Macromolecular Research, Vol. 17, No. 9, pp 709-713 (2009)

Synthesis and Characterization of Novel pH-Sensitive Hydrogels Containing Ibuprofen Pendents for Colon-Specific Drug Delivery

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Received January 2, 2009; Revised February 13, 2009; Accepted February 26, 2009

Abstract: The aim of this study was to develop novel intestinal specific drug delivery systems with pH sensitive swelling and drug release properties. The carboxyl group of ibuprofen was converted to a vinyl ester group by reacting ibuprofen and vinyl acetate as an acylating agent in the presence of catalyst. The glucose-6-acrylate-1, 2, 3, 4-tetraacetate (GATA) monomer was prepared under mild conditions. Cubane-1, 4-dicarboxylic acid (CDA) linked to two 2-hydroxyethyl methacrylate (HEMA) group was used as the crosslinking agent (CA). Methacrylic-type polymeric prodrugs were synthesized by the free radical copolymerization of methacrylic acid, vinyl ester derivative of ibuprofen (VIP) and GATA in the presence of cubane crosslinking agent. The structure of VIP was characterized and confirmed by FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. The composition of the cross-linked three-dimensional polymers was determined by FTIR spectroscopy. The hydrolysis of drug polymer conjugates was carried out in cellophane membrane dialysis bags, and the *in vitro* release profiles were established separately in enzyme-free simulated gastric and intestinal fluids (SGF, pH 1 and SIF, pH 7.4). The detection of a hydrolysis solution by UV spectroscopy at selected intervals showed that the drug can be released by hydrolysis of the ester bond between the drug and polymer backbone at a low rate. Drug release studies showed that increasing the MAA content in the copolymer enhances the rate of hydrolysis in SIF. These results suggest that these polymeric prodrugs can be useful for the release of ibuprofen in controlled release systems.

Keywords: glycopolymers, pH-sensitive, hydrogel, oral drug delivery, ibuprofen.

Introduction

To achieve successful colonic delivery, a drug needs to be protected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for oral delivery. One strategy for targeting orally administered drugs to the colon includes covalent linkage between drug and pH-sensitive hydrogel in such a manner that upon oral administration the moiety remains intact in the stomach and small intestine.[1-10] In other hand, design and application of 'polymeric prodrugs' is an interesting field that is in continuous expansion and development because of the intrinsic advantages offered by specific macromolecular systems in new and risk therapies.[11-14] pH-Sensitive hydrogel containing side-chain carbohydrates are considered as high value polymeric materials because of their potential as biocompatible materials with medical applications. These applications are generally based on the fact that cell-cell interactions between oligosaccharides and lipids play an important role in various life processes.[15] Synthesis of sugar-based hydrogels has attracted biomedical researchers due to the biocompatibility and hydrophilic nature of saccharides.[16-19] Polymeric prodrug, as a conjugation of a drug with a polymer, has many advantages such as increased drug solubility, prolonged drug release, increased stability and decreased toxicity.[20,21] The ibuprofen (propionic acid derivative) is a non-steroid anti-inflammatory drug (NSAIDs) and is widely used for the treatment of rheumatoid arthritis. But, the use of NSAIDs is also limited by their irritant side effects on the gastro-enteric mucous and by their frequent poor water solubility.[22] These problems can be solved by the preparation of polymeric prodrug backbones *via* hydrolyzable bonds. Polymer-drug conjugates of NSAIDs have been developed in order to minimize delivery problems and reduce gastrointestinal side effects by controlling the rate, duration, and site of release. These polymeric prodrugs have been designed for localized and prolonged duration of drug action by parental administration, or as dermal prodrugs.[22,23] Because hydrophilic polymers and hydrogels have displayed bioadhesive properties, design and synthesis of new biodegradable and biocompatible polymeric hydrogel systems based on suger-containing monomers was the primary objective in our study. This

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research work describes an efficient chemical method to design and evaluation of vinyl ester type polymeric prodrugs of ibuprofen. Vinyl 2-(4-isobutylphenyl) propionate (VIP), as a vinyl ester type derivative of ibuprofen was first synthesized by reacting ibuprofen and vinyl acetate in the presence of mercuric acetate. The obtained VIP was then copolymerized with methacrylic acid (MAA) and GATA in the presence of cubane crosslinking agent,[24] by free radical polymerization method. The release of ibuprofen from the obtained polymeric prodrugs was carried out *in vitro* by hydrolysis in buffered solutions at various pH values and the quantity of the released drug detected by UV spectroscopy. The effects of neighboring groups and pH values on release of ibuprofen are discussed.

Experimental

Materials. Cubane-1,4-bis(methacryloyloxyethyl)carboxylate (CA) and glucose-6-acrylate-1,2,3,4-tetraacetate (GATA) were prepared by the methods described in the literatures,[25,26] respectively. Ibuprofen was purchased from Aldrich chemical company. Mercuric acetate, vinyl acetate, sodium acetate, HEMA and MMA were obtained from Merck chemical company and was purified by distillation under vacuum. Azoisobutyronitrile (AIBN) was obtained from Fluka chemical company and recrystallized from methanol. Enzyme-free SGF (pH 1) or SIF (pH 7.4) were prepared according to the method described in the US Pharmacopeia.[27]

Instrumental Measurements. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker 400 AC spectrometer in $CDCl_3$. The IR spectra were recorded on a Shimadzu FT IR-408 spectrophotometer. The amount of released ibuprofen was determined by a Philips PU 8620 UV spectrophotometer at the maximum adsorption of the free drug in aqueous buffered solutions (λ_{max}=264 nm) using a 1-cm quartz cell.

Preparation of Vinyl 2-(4-Isobutylphenyl) Propionate (VIP). The amount of 2.6 g (12.6 mmol) of ibuprofen and 0.3 g of mercuric acetate were dissolved in 30 mL of vinyl acetate and stirred for 30 min at room temperature. Then, 0.2 mL of concentrated sulfuric acid was added into the solution and refluxed for about 3 h. After this time, the solution was cooled to room temperature and 1.0 g of sodium acetate was added to quench the catalyst. The solution was filtered, concentrated and the crude product was then purified by silica gelcolumn chromatography by eluting with petroleum ether/ethyl acetate (30:1, v/v) to give 2.5 g (85%) of VIP as a colorless liquid (Figure 1).

FTIR (KBr, cm^{-1}) 3050 (C-H aromatic and vinylic), 2890 (C-H aliphatic), 1740 (C=O ester),1600, 1480 (C=C).

^{1}H NMR ($CDCl_3$, ppm) 0.8 (d, 6H, -CH(CH_3)$_2$), 1.55 (d, 3H, -ArCHCH_3), 1.9 (m, 1H, -$CHMe_2$), 2.5 (d, 2H, Ar-CH_2-), 3.8 (q,1H, Ar-CH-), 4.5 (dd, 1H, CH_2=C), 4.9 (dd, 1H, CH_2=C), 7.0-7.27(q, 4H, aryl-H), 7.3-7.34 (q, 1H, CH_2=CH).

^{13}C NMR ($CDCl_3$, ppm) 20 (1C, -CH-CH_3), 21 (2C, -CH(CH_3)$_2$), 22 (1C, -$CHMe_2$), 30 (1C, Ar-CH_2-), 45 (1C, -CH-CH_3), 125 (1C, CH_2=CH-), 155 (1C, CH_2=CH-), 126, 129, 138, 140 (6C, aromatic carbons), 172 (1C, C=O).

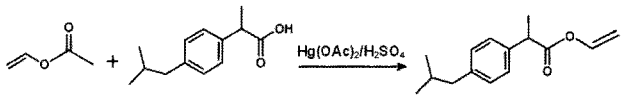


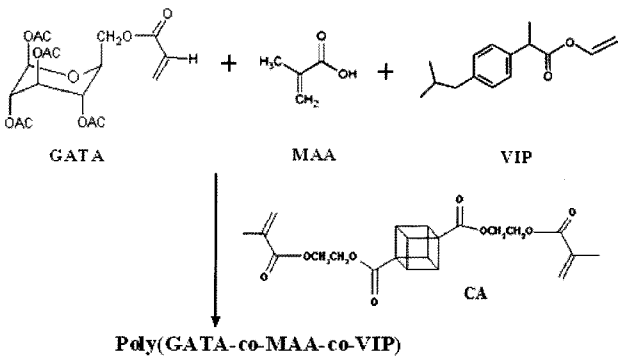
Figure 1. The synthesis route of vinyl ester type derivative of ibuprofen (VIP).

Table I. Composition of Copolymers and Percentage of Particles Adhered onto Rat Intestine

Polymers	Molar Composition of Monomers in the Feed				Percentage Adherence
	GATA	MAA	VIP	CA	
P-1	3	3	1	5	53
P-2	3	3	1	10	52
P-3	3	5	1	5	58
P-4	3	5	1	10	59
P-5	5	3	1	5	63
P-6	5	3	1	10	63

Preparation of pH-Sensitive Hydrogels. Polymer bonded drugs (PBDs) were synthesized by terpolymerization of MAA, GATA and VIP with specific mol percents of CA (5 and 10%) in a solution of dried dioxane with a variable feed ratio as shown in Table I. Terpolymerization was carried out in the presence of 2,2′-azobis isobutyronitrile (AIBN) as an initiator (0.01 molL^{-1}) at 60-70 °C in a thermostatic water bath. All experiments were carried out in Pyrex glass ampoules sealed off under vacuum. After the desired time (48 h) the precipitated network polymer bonded drug was collected, washed with non-solvent for several times, dried under vacuum at room temperature and stored in desiccators until use (Scheme I). IR (KBr): 3350-2530 (broadened, -COOH group), 1735, 1675, 1610, 1470, 1240, 1225 cm^{-1}.

Method of Hydrolysis. The polymer-drug conjugates (200 mg) was poured into 5 mL of aqueous buffered solu-



Scheme I. Preparation of network polydrug.

tion SGF (pH 1) or SIF (pH 7.4) at 37 °C and the mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 mL of same buffer solution maintained at 37 °C. The external solution was continuously stirred and a 3-mL sample was removed at selected intervals and 3 mL of buffer was replaced. The quantity of released drug was analyzed by means of an UV spectrophotometer and determined from the calibration curve obtained previously under the same conditions.

Characterization of Hydrolysis Products. Fifty milligrams of polymer-drug adduct was dispersed in 20 mL of pH 8 buffered solution. The reaction mixture was maintained at 37 °C. After 24 h the hydrolysis solution was sampled and neutralized with 1 molL^{-1} hydrochloric acid and the solvent was evaporated in vacuo. The resulting crude product was treated with 10 mL of acetone and heated. The suspension was then filtered and the acetone solution was evaporated under reduced pressure. The residue was characterized by melting point measurement and IR spectroscopy and showed that the hydrolysis product is ibuprofen.

***In Situ* Bioadhesivity Studies.** Bioadhesivity testing was done by a novel *in situ* method as described by Ranga Rao and Buri.[28] A freshly cut 5-6 cm long piece of small intestine of rat was obtained and cleaned by washing with isotonic saline. The piece was cut open and the mucosal surface was exposed. Known weights of hydrogels were added evenly on the mucosal surface. The intestinal piece was maintained at 80% relative humidity for 30 mts in a desiccator. The piece was taken out and phosphate buffer pH 6 was allowed to flow over the intestinal piece for about 2 mts at a rate of 20 mL/min. The perfusate was collected and dried to get the particles not adhered. The percent of bioadhesion was estimated by the ratio of amount applied to adhere hydrogels. The values are given in Table I.

Results and Discussion

Yang *et al.*[29] and Cai *et al.*[30] have already reported a method for conversion of carboxylic acids to the related vinyl ester by using vinyl acetate as an acylating agent. In this present work, ibuprofen reacted with vinyl acetate in the presence of mercuric acetate as a catalyst, and the related vinyl ester (VIP) was collected in high yield after purification by column chromatography. The resultant FTIR and ^{1}H NMR spectra confirmed the structure of VIP and its purity. The related ^{1}H and ^{13}C NMR spectra of VIP are shown in Figures 2 and 3, respectively. These sugar-based hydrogels have shown same bioadhesive properties as reported for polysaccharides in earlier studies.[31] All the matrices with the presence of GATA and increase in the content of MAA had shown increased bioadhesivity (Table I). The binding of those with sialic acid residues make prolonged contact of the drug with the epithelium, also it was assumed that opening of the intercellular junctions by GATA and MAA could lead to the enhancement of insulin absorption across the mucosa. To achieve successful colonic delivery, a drug needs to be pro-

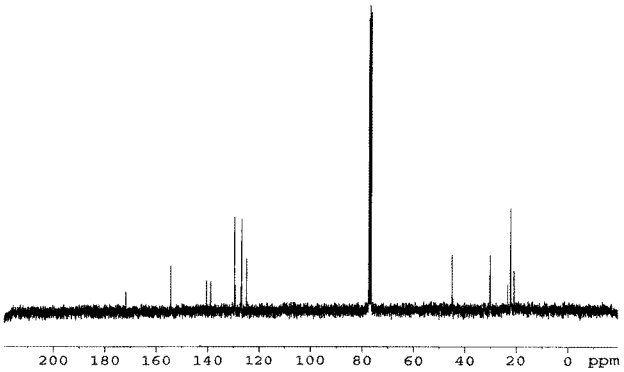


Figure 3. ^{13}C NMR spectrum of VIP in $CDCl_3$.

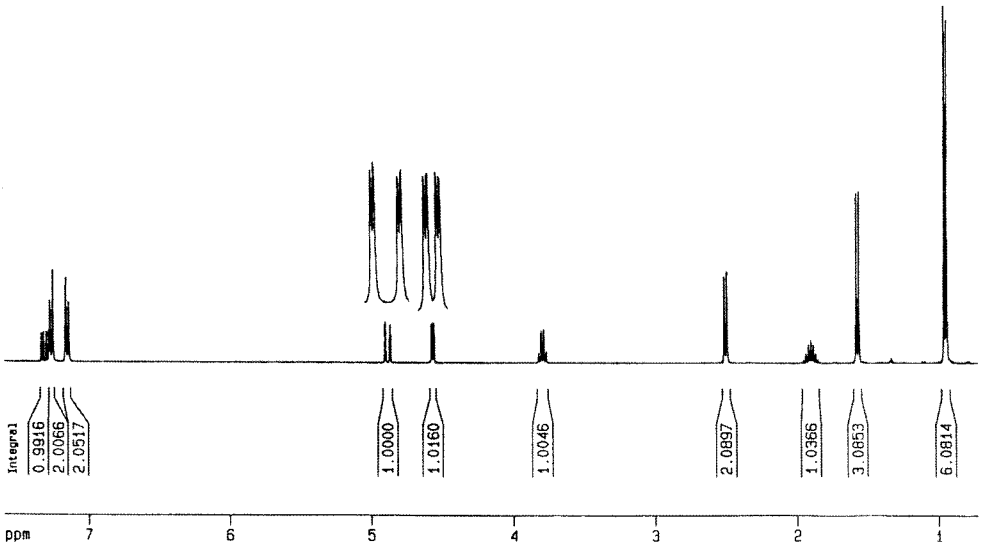


Figure 2. ^{1}H NMR spectrum of VIP in $CDCl_3$.

tected from absorption of the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs. These requirements have prompted the development of polymeric systems that swell minimally under acidic conditions but extensively in basic intestinal medium. 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.[32]

Swelling Ratio. To measure the swelling, preweighed dry drug-free hydrogels were immersed in various buffer solutions (pH 7.4 and pH 1) at 37 °C. After excess water on the surface was removed with the filter paper, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The degree of swelling was calculated according the relation:

$$SW\ (\%) = [(W_s - W_d)/W_d] \times 100$$

Where, W_s and W_d represent the weight of swollen and dry samples, respectively. The study of swelling shows that swelling of hydrogels increases with time, first rapidly and then slowly, reaching maximum constant swelling (mass equilibrium swelling, MES). The swelling value of cross-linked polymers in pH 1 and pH 7.4 at 37 °C are given in Table II.

Drug Release by Hydrolysis of Polymeric Prodrugs. It has been widely demonstrated that the side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug polymer chemical bonds, the structure of the polymer and the surrounding condition. The hydrolysis of a linkage is also dependent on its distance from the polymer backbone. As a sample, the degree of release of ibuprofen from P-5 as a function of time is shown in Figure 4. The degrees of hydrolysis of the network polymer containing ibuprofen in pH 1 and pH 7.4 at 37 °C are given in Table II. As shown in Table II, difference in hydrolysis rate at pH 1 and 7.4 for hydrogels with higher concentration of GATA (P-5, P-6) in SGF and SIF were tiny, which clearly shows that GATA is not very high pH responsive. In the other hand, the increase of glucose content resulted in less collapsed networks at low pH. This led to a relatively large pore size of the networks due to the bulky sugar groups. Thus, ibuprofen could hydrolyze readily from the gel at low pH. It appears that the degree of hydrolysis of network polymers depends on the amount of the MAA units in copolymer and reticulated degree of cross-linking. With increased cross-linking and an increase in the reticulated degree of the polymer, diffusion of the hydrolyzing agents in the network's polymer is reduced and the hydrolysis rate is slower. The drug-release profiles indicated that the amount of drug released depended on the degree of swelling. The swelling value shows that, an increase in the content of MAA in the feed monomer mixtures resulted in less swelling in SGF but greater swelling in SIF. As the content of MAA in the feed monomers increased, hydrolysis rate decreased at pH 1 but increased at pH 7.4. This was because a higher MAA content in the polymer networks led to higher carboxylate anion concentration at high pH. In other words, the existence of hydrogen-bonding interactions between -COOH groups in the polymer matrix results in a complex structure within the network, and so the movement of polymeric segments is restricted. This also accounts for minimum hydrolyzing of the gel in a medium of pH 1. However, when the sample is placed in a medium of pH 7.4, the almost complete ionization of -COOH groups present within the polymer network not only increases the ion osmotic swelling pressure to a great extent but also enhances the relaxation of macromolecular chains because of repulsion among similarly charged -COO- groups. These two factors ultimately result in a greater increase in the water uptake. In pH 7.4 with completed ionization and an increase in the hydrophilicity of the polymer, diffusion of the hydrolyzing

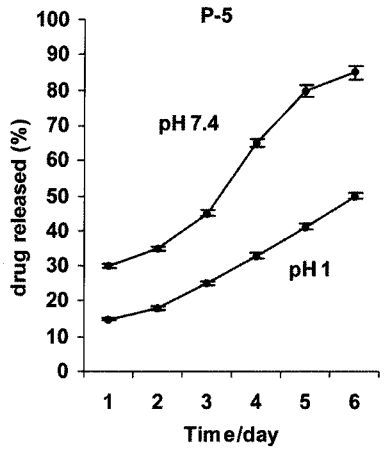


Figure 4. Release of ibuprofen from P-5 as a function of time at 37 °C.

Table II. Percent of Swelling and Ibuprofen Release

Polymers	Maximum Constant Swelling (%)		Maximum Percent of Ibuprofen Release (6 day)	
	pH 1	pH 7.4	pH 1	pH 7.4
P-1	150	550	35	98
P-2	110	480	30	90
P-3	100	750	25	99
P-4	70	680	21	90
P-5	230	560	50	85
P-6	180	500	45	83

agents on polymer is increased and the hydrolysis rate increased.[33] Therefore, in alkaline pH value, the polymers are easily degraded to release of ibuprofen.

Conclusions

As a first part of our developing study about polymeric prodrugs, we have described the synthesis and properties of the pH-sensitive hydrogels consisting of ibuprofen pendent groups by preparation of polymerizable acrylic derivatives. In this work, VIP as a vinyl ester type derivative of ibuprofen was synthesized from reaction between vinyl acetate and ibuprofen in the presence of catalyst. Novel pH-responsive hydrogels containing pendent glucose and ibuprofen were synthesized by free-radical crosslinked copolymerization. By regulating the crosslinking percentage of the MAA copolymers, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained. As the main purpose of polymeric prodrugs is the achievement of controlled drug release or slow release, application of these polymers as a drug delivery system is expected after *in vivo* examinations.

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