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Synthesis and characterization of biodegradable chitosan beads as nano-carriers for local delivery of satranidazole

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ABSTRACT

The main aim of this research is to design a new extended release gastroretentive multiparticulate delivery system by incorporation of the hydrogel beads made of chitosan. As the first part of a continued research on conversion of N-sulfonato-N,O-carboxymethylchitosan(NOCCS) to useful biopolymer-based materials, large numbers of carboxylic functional groups were introduced onto NOCCS by grafting with polymethacrylic acid (PMAA). The free radical graft copolymerization was carried out at 70 °C, bisacrylamide as a cross-linking agent and persulfate as an initiator. The equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids (SGF and SIF, respectively). Also, the satranidazole as a model drug was entrapped in nano-gels and in vitro release profiles were established separately in both enzyme-free SGF and SIF. The drug release was found to be faster in SIF. The drug-release profiles indicate that the drug release depends on their degree of swelling and cross-linking.

1. Introduction

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. In recent years, biodegradable polymeric systems have gained importance for design of surgical devices, artificial organs, drug delivery systems with different routs of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications (BrØndsted & Kopeček, 1990). These polymers are classified as either synthetic (polyesters, polyamides, polyanhydrides) or natural (polyamino acids, polysaccharides) (Giammona et al., 1999; Krogars et al., 2000). Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran, and chitosan. Chitosan[β(1,4)2-amino-2-D-glucose] is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, and krill (Chiu et al., 1999; Jabbari & Nozari, 2000).Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes (Fanta & Doane, 1986; Furda, 1983). Chitosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units links with β-(1-4)bonds. It should be susceptible to glycosidic hydrolysis by microbial enzymes in the colon because it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides. Among diverse approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties (Peppas, 1987; Saboktakin et al., 2007).

Graft copolymerization of vinyl monomers onto polysaccharides using free radical initiators, has attracted the interest of many scientists.

Up to now, considerable works have been devoted to the grafting of vinyl monomers onto the substrates, especially Starch and cellulose (Honghua & Tiejing, 2005; Jabbari & Nozari, 2000). Existence of polar functionally groups as carboxylic acid need not only for bioadhesive properties but also for pH-sensitive properties of polymer (Ratner, 1989; Thierry et al., 2003). The increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH. A part of our research program is chitosan modification to prepare materials with pH-sensitive properties for uses as drug delivery (Bloembergen & Pershan, 1967; Mahfouz et al., 1997; Schmitz et al., 2000). The free radical graft copolymerization polymethacrylic acid onto chitosan was carried

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out at 70 °C, bis-acrylamide as a cross-linking agent and persulfate as an initiator. Polymer bonded drug usually contain one solid drug bonded together in a matrix of a solid polymeric binder. They can be produced by polymerizing a monomer such as methacrylic acid (MAA), mixed with a particulate drug, by means of a chemical polymerization catalyst, such as AIBN or by means of high-energy radiation, such as X-ray or γ-rays. The modified hydrogel and satranidazole as a model drug were converted to nanoparticles by freeze-drying method. The equilibrium swelling studies and in vitro release profiles were carried out in enzyme-free simulated gastric and intestinal fluids [SGF (pH 1) and SIF (pH 7.4) respectively)]. The influences of different factors, such as content of MAA in the feed monomer and swelling were studied (Saboktakin et al., 2008).

2. Materials and methods

2.1. Measurements

The powder morphology NOCCS/PMAA copolymer in the form of pellets (to measure grain size) was investigated using Philips XL-30 E SEM scanning electron microscope(SEM) at 30 kV(max.). The samples were prepared by physical vapor disposition method. The gold layer thickness was about 100 Å at these samples. They were carried out in chemistry Department of Tarbiat Modares University. The DSC curves were obtained on a TGA/SDTA 851 calorimeter at heating and cooling rates of 10 °C/min under N_2. The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali) λ_{max} = 275 nm) using a 1 cm quartz cell. Enzyme-free SGF (pH 1) or SIF (pH 7.4) was prepared according to the method described in the US Pharmacopeia.

2.2. Preparation of N-sulfonato-N,O-carboxymethylchitosan(NOCCS)

Derivatives of NOCCS, N-sulfonato-N,O-carboxymethylchitosan(NOCCS),containing sulfonato groups, $SO_3^-Na^+$, were prepared by the reaction of sulfur trioxide–pyridine complex with NOCCS in alkaline medium at room temperature. Typically, 10 g of NOCCS (0.045 mol) dissolved in 0.60 L of water was treated with repeated additions of 12 g SO_3–pyridine. The SO_3–pyridine was slurried in 50–100 mL of water and added dropwise, over 1 h. Both the NOCCS solution and the sulfur trioxide reagent slurry were maintained at a pH above 9 by the addition of sodium hydroxide (5 M). Following the last addition of the sulfating reagent, the sodium hydroxide solution was added until the pH stabilized (approx. 40 min.).

The pH of the mixture was adjusted to 9, the mixture was heated to 33 °C and held for 15 min. After filtration through a 110 μm nylon screen, the filtered SNOCCS solution was poured into 6 L of 99% isopropanol. The resulting precipitate was collected and air dried overnight. The dried precipitate was dissolved in 0.45 L boiling water, solution was poured into dialysis sacks (M_W = 12,000) and dialyzed for 3–4 days against dionized water. The contents of the sacks were lyophilized to yield the final product (6.2 g).

2.3. The free radical graft copolymerization of NOCCS–methacrylic acid

N-sulfonato-N,O-carboxymethylchitosan with 1:1 molar ratios of methacrylic acid was polymerized at 60–70 °C in a thermostatic water bath, bis-acrylamide as a cross-linking agent (CA), using persulfate as an initiator ([I] = 0.02 M) and water as the solvent (50 mL). The polymeric system was stirred by mechanical stirrer to sticky hydrogel and it was separated from medium without solvent addition. All experiments were carried out in Pyrex glass ampoules. After the specific time (48 h), the precipitated network polymer was collected and dried in vacuum.

2.4. Preparation of nanoparticle

Copolymer (50 mg) and satranidazole (10 mg) were dispersed with stirring in 25 mL deionised water. After approximately 180 min, the sample was sprayed into a liquid nitrogen bath cooled down to 77 K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution.

2.5. Stability of satranidazole during bead preparation

The following procedure was used to assess the stability of satranidazole during the bead preparation process. The prepared beads were extracted twice with a solvent mixture of 1:1 acetonitrile and ethanol (v/v), the extract was evaporated, the residue was injected onto HPLC column. Stability-indicating chromatographic method was adopted for this purpose. The method consisted of a symmetry C18 column (254 mm × 4.6 mm; 5 μm) run using a mobile phase of composition methanol: water (70:30, v/v) at a flow rate of 0.5 mL/min, a Waters pump (600E), and eluants monitored with Water photodiode array detector (996 PDA) at 227 nm.

2.6. Release studies

A definite weight range of 10–15 mg of bead was cut and placed in a 1.5 mL capacity microcentrifuge tube containing 1 mL of release medium of the following composition at 37 °C: phosphate buffered saline (140 mM, pH 7.4) with 0.1% sodium azide and 0.1% Tween 80. At predetermined time points, 100 μL of release medium was sampled with replacement to which 3 mL of scintillation cocktail was added and vortexed before liquid scintillation counting. The cumulative amount of satranidazole released as a function of time was calculated.

2.7. Solid-state characterization

To study the molecular properties of satranidazole and N-sulfonato-N,O-carboxymethylchitosan/PMAA, the solid-state characterization of samples were done by the application of thermal, X-ray diffraction, and microscopy technique.

During this study, the characteristics of satranidazole and N-sulfonato-N,O-carboxymethylchitosan/PMAA were compared with the beads to reveal any changes occurring as a result of bead preparation.

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed with a Mettler Toledo 821 thermal analyzer (Greifensee,Switzerland) calibrated with indium as standard. For thermogram acquisition, sample sizes of 1–5 mg were scanned with a heating rate of 5 °C/min over a temperature range of 25–300 °C. In order to check the reversibility of transition, samples were heated to a point just above the corresponding transition temperature, cooled to room temperature, and reheated up to 300 °C.

2.9. Scanning electron microscopy

Satranidazole samples and N-sulfonato-N,O-carboxymethylchitosan/PMAA beads were viewed using a Philips XL-30 E SEM scanning electron microscope (SEM) at 30 kV (max.) for morphological examination. Powder samples of satranidazole and beads

were mounted onto aluminium stubs using double-sided adhesive tape and then sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of 1.2 kV acceleration potential, and images were collected in collected in secondary electron mode.

2.9.1. X-ray diffraction studies

Molecular arrangement of satranidazole and N-sulfonato-N,O-carboxymethylchitosan/PMAA in powder as well as in beads was compared by powder X-ray diffraction patterns acquired at room temperature on a Philips PW 1729 diffractometer (Eindhoven, Netherlands) using Cu-Kα radiation. The data were collected over an angular range from 3° to 50° 2θ in continuous mode using a step size of 0.02° 2θ and step time of 5 s.

3. Results

3.1. Chemical stability of satranidazole

In the present study, the beads were prepared by the classical method, which involves spreading a uniform layer of polymer dispersion followed by a drying step for removal of solvent system. Since the methodology of bead preparation involved a heating step, it may have had a detrimental effect on the chemical stability of drug. Hence, the stability assessment of satranidazole impregnated in bead was done using stability-indicating method. For this purpose, satranidazole was extracted from bead and analyzed by HPLC. A single peak at 21.2 m representing satranidazole (with no additional peaks) was detected in the chromatogram, suggesting that the molecule was stable during preparation of beads (chromatograms not shown).

3.2. Content uniformity

Satranidazole was extracted from different regions of N-sulfonato-N,O-carboxymethylchitosan/PMAA bead using acetonitrile:ETOH (1:1, v/v) solvent system. After normalization of satranidazole concentration on weight basis of bead, the variation in distribution of satranidazole in different regions of bead was <16%.

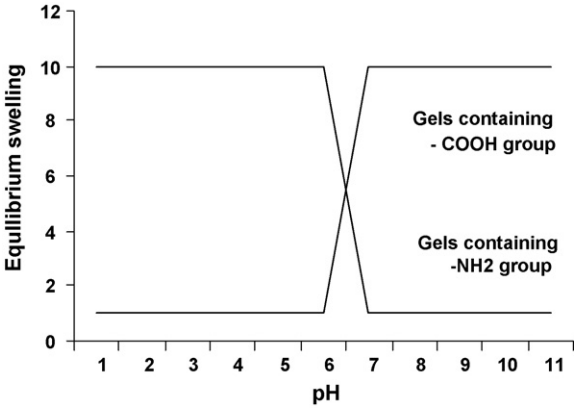

Fig. 1. Equilibrium degree of swelling in response to pH.

3.3. Bead morphology

The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics.

Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendent functional groups. The equilibrium swelling behavior of ionic hydrogels containing acidic and/or basic functional groups is illustrated in Fig. 1. Hydrogels containing basic functional groups is found increased swelling activity in acidic conditions and reduced in basic conditions .The pH-sensitive

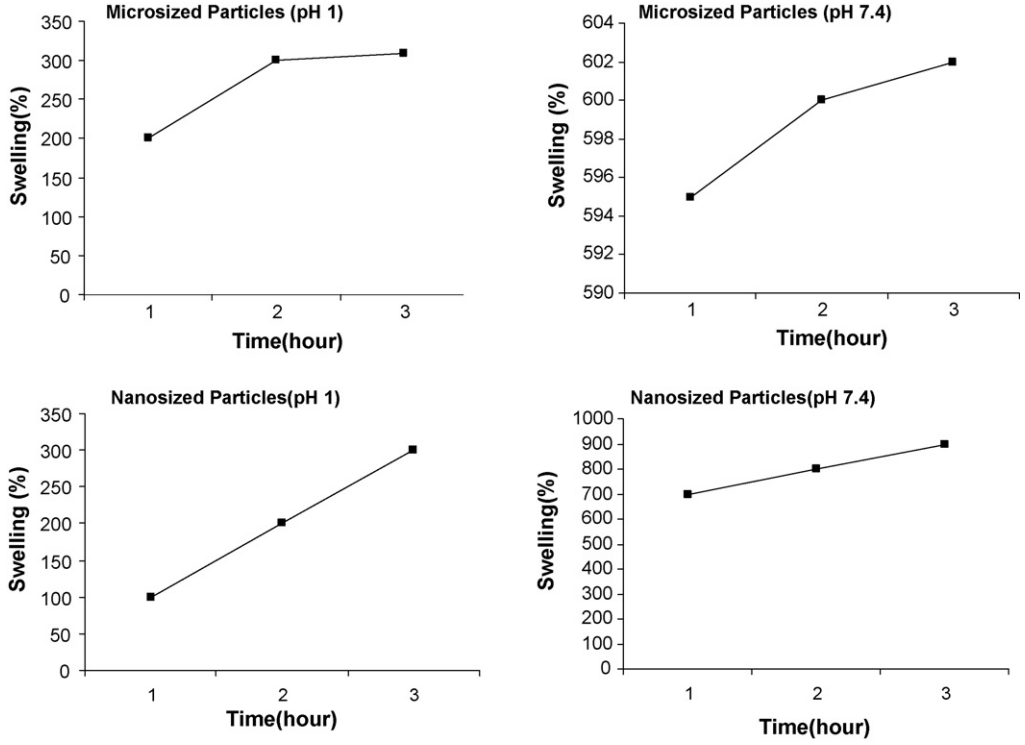

Fig. 2. Time-dependent swelling behavior of micro- and nano-carriers for satranidazole drug model as a function of time at 37 °C.

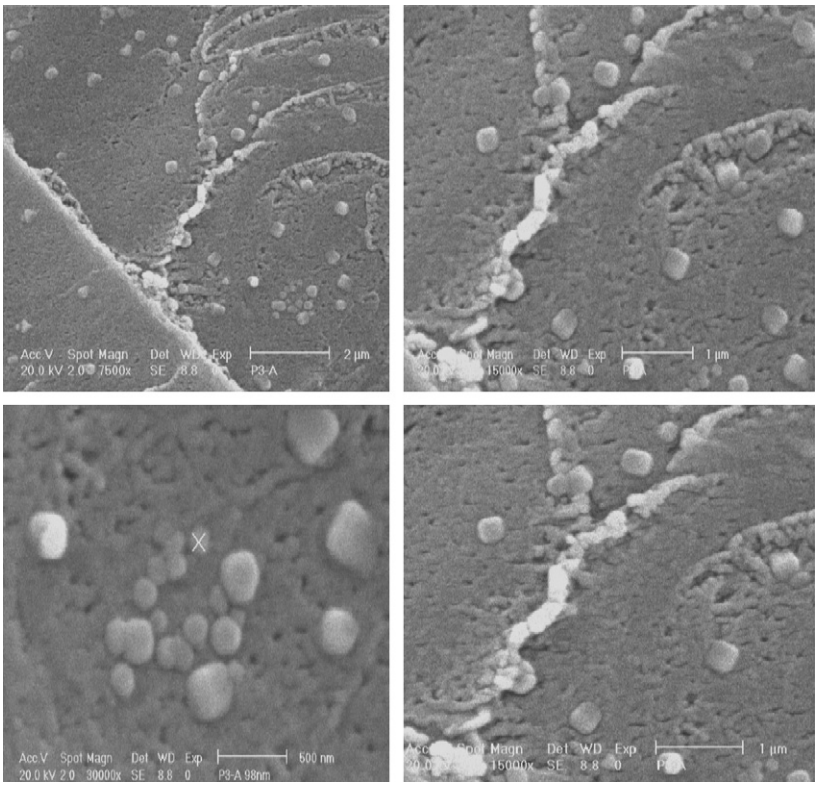

Fig. 3. SEM of NOCCS–polymethacrylic acid hydrogel beads with satranidazole.

anionic hydrogels show low swelling activity in acidic medium and very high activity in basic medium. As shown in Fig. 2, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in simulated gastric fluid but greater swelling in and simulated intestinal fluids. This is because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

Fig. 3 shows the scanning electron microscope (SEM) of graft NOCCS copolymer with polymethacrylic acid and nano-polymer bonded drug, respectively.

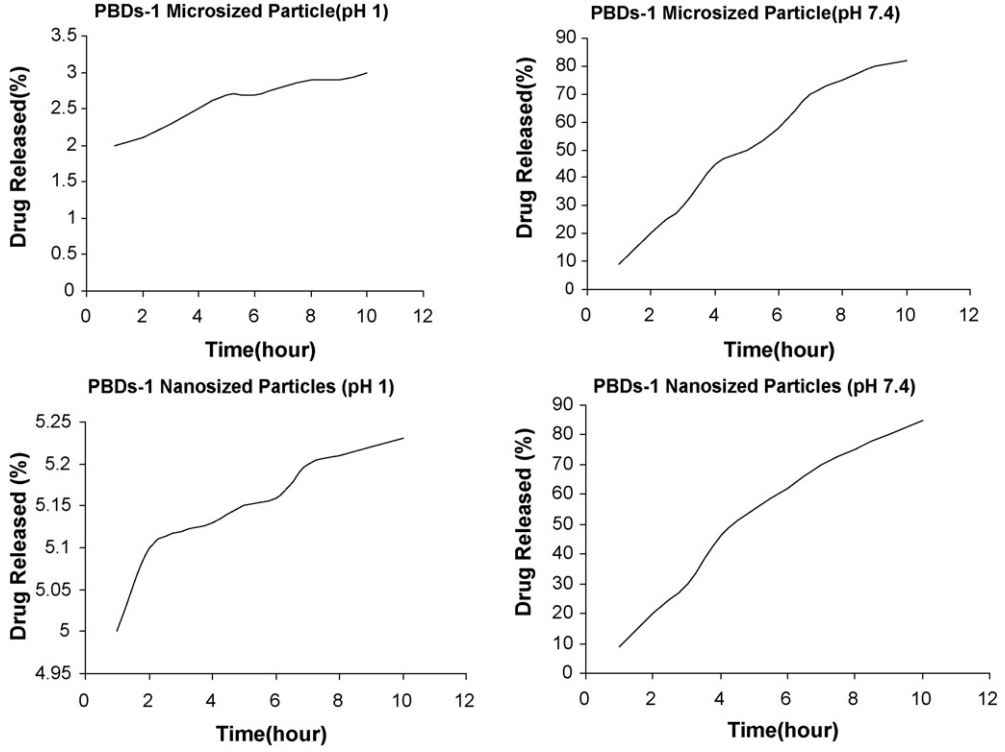

Fig. 4. Release of satranidazole drug from micro- and nano-polymeric carriers as a function of time at 37 °C.

Table 1
DSC data and composition of copolymer.

Polymer samples	Molar composition of monomers in the feed				Degree of substitution (DS)	T_g (°C)
	NOCCS (g)	MAA (g)	CA (g)	IN (g)		
P-1	1	3	0.05	0.05	0.52	135
P-2	1	2	0.05	0.05	0.49	142

3.4. In vitro release studies

Nano- and micro-polymer bonded drugs (50 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4) (Fig. 4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37 °C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The removed volume was replaced with SGF or SIF Fig. 5.

The triplicate samples were analyzed by UV spectrophotometer, and the quantity of satranidazole were determined using a standard calibration curve obtained under the same conditions.

3.5. Compare of swelling ratio for nano and microparticles

It appears that the degree of swelling depends on their particle size, a decrease in the molecular size of carriers increased the swelling rate.

3.6. Thermal analysis

The thermal behavior of a polymer is important for controlling the release rate in order to have a suitable drug dosage form. The glass transition temperature (T_g) was determined from the DSC thermograms. The values are given in Table 1. The higher T_g values probably related to the introduction of cross-links, which would decrease the flexibility of the chains and the ability of the chains to undergo segmental motion, which would increase the T_g values. On the other hand, the introduction of a strongly polar carboxylic acid group can increase the T_g value because of the formation of internal hydrogen bonds between the polymer chains.

3.7. X-ray diffraction studies

X-ray diffraction is a proven tool to study crystal lattice arrangements and it yields very useful information on degree of crystallinity. The X-ray diffraction patterns of hydrogel with satranidazole have several high-angle bead at the following 2θ values: 21°, 22°, 25°, 26.5°, 28°, and 32°.

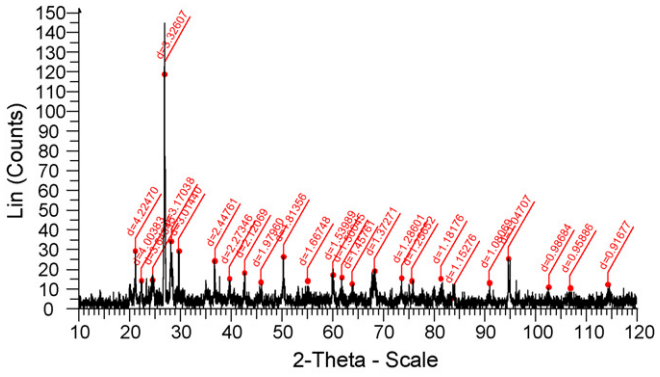

Fig. 5. XRD patterns of NOCCS–polymethacrylic acid hydrogel beads.

The 26.5° 2θ peak had the highest intensity,and the hump in the baseline occurred from 7° to 45° 2θ, as observed for chitosan bead.

4. Discussion

In order to develop a local delivery system for satranidazole, a biodegradable N-sulfonato-N,O-carboxymethylchitosan–PMAA bead was attempted, which to our knowledge in the first effort of its kind. The potential formulation problems were anticipated since chitosan is only soluble in aqueous acidic solutions, whereas satranidazole, being a hydrophobic drug, is insoluble under similar conditions. In early stages of formula optimization studies, the satranidazole was incorporated into bead. The primary mechanisms for release of drugs from matrix systems in vitro are swelling, diffusion, and disintegration. In vitro degradation of chitosan beads were prepared by solution casing method occurred less rapidly as the degree 73% deacetylated showed slower biodegradation. Since the grade of chitosan used in the present study was of high molecular weight with a degree of deacetylation ≥85%, significant retardation of release of satranidazole from bead is attributed to the polymer characteristics. In addition, diffusion of satranidazole may have been hindered by increased tortuosity of polymer accompanied by a swelling mechanism.

As observed from SEM photomicrographs, the crystals of satranidazole have a different appearance than recrystallized satranidazole. These nanoparticles do not have clearly defined crystal morphological features in the SEM photomicrographs. Hence, it appears that the irregularly shaped particle are surface deposited with poloxamer, which gives them an appearance resembling that of coated particles. X-ray diffraction technique is also used to study the degree of crystallinity of pharmaceutical drugs and excipients.

A lower 2θ value indicates larger d-spacings, while an increase in the number of high-angle reflections indicates higher molecular state order. In addition, broadness of reflections, high noise, and low peak intensities are characteristic of a poorly crystalline material. A broad hump in the diffraction pattern of chitosan extending over a large range of 2θ suggests that chitosan is present in amorphous state in the bead.

5. Conclusions

The swelling and hydrolytic behavior of the hydrogels beads were dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Modified chitosan with different contents of MAA and CA by graft copolymerization reactions were carried out under microwave-radiation. The swelling of the hydrogels beads was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Incorporation of MAA made the hydrogels beads pH-dependent and the transition between the swollen and the collapsed states occurred at high and low pH. The swelling ratios of the hydrogels beads increased at pH 7.4, but decreased at pH 1 with increasing incorporation of MAA.

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