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Novel pH-sensitive IPNs of polyacrylamide-*g*-gum ghatti and sodium alginate for gastro-protective drug delivery

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ABSTRACT

This article reports the development of pH-sensitive interpenetrating polymer network (IPN) microbeads using polyacrylamide-*grafted*-gum ghatti (PAAm-*g*-GG) and sodium alginate (SA) for gastro-protective controlled delivery of ketoprofen. We have synthesized PAAm-*grafted*-GG copolymer under microwave irradiation using cerric ammonium nitrate as reaction initiator; further, the PAAm-*g*-GG was converted pH-sensitive copolymer through alkaline hydrolysis. Sophisticated instrumentation techniques were used to characterize PAAm-*g*-GG. The IPN microbeads of PAAm-*g*-GG and SA, pre-loaded with ketoprofen were prepared by dual crosslinking using Ca^{2+} ions and glutaraldehyde (GA). The IPN microbeads demonstrated excellent pH-sensitive behavior as noted in the pulsatile swelling test and scanning electron microscopy. IPN microbeads also showed larger amount of drug release in buffer solution of pH 7.4 as compared to drug release in solution of pH 1.2. The in vivo pharmacokinetic, pharmacodynamic and stomach histopathology studies conducted on wistar rats confirmed the pH-sensitive controlled release of ketoprofen; IPN microbeads retarded the drug release in stomach resulting in reduced adverse effects of ketoprofen.

1. Introduction

Literature on drug delivery witnessed the importance of polymers as backbone of the advanced drug delivery systems for controlled drug release and drug targeting [1]. In recent years, we noticed a tremendous use of natural polymers for the development of controlled drug release and drug targeting [2]. These natural polymers often have few restrictions in their reactivity and processibility; hence there is need for improving functional properties and mechanical strength of natural polymers [3]. To conquer the poor biological performance and to improve mechanical strength, new groups of polymers have been synthesized by the name interpenetrating polymer networks (IPNs). An IPN can be termed as a mixture of two or more polymers in a network form, where they are synthesized and/or crosslinked in the immediate presence of each other. Each polymer network of IPNs retains its individual properties leading to synergistic improvements in mechanical strength and biological performance [4]. Such IPNs are now attaining place in controlled and targeted drug delivery domain. Many research reports have shown that the wide range of drugs can be delivered successfully using IPN based delivery systems [5].

Sodium alginate (SA) is a linear polymer with the composition of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid residues. It is obtained from brown seaweed and marine algae [6]. The utilization of SA depends on its capability to form stable and strong gels with divalent or trivalent cations, preferably Ca^{2+} ions. The SA can form a stable network upon covalent crosslinking with glutaraldehyde; thus, it finds application many fields including controlled release of drugs [7–9].

Gum ghatti (GG) is an anionic polysaccharide, obtained from the exudation of Anogeis-suslatifolia tree pertaining to combretaceae family. The composition of GG includes 1 → 6 linked-D-galactopyranose major units and alternating 4-O-subsituted and 2-O-subsituted-D-mannopyranose units along with a single L-arabinofuranose unit as side chain [10]. GG is commonly used in food and biotechnology industries, but little attention was paid on the use of GG in drug delivery application [11].

The grafting of synthetic polymers with natural polymers is a vital approach to use natural polymers for controlled and targeted drug delivery application. The graft polymerization of

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polyacrylamide (PAAm) on GG will result in an innovative copolymer including the properties of GG and PAAm polymers. Such a grafted copolymer can be converted to pH-sensitive carrier by the way of alkaline hydrolysis, where —$CONH_2$ functional groups of PAAm-*g*-GG will be converting to —COO^- groups [12]. Further, synthesis of IPNs of PAAm-g-GG and SA is to our advantage because it comprises of two crosslinked polymer chains in complex structure resulting in three-dimensional pH-sensitive matrix with more free volume for increased entrapment efficiency and better mechanical strength.

In a research report, Mittal et al., have synthesized biodegradable flocculants of polyacrylamide grafted GG, which was found to be pH and temperature responsive biomaterial [13]. Rani et al., have synthesized the graft copolymers of gum ghatti and polyacrylamide by microwave assisted method toward the possible application as flocculant for wastewater treatment [14]. In another report, Reddy et al. have reported the ghatti gum and chitosan IPN microparticles by emulsion-crosslinking method for diclofenac sodium [15]. However, to the best of our understanding, no work is available on the application of pH-sensitive IPNs of PAAm-g-GG and SA in drug delivery. In continuance of our previous reports on graft copolymers and their IPNs for drug delivery application [16–20], we now report the synthesis, development and evaluation of novel IPN microbeads of pH-sensitive PAAm-*g*-GG and SA for gastro-protective controlled delivery of ketoprofen.

Ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) is being used to treat musculo-skeletal and joint disorder. It readily absorbs through gastrointestinal tract and it has a shorter half-life of 2 h. Upon oral administration, it produces adverse effects in the stomach like gastric irritation, ulceration, hemorrhage, etc. Hence, there is a need for controlled release of ketoprofen avoiding its release in stomach for the effective treatment of musculoskeletal disorder and patient compliance [21].

Purpose of this investigation was to synthesize pH-sensitive IPN microbeads using PAAm-*g*-GG and SA for gastro-protective controlled delivery of ketoprofen. As IPN microbeads hold —COO^- functional groups, they will be unionized in the stomach pH leading to small amount of drug release; on the other hand, the microbeads undergo ionization in intestinal pH leading to greater drug release. This may perhaps decrease the side effects of ketoprofen.

2. Materials and methods

2.1. Materials

Ketoprofen was kindly gifted by Rhone-Poulenc (Mumbai, India). Gum ghatti, sodium alginate, ceric ammonium nitrate, acrylamide (AAm), acetonitrile (HPLC), methanol (HPLC) and potassium dihydrogen phosphate were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Glutaraldehyde (25%, w/v), calcium chloride dihydrate and sodium hydroxide were got from SD fine Chemicals (Mumbai, India). Distilled water was used during the study. All the chemicals were of analytical grade and used as received without further purification.

Institutional Animal Ethics Committee approval was obtained for animal study protocols as per the guidelines of CPCSEA.

2.2. Microwave assisted synthesis of PAAm-g-GG copolymer

Microwave assisted synthesis of PAAm-*g*-GG was carried out as per the method reported earlier with little modification [14]. In brief, GG (2 g) was soaked in 70 ml distilled water at 40 °C in a reaction vessel by overnight stirring at 50 rpm. Precisely weighed quantities of acrylamide monomer (8.53 g) and 0.5 g of ceric ammonium nitrate (CAN) were dissolved in 10 ml distilled water and added to GG solution. The reaction mixture was kept in microwave oven (GMG 17E 07 SLGX, Godrej, India) and microwave irradiation was performed at 900 W. The irradiation was stopped at the onset of boiling (~70 °C) at different time intervals and the reaction mixture was cooled in ice-cold water. The microwave irradiation-cooling cycle was continued until polymer solution was converted to gel. Then the reaction mixture was kept undisturbed for 1 h for complete reaction. Later, the reaction mixture was transferred to methanol and kept for 24 h; further, it was washed with methanol and distilled water. The resulting graft copolymer was filtered and dried at 50 °C for 12 h, pulverized and stored in a moisture free container.

2.3. Hydrolysis of PAAm-g-GG

Accurately weighed amount (2 g) of PAAm-g-GG was added to 100 ml of 0.9 M NaOH solution with stirring. The reaction was carried out for 1 h at 75 °C with continuous stirring on a temperature controlled water bath. After 1 h, the product was cooled to ambient temperature and 200 ml of methanol was added and left for 12 h to dewater. The product was filtered, rinsed three times with methanol and dried at 50 °C for 12 h.

The percent grafting efficiency of PAAm-*g*-GG was determined using the equation:

$$\%\,\text{Grafting efficiency} = \left(\frac{W_1 - W_0}{W_2}\right) \times 100$$

W_0, W_1 and W_2 denote the weights of GG, PAAm-*g*-GG, and AAm, respectively.

2.4. Characterization of PAAm-g-GG

The FTIR spectra of pristine GG, PAAm-g-GG and alkaline hydrolyzed PAAm-*g*-GG were taken using FTIR spectrophotometer (8400S Shimadzu, Japan). The samples were prepared by crushing with potassium bromide to convert into pellets under hydraulic pressure of 600 kg and they were scanned in the range of 500 and 4000 cm^{-1}.

The ^1H NMR study was performed on pristine GG, AAm and PAAm-g-GG using tetramethylsilane (TMS) as an internal standard. The samples (10 mg/dm^3) were dissolved in dimethylsulfoxide (DMSO) and spectra were obtained using Varian spectrophotometer (Mercury plus 300 MHz NMR, WA, USA) at 60 °C.

The pristine GG, PAAm-*g*-GG and alkaline hydrolyzed PAAm-*g*-GG were subjected for estimation of elements, which was performed using CHN analyzer (Flash EA 1112, Thermo Finnigan, Italy). The result was expressed as percentage of nitrogen, carbon and hydrogen.

2.5. Preparation of IPN microbeads

The polymeric solution of PAAm-g-GG and SA at a concentration of 4%, w/v was prepared in distilled water with continuous stirring for 4 h. An exactly weighed quantity of ketoprofen was dispersed in the polymeric solution and mixed uniformly on magnetic stirrer for 30 min. This polymeric dispersion was loaded into 20 ml hypodermic syringe and slowly extruded into an aqueous solution of $CaCl_2$ through a # 23 needle while stirring. The obtained microbeads were removed from the $CaCl_2$ solution; washed two times with 50 ml distilled water, kept at room temperature for 24 h and then dried at 40 °C for 12 h. Thus, we produced Ca^{2+} ion cross-linked IPN microbeads. Further, these IPN microbeads were subjected for covalent crosslinking by placing them into methanol containing different concentrations of GA and 1 N HCl at 50 °C for 30 min. Thus, prepared dual cross-linked IPN microbeads were constantly washed with distilled water to take away un-reacted GA