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A Novel pH-Responsive Super Absorbent Hydrogel Based on Collagen for Ephedrine Controlled Release

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In this work, a novel family of pH-responsive polymeric hydrogel based on collagen was prepared for controlled delivery of ephedrine. Acrylic monomers, acrylic acid and itaconic acid were simultaneously graft copolymerized onto collagen backbones by a free radical polymerization technique using ammonium persulfate as initiator and methylene bisacrylamide as a crosslinker. Hydrogel formation was confirmed by FTIR spectroscopy. Thermogravimetric analysis showed that the thermal stabilities of the hydrogels. Results from scanning electron microscopy observation also showed a porous structure with smooth surface morphology of the hydrogel. Swelling profiles obtained clearly indicated that these hydrogels swell slightly in a simulated gastric fluid and strongly in a simulated intestinal fluid. The model drug, ephedrine, was successfully loaded into the hydrogels and *in vitro* release studies were performed in simulated gastric fluid for the initial 122 min, followed by simulated intestinal fluid until complete dissolution. The release of ephedrine was continued up to 215 min. The release mechanism of the hydrogels was also studied using the Ritger-Peppas model.

Key Words: Collagen, Hydrogel, Acrylic acid, Itaconic acid, Ephedrine, Controlled release.

INTRODUCTION

Drug delivery systems (DDSs) are regarded as a promising means to control post-operative inflammation[1], although design improvements are needed to increase biocompatibility and effectiveness, as well to prolong controlled release of the drug[2]. Interest in biodegradable polymers and specifically in a drug delivery system matrix, has been growing. The main reason for this is that delivery systems based on biodegradable polymers do not require removal of the polymers from the body at the end of the treatment period, as they degrade into physiologically occurring compounds that can be readily excreted from the body[3].

In recent years, much interest has been shown in the development of synthesis of natural-based super absorbent hydrogels[4,5]. These biopolymer materials are crosslinked hydrophilic polymers, capable of absorbing large quantities of water, saline or physiological fluids[4]. Because of their non-toxicity, biocompatibility and biodegradability, natural-based hydrogels have attracted in many fields such as hygienic, cosmetics and agriculture[6]. Stimuli-responsive smart hydrogels that can respond to environmental physical and chemical stimuli, such as temperature, light, electric field and magnetic field have attracted great interests in recent years due to their versatile applications such as controlled drug and gene delivery systems[6], chemical-/bio-separations and sensors and/or actuators. Among those smart hydrogels, pH-responsive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastro-intestinal tract and have been prepared for delivery of low molecular weight drugs.

Proteins are widely distributed in nature and are synthesized mainly in animals, *i.e.*, collagen and keratin *etc*. and in a few plants such as soya. In general, proteins are high molecular weight polymers and their solubility in aqueous solutions is difficult. Two efficient methods for preparation of aqueous soluble proteins are alkaline and enzymatic hydrolysis. According to the literature survey based on Chemical Abstract Service, a few studies have been reported in the case of protein-based hydrogels[7]. Hence, the target of the current study was to exploit novel pH-sensitive collagen-based hydrogels for the effective ephedrine controlled release system. Drug absorption and release capacities of hydrogel systems were also examined.

Ephedrine (L-erythro-1-phenyl-2-methylaminopropanol-1) (Fig. 1), is an alkaloid that is present in various forms of the ephedrine family and which is still extracted from *Ephedra*

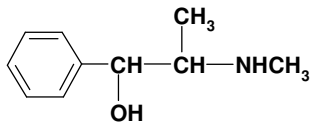


Fig. 1. Chemical structure of drug ephedrine

sinica and *Ephedra equisetina*. The pharmacological action of ephedrine is typical of noncatecholamine sympathomimetics of mixed action. It is mainly used for bronchial asthma, allergic illnesses, as an antiedemic for mucous membranes in rhinitis and also as a drug to increase blood pressure during surgical interventions. It is used locally in ophthalmology as a vasoconstricting agent for dilating pupils[8-10].

EXPERIMENTAL

Encapsulation of model drug: Loading of ephedrine (20 % w/w, based on the total weight of the hydrogel) was carried out by swelling of dried polymeric hydrogel sample in phosphate buffer solution (pH 7.4) at 37 ºC. After immersing the vacuum dried powdered samples (0.1 g) for 24 h, it was taken out, dried and accurately reweighed. The increase in the weight of the hydrogel was taken as the amount of drug loaded, ephedrine encapsulation efficiency percentage, (EE %). The swollen hydrogels loaded with drug were placed in a vacuum oven, dried under vacuum at 37 ºC and stored until further investigation.

It should be pointed out that the phosphate may be lightly absorbed into the hydrogel along with the model drug. But the absorbed phosphate was very little. The anionic phosphate can't be largely absorbed by anionic hydrogel. However, we have washed the loaded hydrogel by distilled water for removing the residues.

Spectrophotometric analysis of model drug: A UV/visible spectrophotometer (Shimadzu, UV-2550) was used to determine the maximum spectra of the drug. Model drug in aqueous solution was prepared for determining the maximum absorption wavelength. The absorbance value at the maximum wavelength of 276 nm of the model drug was read and the corresponding model drug concentrations were calculated from the calibration curve.

Determination of the amount of drug entrapped: The amount of ephedrine entrapped into the hydrogels was calculated by measuring the absorbance of the gelling medium at 276 nm. The amount of ephedrine entrapped was estimated by the difference between the initial and the final amount of drug in gelling media. Encapsulation efficiency percentage was expressed as the weight of drug entrapped in the polymeric hydrogel divided by the initial weight of ephedrine in solution. Moreover, it is important to notice that the drug exhibited the same λ_{max} for whatever the release medium used in this study, as the free drug in water and the presence of dissolved polymers did not interfere with the absorbance of the drug at this wavelength[11].

Release studies: *In vitro* release studies were performed in simulated gastric fluid (SGF) and strongly in a simulated intestinal fluid (SIF). at 37 ºC. Accurately weighed amounts of dried drug-loaded polymeric hydrogel (ranging from 0.1-0.2 g) were placed in beakers containing 1 L of the release medium at 37 ºC. At periodic intervals 5 mL of aliquots were collected from the release medium and the ephedrine concentrations were measured using a spectrophotometer at λ_{max} 276 nm. The percentage of cumulative amount of released ephedrine, obtained from three experiments, was calculated and plotted against time[12].

RESULTS AND DISCUSSION

Synthesis of hydrogels: A general reaction mechanism for collagen-based hydrogel formation is shown in **Scheme-I**. At the first step, the thermally dissociating initiator, *i.e.*, ammonium persulfate, is decomposed under heating to produce sulfate anion-radical. Then, the anion-radical abstracts hydrogen from one of the functional groups in side chains (*i.e.*, COOH, SH, OH and NH_2) of the substrate to form corresponding radical. So, these macroradicals initiated monomers grafting onto collagen backbones led to a graft copolymer. In addition, crosslinking reaction was carried out in the presence of a crosslinker, *i.e.*, MBA, so that a three dimensional network was obtained[13,14].

FTIR spectroscopy: The grafting was confirmed by comparing the FTIR spectra of the collagen substrate with that of the grafted products. The band observed at 1644 cm^{-1} can be attributed to C=O stretching in carboxamide functional groups of substrate backbone (Fig. 2a). The super absorbent hydrogel product comprises a collagen backbone with side chains that carry sodium carboxylate functional groups that are evidenced by a peak at 1561 cm^{-1} (Fig. 2b). This characteristic band is due to asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1422 cm^{-1} which is related to the symmetric stretching mode of the carboxylate anion.

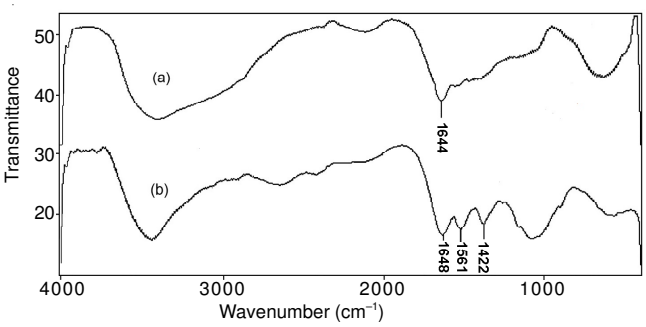
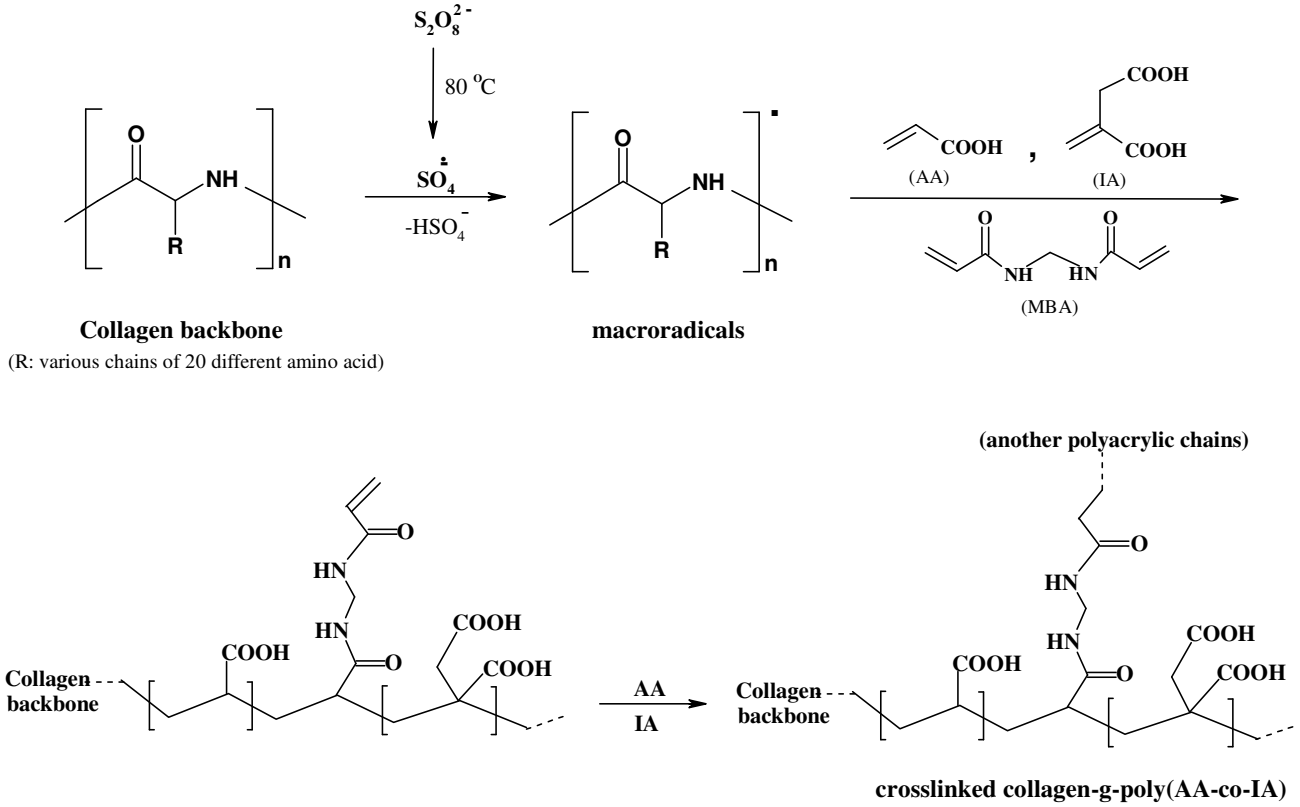


Fig. 2. FTIR spectra of collagen (a) and collagen-g-poly(AA-co-IA) hydrogel (b)

Amount of drug encapsulated: The amount of ephedrine encapsulated in the polymeric hydrogels increased with increasing drug concentration. The ephedrine encapsulation efficiency percentages (EE %) are 63, 74 and 88 % according to the concentrations 0.15, 0.75 and 1.2 % of ephedrine, respectively.

***In vitro* release behaviour of hydrogels:** In order to simulate the possible effect of pH on drug release rate, a swelling study was conducted in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) at physiological temperature of 37 ºC (Fig. 3). It should be pointed out that there are no differences between simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) besides the pH. At pH 7.4,



Scheme-I: Proposed mechanistic pathway for synthesis of the collagen-based hydrogels

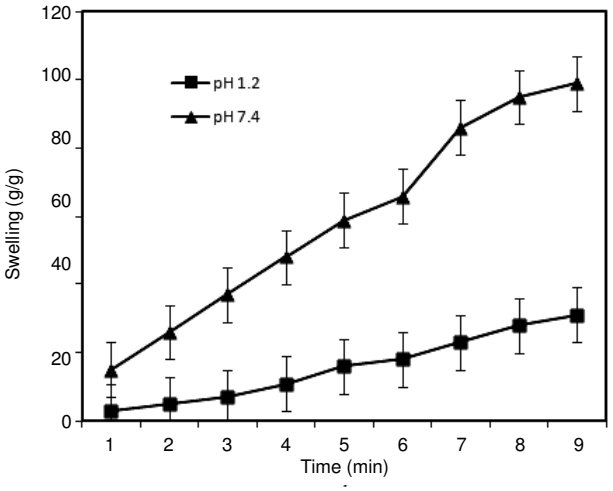


Fig. 3. Effect of pH of solution on swelling of collagen-g-poly(AA-co-IA) hydrogel

the hydrogel swells due to anion-anion repulsive electrostatic forces, while at pH 1.2, it shrinks within a few minutes due to protonation of the carboxylate anions. This swelling behaviour of the hydrogels makes them as suitable candidate for designing drug delivery systems.

The most challenging task in the development of drug pharmaceuticals is to deal with instabilities of drugs in the harsh environment of the stomach. Drug encapsulation processes that require the use of organic solvents or heating might potentially physically modify or denature the therapeutic proteins. Encapsulation processes that require chemical bond formation among the encapsulation reagents might unintentionally chemically modify the therapeutic proteins. However, our drug loading process was desirable as the encapsulation of ephedrine was performed avoiding any organic solvent, high temperature, unfavorable pH and other harsh environmental conditions[13-15]. The conditions were benign sufficiently as the resulting hydrogel physically entrapped the ephedrine drug. Fig. 4 shows the ephedrine release profile of the test hydrogels at pH 1.2 and subsequently at pH 7.4. The amount of ephedrine released at pH 1.2 was low; only *ca.* 15 % ephedrine was released from the test hydrogel, whereas that released at pH 7.4 increased significantly (94 %). The favorable ephedrine release performance could be attributed to the pH-sensitivity of the hydrogel. Swelling of such hydrogel in the stomach was minimal and thus the drug release was also minimal. Due to increase in pH, the extent of swelling increased as the hydrogel passed down the intestinal tract, the hydrogel swelled and the controlled release of ephedrine was affected. Fig. 5 shows the schematic of actuation at a distance and resultant squeezing effect for the pH-responsive collagen-based system. Because of the high matrix porosity of the hydrogel, the capillary forces could reinforce the diffusion of solvent into the hydrogel; thereby the ephedrine release from the hydrogel matrix occurred mainly due to the diffusion of the drug though the pores of the swelled matrix in the intestinal pH[16].

The dependence of the extent of crosslinking on *in vitro* release was also displayed in Fig. 6. It is observed that release rates depend upon the amount of MBA used as crosslinking agent. The cumulative drug release of ephedrine from the hydrogels was decreased with increasing MBA content[17-20]. This could be due to the fact that at higher crosslinking, free volume of the matrix will decrease, thereby hindering the transport of drug molecules through the matrix.

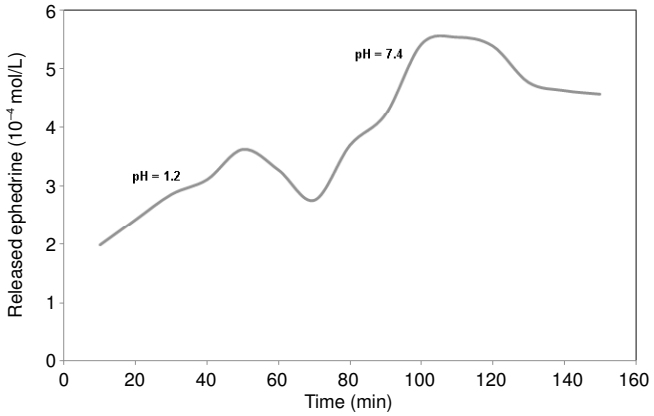


Fig. 4. Ephedrine release profile in simulated gastric fluid (pH 1.2) and subsequently in simulated intestinal fluid (pH 7.4) at 37 ºC

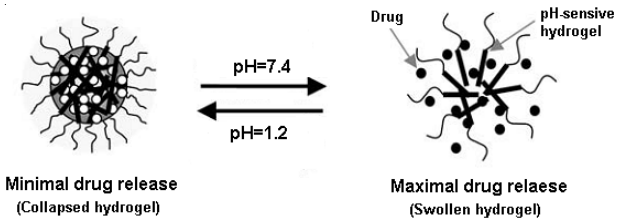


Fig. 5. Schematic showing the effect of ON-OFF cycles of pH on swelling behaviour. It shows the pH triggered collapse and resultant burst release due to squeezing effect

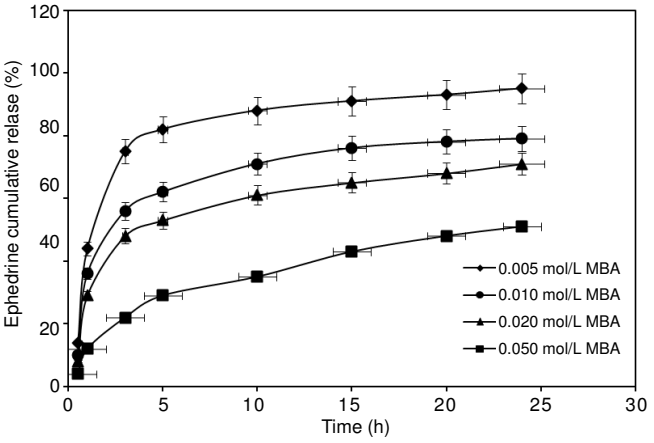


Fig. 6. *In vitro* cumulative release of ephedrine from the hydrogel with different crosslinker content at pH 7.4 and 37 ºC

Drug release mechanism: A simple semi-empirical equation has been introduced to express general drug release behaviour depending on the geometry of a system[20]:

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where and M_t and M_∞ are the absolute cumulative amounts of drug released at time *t* and after the finish of release respectively, k is a diffusional kinetic constant for the characteristics of a polymer network system and n is a diffusional exponent representing the release mechanism.

When log M_t/M_∞ is plotted against log t the value of n is obtained. The case of n = 0.5 is for purely diffusion-controlled drug release (Fickian release) and the case of n = 1 is for a drug release rate independent of time, corresponding to zero-order release kinetics (case II transport). Other values for n are for anomalous transport kinetics and combined mechanisms of pure diffusion and case II transport. In our experiments the diffusion coefficients were calculated based on fitting 20 % and 60 % of drug release, respectively. When 20 % of drug had been released n was 9.74, indicating case II transport close to zero order release. In the 60 % case n was 0.71, related to non-Fickian or anomalous transport.

Conclusion

A new pH-responsive drug delivery system based on collagen hydrogel was developed for oral drug delivery of a poorly water-soluble drug to the intestinal environment. Ephedrine was encapsulated as a model drug and *in vitro* release studies were carried out in SGF and SIF. These studies indicated that the model drug encapsulation efficiency was increased with increasing the concentration of ephedrine. We have also evidenced that the release of ephedrine from these systems was influenced not only by the pH of swelling medium, but also by crosslinking content. The release value of ephedrine from hydrogels at pH 7.4 was higher than that at pH 1.2 due to the electrostatic repulsion between carboxylate groups. Moreover, the drug release from the hydrogels was decreased with increasing MBA content. It is concluded that by varying crosslinking density or especially by changing the pH of solution, we can control and modulate the drug release rate.

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