Synthesis and *In Vitro* Studies of Cross-Linked Hydrogel Nanoparticles Containing Amoxicillin

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ABSTRACT: In this paper, we report the synthesis and characterization of a novel cross-linked *N*-isopropylacrylamide-acrylic acid-hydroxyethyl methacrylate [P (NIPASM-AA-HEM)] hydrogel nanoparticles (NPs) containing amoxicillin. The aim of present study was to investigate whether these hydrogel NPs have the potential to be used in antibiotic delivery to stomach for treatment of *Helicobacter pylori*. Amoxicillin-loaded hydrogel NPs were prepared using cross-linked P (NIPASM-AA-HEM) as mucoadhesive polymer for the potential use of treating gastric and duodenal ulcers. Aiming at predicting the *in vivo* behavior of the amoxicillin-loaded NPs, the physicochemical properties in terms of entrapment efficiency (EE%), mean diameter, and morphology of NPs was evaluated. The dependence of the EE% of the drug on the organic to aqueous phase ratio was also studied. The profile of amoxicillin release from P (NIPASM-AA-HEM) NPs system was studied under various conditions. In all these experiments, amoxicillin release in the free form was studied by ultraviolet (UV) spectrophotometric analysis. Experimental results showed that at pH 7.4, drug release rises when polymer concentration in the formulation increases; in human plasma on the contrary, drug release is reduced as concentration of the polymer in the formulation rises. *In vitro* amoxicillin release rate was also higher in pH 1 than that in pH 7.4. About 88.5% of amoxicillin entrapped in the NPs was released in 4 h in the pH 1.0 medium, whereas in phosphate buffer at pH 7.4 no more than 45% was released after 4 h incubation at 37°C. Amoxicillin concentration in rat's gastric tissue was determined. The results of *in vivo* studies showed that the hydrogel NPs enhance drug concentration at topical site than powder amoxicillin. Thus, amoxicillin-loaded hydrogel NPs may provide therapeutic concentration at a much lower dose that may reduce the adverse effects of amoxicillin in high doses. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:1057–1066, 2011

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INTRODUCTION

Over the last two decades, numerous gastroretentive dosage forms have been designed to prolong gastric residence time. These methods may be classified into: (a) high density systems (sinking), (b) low density systems (floating), (c) super porous hydrogels, mucoadhesives, and (d) magnetic systems.[1] These systems enable oral therapy by drugs with a narrow absorption window in the upper part of gastrointestinal tract. Preparation of suitable gastroretentive dosage forms is especially important in treatment of microorganisms, which colonized in the stomach. Basically, three main factors can limit luminal delivery of drugs: (a) gastric emptying, (b) gastric acidity, and (c) the epithelial mucus layer. In particular, *Helicobacter pylori* (*H. pylori*) lives deep within the gastric mucus and prolonged local application of drug is needed to diffuse sufficiently to the bacteria.[1]

No single antibiotic is effective in eradication of *H.pylori* when administered *in vivo,* the treatment of such infectious disease in peptic ulcer usually requires a triple therapy that includes antibiotic, antibacterial, and proton pump inhibitors. The failure of a single antibiotic therapy could be due to poor stability of the drug in the acidic pH of the stomach,

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poor permeability of antibiotics across the mucus layer, or due to the availability of subtherapeutic antibiotic concentrations at the infection site after oral administration in a conventional dosage form.[2–4]

To improve the efficiency of such therapeutic modality, site-specific antibiotic drug delivery systems have been developed for localized treatment of the *H.pylori* infections of the stomach.[5–13] Some attempts have been made to develop a sustained release dosage form to localize the antibiotic delivery system in the acidic environment of the stomach.[14,15]

Amoxicillin (α-amino-hydroxybenzyl penicillin) is a semi synthetic, orally absorbed, and broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H.pylori* infection. Some researchers have used new amoxicillin formulations such as float tablet, pH-sensitive hydrogels, and mucoadhesive microparticles, which were able to reside in the gastrointestinal (GI) tract for an extended period of time resulting in more effective eradication of *H.pylori*.[16]

Furthermore, hydrogel nanoparticles (NPs) [nanogels] have gained considerable attention in recent years as one of the most promising drug delivery systems. So far, many researchers have focused on the preparation of NPs using synthetic polymers in addition to some commonly used naturally occurring hydrophilic polymers (e.g., chitosane-based hydrogel NPs and alginate-based hydrogel NPs). Among the synthetic group polymers, poly (vinyl pyrrolidone) and poly (*N*-isopropylacrylamide) have been reported to display good potential to serve as drug delivery system.[17]

Poly *N*-isopropylacrylamide (PNIPAM) appears to be the most well-known member of the thermosensitive polymers group. PNIPAM is an uncharged polymer that is highly water soluble at low temperatures but forms gel, when the temperature rises above 31°C–32°C. The sol–gel transition temperature is referred to as lower critical solution temperature (LCST), which is attributed to the disruption of hydrogen bonding of water molecules around the amide of side pendant groups of polymer chain. The LCST of NIPAM copolymers depends on the nature of the comonomers, copolymer composition, and copolymer architecture. The copolymeric NPs networks of PNIPAM can be formed by covalently cross-linking.[18] Thermosensitive core-shell NIPAM NPs have been synthesized via seeding and feeding precipitation copolymerization.[19] Accordingly, thermo- and pH-responsive PNIPAM-based copolymers for drug delivery systems have been previously reported.[20–25]

Furthermore, poly acrylic acid (PAA, Carbopol, polycarbophil) is deemed to be a polymer with bioadhesive and pH-dependence properties, even though it is difficult to maintain its effectiveness in GI tract. Using polyglycerol esters of fatty acids and PAA derivatives, mucoadhesive microparticles have been prepared and examined as *in vivo* and *invitro* in rats showing prolonged gastrointestinal residence.[26] Interestingly, it has been reported that cross-linked chitosane microspheres are more resistant to gastric acidity than cross-linked chitosane.[27]

Taken all these together, it seems that oral drug delivery systems present their own advantages and drawbacks; nevertheless, developing an effective gastroretentive dosage form is yet to be accomplished. To pursue such aim, in the current investigation, we have synthesized and characterized a novel cross-linked *N*-isopropylacrylamide-acrylic acid-hydroxyethyl methacrylate [P (NIPASM-AA-HEM)] hydrogel NPs containing amoxicillin that can be used as an effective delivery system to shuttle desired antibiotic to target *H.pylori*.

MATERIALS AND METHODS

Materials

Acrylic acid (AA), hydroxyethyl methacrylate (HEM), triethyleneglycol dimethacrylate (TEGDMA) and ammonium persulphate (APS) were obtained from Merck Chemical Co. (Darmstadt, Germany). *N*-Isopropylacrylamide (NIPAM) was purchased from Across Organics (New Jersey, USA) and purified by recrystallization from n-hexane-toluene (90:10, v/v). Amoxicillin was kindly provided by Daana Pharmaceutical Co. (Tabriz, Iran).

Synthesis of Poly (*N*-Isopropylacrylamide-Acrylic Acid-hydroxyethyl Methacrylate) Copolymer

Poly (*N*-isopropylacrylamide-acrylic acid-hydroxyethyl methacrylate) [Poly (NIPAM-AA-HEM)] copolymer was synthesized by free radical copolymerization of monomers in 1,4-dioxane under N_2 atmosphere. The ratio of NIPAM:AA:HEM was 85:5:10. Monomers were dissolved in 1,4-dioxane to form a 5 wt% solution containing PBO (7.5×10^{-3}) and TEGDM (0.1 wt%) as a cross-linking agent. The polymerization was carried out at 70°C for 10 h under N_2 atmosphere. The resulting copolymer was precipitated in excess cold n-hexane. The crude polymer was purified by dissolving in tetrahydrofurane (THF) and reprecipitation in diethyl ether to remove the reactant residues. The copolymer was finally dried by pumping under reduced pressure. Chemical structure of copolymers was determined by FT-IR (Shimadzu 8400, Kyoto, Japan) and ^{1}H-NMR (Bruker AC 80, Rheinsteten, Germany) spectroscopies.

Characterization of the Hydrogel

Calorimetric Studies.

Glass transition temperature (T_g) was determined using differential scanning calorimetric measurements