone marrow cells.²¹⁻²²

The above mentioned side effects associated with conventional therapy of 5-FU can be reduced significantly by targeting 5-FU in colon cancer. Therefore, here, we developed and optimized a new β -CD based graft polymer of MAA crosslinked with ethylene glycol dimethacrylate EGDMA. The new graft polymeric network showed a promising ability for delivery of 5-FU targeted to colon.

EXPERIMENTAL

Chemicals

Beta cyclodextrin (MW 80,000 amu), methacrylic acid, benzoyl peroxide and ethylene glycol dimethacrylate were purchased from Sigma Aldrich, Germany. Double distilled water used in this study was prepared in our Pharmaceutics Research Laboratory.

Hydrochloric acid, sodium hydroxide, sodium chloride, ethanol and potassium dihydrogen phosphate were obtained from Merck Marker, Germany. Active Pharmaceutical Ingredient (5-Fluorouracil) was provided as a gift sample from Pharmedic Pharmaceutical Laboratories (Lahore, Pakistan).

Copolymerization reaction

Aqueous based free radical graft copolymerization was applied to synthesize β CD-MAA graft polymer hydrogels. The composition and feed ratio for synthesis is shown in Table 1. All aqueous solutions of β -CD were prepared using continuous stirring and EGDMA was mixed in MAA. Then β -CD solution and monomer-crosslinker mixture were mixed and stirred together for 30 minutes. Finally, the initiator, benzoyl peroxide was added, mixed well, and the reaction mixture was transferred to glass molds of uniform diameter. Graft copolymerization was carried out in thermo controlled water-bath. The temperature scheme for reaction was adopted as follows: 50 °C for 1 h, 55 °C for 2 h, 60 °C for 3 h and 65 °C for 8 h. The three dimensional rigid non-dispersible rolls were removed from molds and cut into 4-5 mm discs.

Gel fraction

Sol-gel analysis helps to find out the extent of chemical reaction. Sol-gel analysis was carried out in a Soxhlet apparatus. Thin hydrogel discs (2-3 mm thickness) without any previous treatment were used to study the sol-gel fraction. Each disc was weighed and labeled before placing it into deionized water.

Each disc was extracted at 90 °C for twelve hours. Finally, the extracted gel pieces were removed, first air dried and then dried in an oven at 45 °C for one week. The following relations (Eqs. 1 and 2) were used to calculate sol and gel contents:

$$\text{Sol Fraction (\%)} = \left[\frac{M_0 - M_i}{M_0} \right] \times 100 \quad (1)$$

$$\text{Gel Fraction} = 100 - \text{Sol Fraction} \quad (2)$$

where M_0 indicates the weight of dry hydrogel before the extraction process and M_i represents the weight of copolymer dried after completion of the extraction process.

Swelling kinetics in simulated gastric fluids

Swelling of crosslinked hydrogels is an important phenomenon that determines the efficiency of drug loading and predicts the release behavior. Since the pH of the gastrointestinal tract varies from highly acidic in the stomach to basic in the colon, the swelling kinetics was measured in simulated gastric fluids of pH 1.2 and 7.4. Washed and dry hydrogel discs were weighed on an analytical balance and placed on the bottom of a glass jar containing 100 ml of buffer solution at room temperature. Swelling kinetics was measured by removing the swollen gel.

Table 1
Polymer and monomer contents in β -CD MAA hydrogel formulations

Formulation	β -cyclodextrin (g/100 g)	Methacrylic acid (g/100 g)	EGDMA (g/100 g)	β -CD/MAA (wt%)
CDF-1	1.00	30.0	0.2	3.225/96.77
CDF-2	2.00	30.0	0.2	6.25/93.75
CDF-3	5.00	30.0	0.2	14.28/85.71
CDF-4	10.0	30.0	0.2	25.00/75.00
CDF-5	2.00	10.0	0.2	16.66/83.33
CDF-6	2.00	20.0	0.2	9.09/90.91
CDF-7	2.00	30.0	0.2	6.25/93.75
CDF-8	2.00	40.0	0.2	4.79/95.24

The swollen gels were removed at predetermined time intervals, gently wiped with tissue paper, weighed on an analytical balance and again placed in the same buffer solution. Swelling experiments on β -CD MAA hydrogels were continued till equilibrium was achieved. The following equation (Eq. 3) was used to calculate the swelling index:

$$q = \frac{W_h}{W_d} \quad (3)$$

where W_h shows the weight after swelling and W_d represents the weight of the dry hydrogel before the start of swelling. The equilibrium swelling index was measured by the same equation as mentioned above at equilibrium swelling. Swelling experiments were performed thrice for each hydrogel formulation.

Porosity

Porosity represents the overall space available in three dimensional networks of hydrogel discs. Dry hydrogel discs of known weight, diameter, and thickness were immersed and allowed to swell in absolute ethanol for 16 h. The polymer discs were removed, the excess surface liquid was carefully blotted, weighed on an analytical balance, and the diameter was measured. The following equation (Eq. 4) was used to calculate percent porosity:

$$\text{Porosity} = \left[\frac{M_2 - M_1}{\rho V} \right] \times 100 \quad (4)$$

where M_1 and M_2 represent the weights of a copolymer disc before and after immersion in absolute ethanol respectively, ρ is the density of absolute ethanol and V is the volume of hydrogel disc after swelling.

Drug uptake capacity

In chemically crosslinked hydrogels, generally two methods are used to incorporate drug molecules in the network. The first method involves the addition of drug in the synthesis solution of hydrogels. But this method has some serious drawbacks especially for the drug molecules having reactive sites. The drug molecule may be chemically attached to any one constituents used for hydrogel synthesis. Therefore, keeping the above facts in view, the diffusion method was applied to entrap 5-FU. Dry hydrogel discs of uniform weight were soaked in 1% solution of 5-FU in

phosphate buffer of pH 7.4. Hydrogel discs were allowed to absorb drug solution for 72 h. Then, hydrogel discs were removed, wiped gently, weighed on an analytical balance, and the diameter and thickness of the hydrogels were measured. The loaded hydrogels were first allowed to air dry for 24 h and then were dried to equilibrium at 45 °C in a hot air oven. Drug loading and percent drug loading were measured by using the following relations:

$$\text{Amount of drug loaded} = W_D - W_d \quad (5)$$

$$\text{Drug loading (\%)} = [(W_D - W_d)/W_d] \times 100 \quad (6)$$

where W_d and W_D are the weights of dried hydrogels before and after immersion in drug solution, respectively.

Drug release studies in simulated gastrointestinal fluids

In vitro drug release experiments were performed in a USP dissolution apparatus II. In order to evaluate the site specific drug release potential, simulated physiological media of pH 1.2 and pH 7.4 were prepared and used for dissolution experiments. The volume of dissolution was 500 ml in each vessel, the temperature of the vessels was maintained at 37±0.5 °C and the paddle speed was maintained at 50 rpm throughout the release experiments. A 5 ml aliquot was collected from the dissolution medium at predetermined time intervals and replaced every time with fresh buffer to maintain the volume constant throughout the experiment. The aliquot was analyzed spectrophotometrically at $\lambda_{\text{max}} = 260$ nm and the cumulative drug release was calculated at different time intervals up to 50 h.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of MAA, β -CD, 5-FU, benzoyl peroxide and β -CD MAA hydrogels were recorded. The samples were thoroughly ground and analyzed by an attenuated total reflectance Fourier transform infrared spectrometer (ATR-FTIR, Shimadzu, Germany) in the range of 4000-650 cm^{-1} . All hydrogel formulations were evaluated by recording FTIR spectra.

Scanning Electron Microscopy (SEM)

Quanta 400 SEM (FEI Company, Cambridge, UK) was used to analyze the shape and surface morphology of the synthesized hydrogels. The samples for SEM study were prepared by fixing the hydrogel disks on a double-adhesive tape stuck onto an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under 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.

Thermogravimetric Analysis (TGA)

Thermal analyses were performed by a thermogravimetric analyzer (TGA) and by 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 0.5-5 mg of MAA, β-CD and β-CD MAA hydrogel samples was placed in an open pan (platinum 100 µl) attached to a microbalance. The samples were heated under nitrogen flow at 20-600 °C at the rate of 20 °C/min. All the measurements were made in triplicate.

Differential Scanning Calorimetry (DSC)

A TA instruments Q2000 Series Thermal Analysis system (TA Instrument West (UK) was used for DSC analysis. The DSC instrument was calibrated by indium (99%, melting point 156.6). Confirmation of the calibration was performed using a zinc standard with a melting point of 419.5 °C. Small quantities (0.5-3 mg) of polymer, monomer and new copolymer were precisely weighed into an aluminum pan onto which aluminum lid with a central pierced hole was crimped. The scanning of samples was carried out in nitrogen environment from 10-300 °C using a heating rate of 20

°C/min. Each sample was subjected to DSC scanning for three times.

Powder X-ray Diffractometry

A Bruker D-8 powder diffractometer (Bruker Kahlsruhl, Germany) was used to measure x-ray diffraction at room temperature. Sample preparation involved filling a plastic sample holder with the powder of pure β-CD and ground β-CD MAA hydrogel. The samples were scanned over the range 5-60° 2θ at a rate of 1° 2θ/min, using a copper Ka radiation source with a wavelength of 1.542 Å and 1 mm slits. All formulations were analyzed in triplicates.

RESULTS AND DISCUSSION

Effect of polymer/monomer ratio on gel contents

Higher polymer to monomer ratio contributed to an increased gel ratio of β-CD MAA hydrogels. Importantly, the effect of β-CD ratio was not prominent when compared to the effect of MAA. At high concentrations of polymer, 5% or higher (CD-F3 & CD-F4), undissolved polymer crystals were observed. This effect might be due to the saturation of the reaction sites on the polymer after the reaction with the monomer (MAA). However, all formulations showed a gel ratio greater than 90%. It indicates that new three dimensional crosslinked network structures are successfully developed and the reaction rate could be optimized by careful selection of monomer, crosslinker and polymer ratio. The values of gel, sol fractions and drug uptake (loading) are provided in Table 2.

Table 2
Effect of reaction variables on porosity, swelling index, gel fraction and 5-FU loading

Formulation	Porosity	Swelling index		Gel fraction	5-FU loading (mg/mg of dry hydrogel)
		pH 1.2	pH 7.4		
CDF-1	29.74	1.45	10.27	92.57	0.124
CDF-2	30.05	1.69	13.34	92.37	0.141
CDF-3	31.69	2.23	b	93.29	NA
CDF-4	31.96	2.83	b	94.07	NA
CDF-5	32.37	1.33	9.37	90.49	0.088
CDF-6	34.66	2.27	13.39	93.71	0.095
CDF-7	35.51	3.15	16.19	95.46	0.152
CDF-8	37.19	4.11	21.68	96.11	0.158

^b Samples broke during swelling

^{NA} Not applicable

Effect of polymer/monomer ratio and pH on swelling

Table 2 explains the effect of polymer/monomer ratio on the swelling behavior

of the hydrogels. Swelling of β-CD MAA hydrogels was highly influenced by the monomer to polymer ratio. The swelling index increased gradually on increasing the ratio of MAA. On an

increase of the MAA ratio in the formulation, an increase in grafted carboxylic groups on the polymer was observed. The higher the numbers of ionizable groups grafted on the backbone, the higher is the swelling potential. Sadeghi and Hosseinzadeh prepared methylene bisacrylamide crosslinked hydrolyzed collagen graft polymethacrylic acid hydrogels and reported a similar effect of increasing MAA ratio on the swelling dynamics of crosslinked hydrogels.²³

Higher swelling in hydrogels has been observed on an increase of the β -CD ratio, but the net effect was not significant. This increase in the swelling index may be attributed to the hydrophilic nature of β -CD and increased number of functional units available for grafting MAA. Graft networks containing 5% or higher β -CD concentrations did not stand well with swelling cycles and broke apart into pieces before approaching the equilibrium swelling. These formulations (CD-F3 & CD-F4) were not used for loading 5-FU.

All β -CD MAA hydrogel formulations showed high pH-sensitivity (Table 2). The swelling index of the hydrogels was very low at pH 1.2, but rapidly increased at pH 7.4. At pH 1.2, the presence of protonated pendent carboxylic groups restricts the swelling of the hydrogels. As the pH of the buffer is increased above 1.2, the ionization of carboxylic groups grafted on the polymeric backbone is initiated. The ionized carboxylic groups repel each other as a result of anion-anion repulsive forces, thereby expanding the crosslinked structure. The stretched hydrogel structure facilitates a rapid uptake of the surrounding fluid and rapid swelling of the hydrogels takes place. Swelling experiments into different simulated fluids are good indicators for *in vitro* and *in vivo* performance of the hydrogels for site specific delivery. Therefore, it can be predicted that β -CD MAA hydrogels possess a strong potential for delivery of 5-FU to colon with minimal invasion of upper gastrointestinal tract. Similar pH-responsive swelling behaviour of polymethacrylic acid hydrogels has been reported by Guo Tao & coworkers.²⁴

Effect of polymer/monomer ratio on drug incorporation

Drug loaded amounts in different hydrogel formulations are shown in Table 2. 5-FU entrapment gradually increases on increasing the MAA and β -CD ratio. The amounts of drug loaded into the hydrogels are in good association

with the swelling kinetics of the hydrogels. Here, the drug incorporation is based on the diffusion of the drug solution. Hydrogel formulations that showed rapid swelling also presented higher amounts of drug incorporation. Therefore, the highest amount of drug was entrapped in CDF-8 having the highest ratio of MAA and showing the highest swelling coefficient.

Porosity

A high feed ratio of MAA and β -CD contributed to higher porosity of the β -CD MAA hydrogel structure. Hydrogel swelling is accomplished due to the porous nature or the free spaces in the crosslinked network structure. The diffusion of water molecules in and out of the porous crosslinked structure is responsible for drug incorporation and for drug release as well. The values of porosity are presented in Table 2.

Drug release properties

The kinetics of drug release was highly dependent on the monomer and polymer ratio. Environmental pH is another key parameter, which greatly influences the release rate of the drug. The *in vitro* drug release behavior was evaluated to predict the release performance of β -CD MAA hydrogels in actual physiological environment. In a buffer of pH 1.2, the cumulative release of 5-FU remained less than 20% after 24 h dissolution. However, at pH 7.4, more than 80% of loaded drug was released in the same time period under similar sink conditions. The release rate was significantly more rapid at pH 7.4 compared to that at pH 1.2. The mean values of the drug release profile of CDF-8 hydrogel formulations at pH 1.2 and 7.4 are shown in Fig. 1.

Structure and morphology

Fig. 2 shows the FTIR spectra of MAA, β -CD, EGDMA, benzoyl peroxide and β -CD MAA hydrogel. The presence of an intense peak at 1716 cm^{-1} in the spectra of β -CD-MAA hydrogels ensures the presence of carboxylic groups grafted on the polymeric backbone. A broad peak corresponding to hydroxyl groups between 3350-3450 cm^{-1} represents the presence of β -CD in the network of the newly synthesized polymer.⁷ The peaks at 1080 and 1018 cm^{-1} show the esterification of β -CD with MAA. The peaks at 1560 and 1408 cm^{-1} represent the asymmetrical and symmetrical stretching vibrations of $-\text{COO}^-$.²⁵ The characteristic pH-responsive behavior of β -

CD MAA hydrogels is due to grafting or esterification of MAA on the backbone of β -CD crosslinked with EGDMA.

SEM micrographs taken from open surfaces and cross-section surfaces are shown in Fig. 3. The morphological analysis of the cross-section of β -CD MAA hydrogels reveals a highly interconnected porous network structure. However, the outer surfaces of the hydrogels show a relatively smoother texture. Large pin holes present inside the hydrogel disc are responsible not only for the absorption of physiological solutions, but also for loading and delivery of drugs. The porous interior morphology of the hydrogel discs closely resembles honey comb architecture. The interconnected network structure can be explained on the basis of the phase separation phenomenon. As the polymerization reaction proceeds, the solubility of the newly formed polymeric network decreases. This results in the evaporation of water molecules

and a compact interconnected network structure of β -CD MAA is developed at the end of the copolymerization reaction. The swelling capacity of the hydrogels is dependent on the porosity of the interconnected network structure.²⁶

Thermal properties

Fig. 4 presents TGA thermograms of MAA, β -CD and β -CD MAA. A sharp weight loss peak of MAA at 70 °C is due to thermal decomposition of the monomer. The TGA curve of β -CD shows an initial weight loss peak due to loss of moisture at 93 °C and a large weight loss peak at 315 °C is due to thermal decomposition of β -CD. The TGA curve of β -CD MAA hydrogel shows a three phase thermal degradation. Initially, a small weight loss peak at 98 °C due to loss of moisture, then a weight loss peak due to glassy rubbery transition, and finally the decomposition of the new polymer starts at 410 °C due to the thermal decomposition of the graft copolymer.

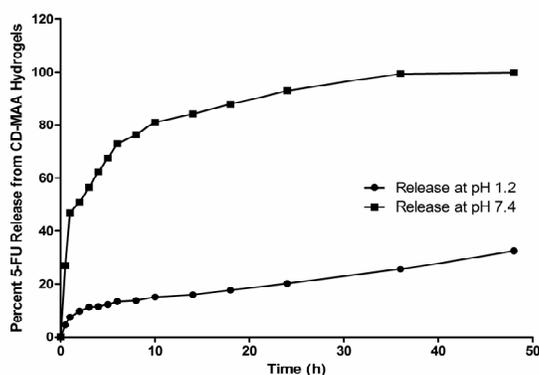


Figure 1: 5-FU release profile of CDF-8 hydrogel formulations at pH 1.2 and 7.4

The findings of the study clearly indicate an increase in thermal stability of the modified three dimensional polymeric network of β -CD. Crosslinking of polymers can enhance the stability of graft networks. Dos Santos *et al.* reported a similar effect of crosslinking and observed an increase in T_g after copolymerization of hydroxyethyl methacrylate (HEMA) with methacrylated-derivative of β -cyclodextrin.¹⁴

The DSC curves of MAA, β -CD and newly synthesized β -CD MAA hydrogel are shown in

Fig. 5. The DSC curve of MAA shows a deep endothermic peak at 15 °C. In the DSC curve of β -CD, a weak endothermic peak at 82 °C corresponds to loss of water and a deep peak at 140 °C represents the glass transitional temperature of the polymer. The DSC curve of the β -CD MAA hydrogel shows an endothermic peak at 81 °C, and the transitional state peak of pure β -CD at 140 °C is shifted to 230 °C, indicating the development of a new polymeric structure with high thermal stability.

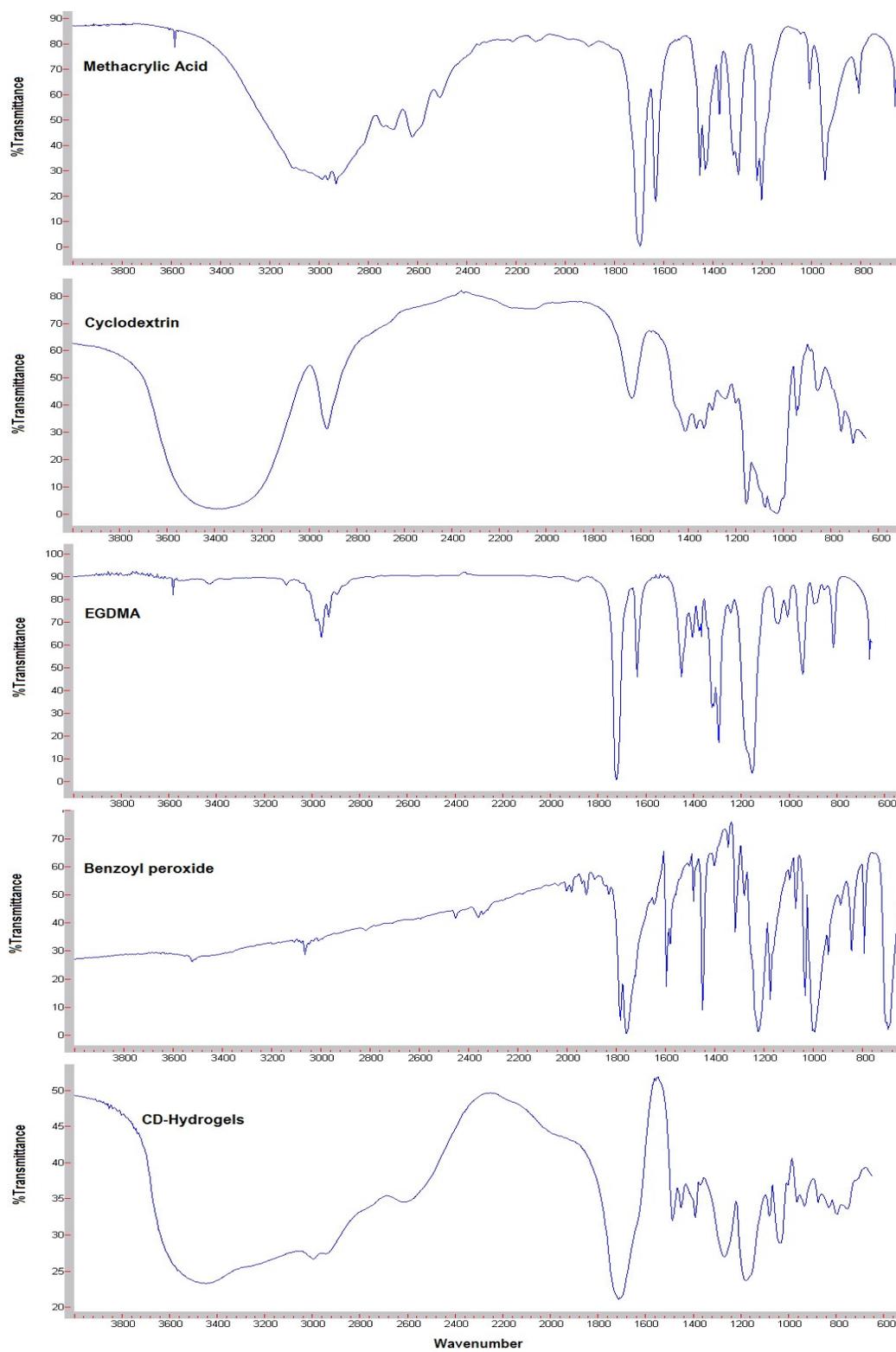


Figure 2: FTIR spectra of MAA, β -CD, EGDMA, benzoyl peroxide and β -CD MAA hydrogel

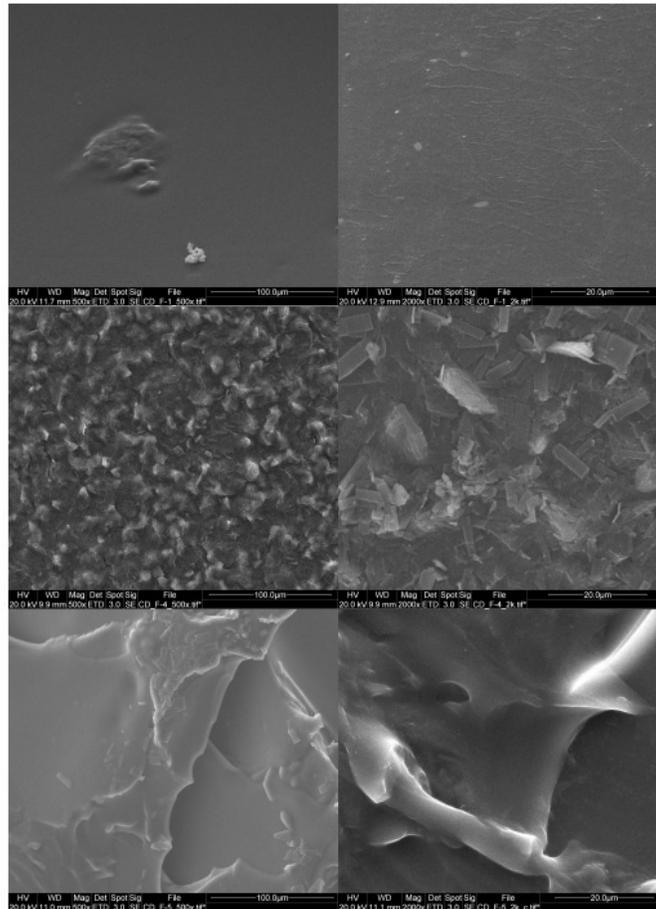


Figure 3: SEM micrographs of β -CD MAA hydrogels

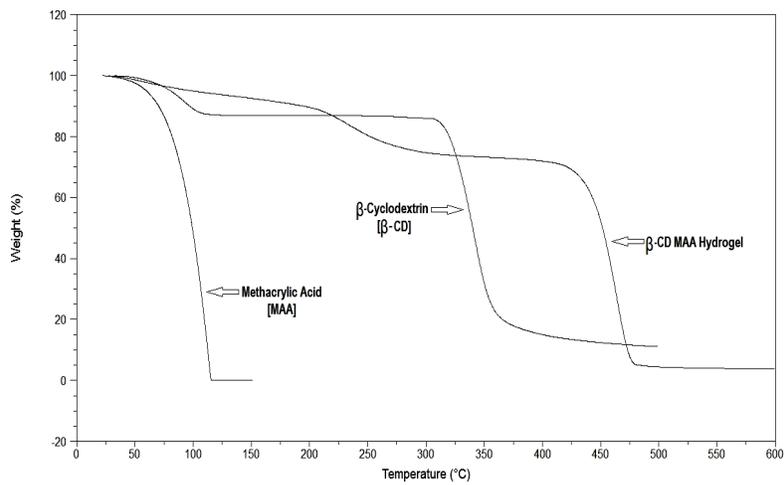


Figure 4: TGA thermograms of MAA, β -CD and β -CD MAA hydrogel

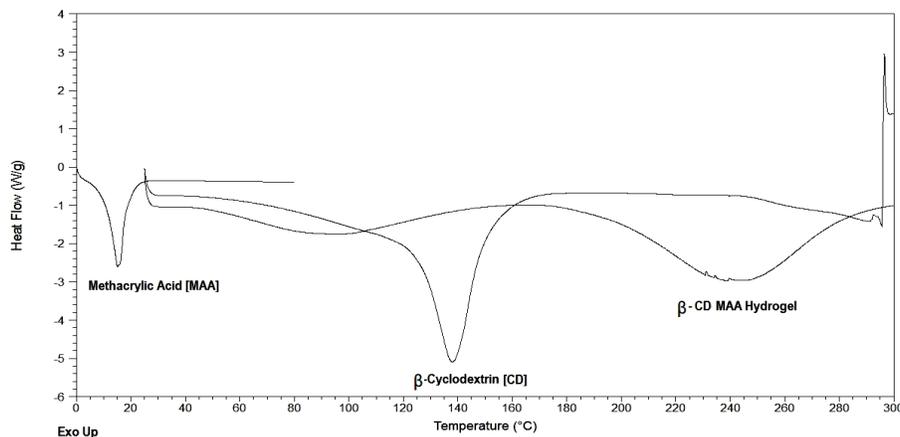


Figure 5: DSC curves of MAA, β -CD and β -CD MAA hydrogel

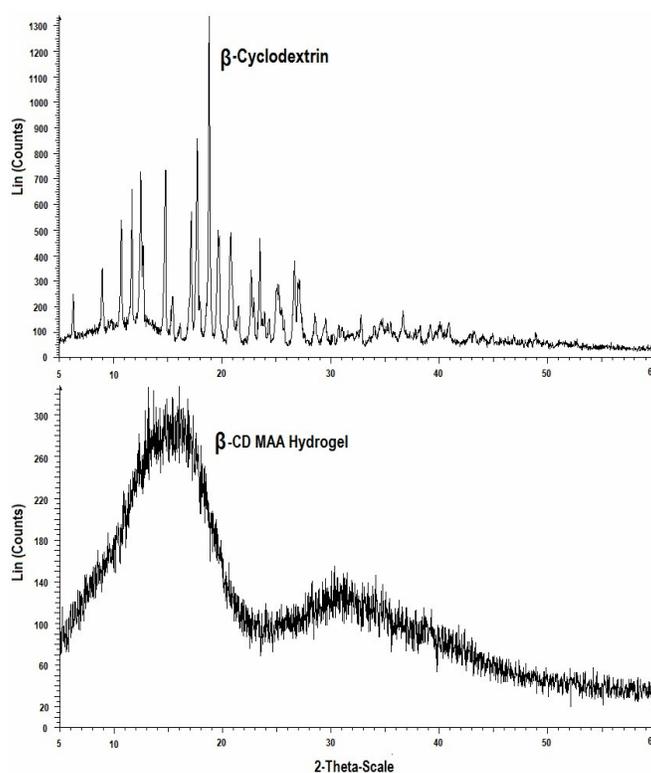


Figure 6: X-ray diffractograms of β -CD and β -CD MAA hydrogel

X-ray Diffractometry

The findings of PXRD spectra for pure β -CD and β -CD MAA hydrogel are shown in Fig. 6. There was a sharp decrease in the crystallinity of β -CD MAA hydrogels, as compared to that of pure β -CD. This decrease in crystallinity also verifies the development of a new structure. A decrease in crystallinity after copolymerization of various polymers has been also reported by previous studies.²⁷

CONCLUSION

A colon specific hydrogel structure was synthesized by free radical graft copolymerization of β -CD, MAA and EGDMA. Excellent gelling between polymer and monomer contents was observed by controlling reaction ingredients and process parameters. FTIR, DSC, TGA, SEM and PXRD supported the formation of a new three dimensional β -CD based crosslinking. The swelling behavior of β -CD MAA hydrogels in

different simulated physiological media revealed a potentially excellent colon specific drug delivery system. Higher 5-FU incorporation was achieved through diffusion in pH 7.4 buffer. The cumulative release of drug was insignificant at pH 1.2 and higher, but a sustained drug release pattern was found at pH 7.4. The stability of the prepared hydrogel and its dissolution behavior in simulated pH medium are major determinants that indicate the development of a stable polymeric transporting system of 5-FU for colon targeting.

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