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Insulin Release Behavior of Poly(methacrylamide-co-N-vinyl-2-pyrrolidone-co-itaconic acid) Hydrogel: An Interesting Probe. Part II

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In this work, a terpolymeric hydrogel, consisting of N-vinyl-2-pyrrolidone, methacrylamide and itaconic acid, has been investigated for its blood compatibility. The hemolytic activity and hemoglobin binding capacity of the hydrogel has been investigated. Finally, release of insulin from the hydrogel has been studied in the media of varying pH to mimic transition from mouth to colon. The mesh sizes of the swelling hydrogel, at different time-intervals, has been calculated and correlated with the percent insulin released in simulated gastric and intestinal fluid at 37°C. The percent protection efficiency (PPE) value for the samples prepared with 0.324 and 0.454 mM of crosslinker was found to be 10.5 and 33.4, respectively.

Keywords: insulin; blood compatibility; hydrogel; mesh size

1 Introduction

The advent of insulin revolutionized the treatment of diabetes and must be one of the most outstanding achievements of the 20th century in the field of medical science (1). For the past 75 years, subcutaneous injections have been the only route of delivery of insulin therapy to diabetic patients. However, in the last few decades considerable research has been done in the design of systems for delivery of insulin via the oral route. The major difficulties encountered for delivery of insulin-like protein drugs result mainly from their delicate physico-chemical properties, their degradation by proteolytic enzyme and their poor permeability through the intestinal tissue.

In our laboratory, we have developed some pH-sensitive drug delivery systems (2–5) that are supposed to function on the generally accepted principle that they protect the encapsulated drug in the highly acid and proteolytic-enzymes containing environment of the stomach. Furthermore, the systems demonstrate minimum swelling and release most of the drug when they reach the slightly alkaline environment of the large intestine, where they exhibit maximum swelling.

Recently we synthesized a terpolymeric hydrogel system, consisting of N-vinyl-2-pyrrolidone, methacrylamide and itaconic acid, and reported its water uptake behavior (6). We found that the gels showed excellent pH dependent swelling behavior. Therefore, it was thought that they could prove to be a potential candidate for oral delivery of insulin-like drugs to the colon (i.e., large intestine). In the present work, we have investigated blood compatibility of this newly developed system. In addition, the release of insulin has been studied in the media of varying pH at physiological temperature and we have attempted to investigate whether the device can really provide protection to entrapped insulin in gastric fluid medium.

2 Experimental

2.1 Chemicals

The monomers N-vinyl-2-pyrrolidone (NVP; Sigma, St.Louis, MO), methacrylamide (MAAm; High Media, Mumbai, India) and itaconic acid (IA, Research Lab, Pune, India) were of analytical grade. The crosslinker N,N′-methylene bisacrylamide (MB), initiator potassium persulfate (KPS), and tetramethylethylenediamine (TEMED) catalyst were all purchased from HiMedia Laboratories, Mumbai, India.

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The monomer MAAm was crystallized in methanol to remove the inhibitor. The injections of insulin were purchased from Torrent Pharmaceuticals. Double distilled water was used throughout the investigations.

2.2 Synthesis of Drug-Loaded Hydrogels

The insulin loaded cylindrical gels were prepared by carrying out free radical, aqueous polymerization of NVP, MAAm, and IA, in the presence of a pre-calculated quantity of insulin, using MB as crosslinker, KPS and TEMED as initiator and catalyst, respectively (6). In order to preserve the activity of insulin, the polymerization was carried out at room temperature for a period of 24 h. The cylindrical gels, with a length of 16.8 ± 0.2 mm and a diameter of 3.86 ± 0.02 mm, were washed with distilled water and kept in a dust free chamber at room temperature until they dried completely and attained constant weight. For biocompatibility testing, hydrogels were also prepared in the form of films by pouring the reaction mixture on a teflon-coated Petri-dish (Borosil, India) of a 6" diameter.

2.3 IR Spectra of Polymer and Drug

The FTIR spectra of plain polymer, drug-loaded polymer and drug insulin were recorded on a Shimadzu 8201 PC spectrophotometer.

2.4 Hemolysis Assay

Hydrogel film (1 cm^2) was equilibrated in normal saline water for 60 min at 37°C and human ACD blood (0.25 ml) was added on the film. After 20 min, 2.0 ml of 0.9% sodium chloride saline was added to stop the hemolysis and the sample was incubated for 60 min at 37°C. Positive and negative controls were obtained by adding 0.25 ml of human ACD blood and 0.9% NaCl saline, respectively, to 2.0 ml of double distilled water. Incubated samples were centrifuged for 45 min, the supernatant was taken and absorbance was recorded on a spectrophotometer 2201, Systronics, India) at 545 nm. The percent hemolysis was calculated using the following relationship (7):

$$\%\ hemolysis = \frac{A\ test\ sample - A\ (-)\ control}{A\ (+)\ control - A\ (-)\ control} \times 100$$

where A = absorbance.

2.5 Adsorption of Hemoglobin

Hydrogel film of definite weight was put in the hemoglobin solution for a period of 24 h at 37°C. Then, the films were taken out and the absorbance of the remaining solution was measured and the amount of hemoglobin adsorbed was determined using a standard calibration curve prepared with solutions of know concentrations.

2.6 Swelling Studies

Completely dried pre-weighed gels were put in 250 ml buffer solutions of desired pH at 37°C and their mass was measured at different time-intervals, until the gels attained constant weight. The percent mass swelling was determined using the following expression:

$$\%\ S_M = \frac{M_t - M_o}{M_o} \times 100$$

where M_t and M_o are the initial mass and mass at different time-intervals, respectively. All the experiments were carried out in triplicate and average values have been reported in the data.

2.7 Determination of Mesh Size (ξ)

The mesh size of the swelling hydrogel was determined from water uptake measurements, using mathematical formulations given by Saraydin et al. (8) for diprotic acid containing hydrogels. The percent mass swelling of the hydrogel was measured at different time-intervals and the data was used for the determination of mesh sizes of the swelling network at different stages.

2.8 In Vitro Drug Release Studies in Media of Varying pH

Relying on the experimental data provided by Satyanarayan et al. (9), on the basis of their gamma scintigraphic studies on guar gum tablets, we considered the gastric transit time as 2 h for an oral formulation and so the release studies were carried out by putting the insulin loaded hydrogel sample for 2 h in 500 ml simulating gastric fluid (SGF, pH 1.2) and then transferring them into 25 ml of simulating intestinal fluid (SIF, pH 7.4) for the remaining time. After every 1 h, the release media was replaced by fresh buffer. The agitation speed was maintained at 50 rpm. The amount of insulin released was determined spectrophotometricaly at 237 nm (10).

2.9 Percent Protection Efficiency (PPE) of Hydrogel

To determine the protective ability of the hydrogel for insulin under conditions simulating the human stomach environment (11), the pre-weighed insulin-loaded samples ($\underline{S_1}$) was put in simulated gastric fluid. The simulated fluid was prepared by dissolving 2.0 g of NaCl and 3.2 g of pepsin in 7.0 ml of HCL and water to make 1000 ml. After 2 h, the gel was taken out and placed in simulated intestinal fluid (SIF) of pH7.4 for a period of 3 h. One more pre-weighed drug-loaded sample (S_2) of the same composition was directly put in SIF for a period of 3 h. The percent protection of

insulin was given as follows:

$$\% \text{ PPE} = \frac{\text{Insulin released in 3h in SIF from Sample } S_1}{\text{Insulin released in 3h in SIF from Sample } S_2} \times 100$$

The above expression has been formulated with the presumption that if the hydrogel is able to provide 100% protection to the encapsulated insulin in gastric environment by exhibiting minimum swelling and not permitting insulin molecules to come out then the sample S_1 (which was previously put in SGF of pH1.2 for 2 h) should release almost the same amount of insulin in SIF in 3 h as by the sample S_2 (which has not been acid-treated, but directly put for 3 h in SIF of pH 7.4).

3 Results and Discussion

3.1 IR Spectra Analysis

Figure 1 (a), (b) and (c) show the FTIR spectra of plain polymer, insulin loaded polymer and native insulin, respectively. The peak appearing at 1667 cm^{-1} in the drug loaded polymer spectrum_may be the result of overlapping of the two peaks; one appeared in IR spectrum of plain polymer at 1663 cm^{-1} due to $-COO^-$asymmetrical stretching and the other appeared in the spectrum of insulin due to –C═O stretching at 1655cm^{-1}. Similarly, in Figure (b), the peak at 3444 cm^{-1} is due to –OH stretching in the polymer and the peak at 3430 cm^{-1}, appeared in the IR spectrum of insulin (Figure 1(c)) is due to –NH vibration. These two peaks also overlap to yield the resultant peak at 3431 cm^{-1} in the drug-loaded polymer sample in Figure 1(b). Finally, the peak appearing in the spectrum of drug loaded polymer at 2961 cm^{-1} also confirms the presence of methylene group, which is already present in the polymer as well as in the insulin drug.

3.2 Blood Compatibility

Although the present hydrogel system is intended to be used as an insulin-delivery system via the oral route, we investigated its blood compatibility so that chances of its possible use as an implant material could be explored in the human body, i.e., where it would be in extended contact with the

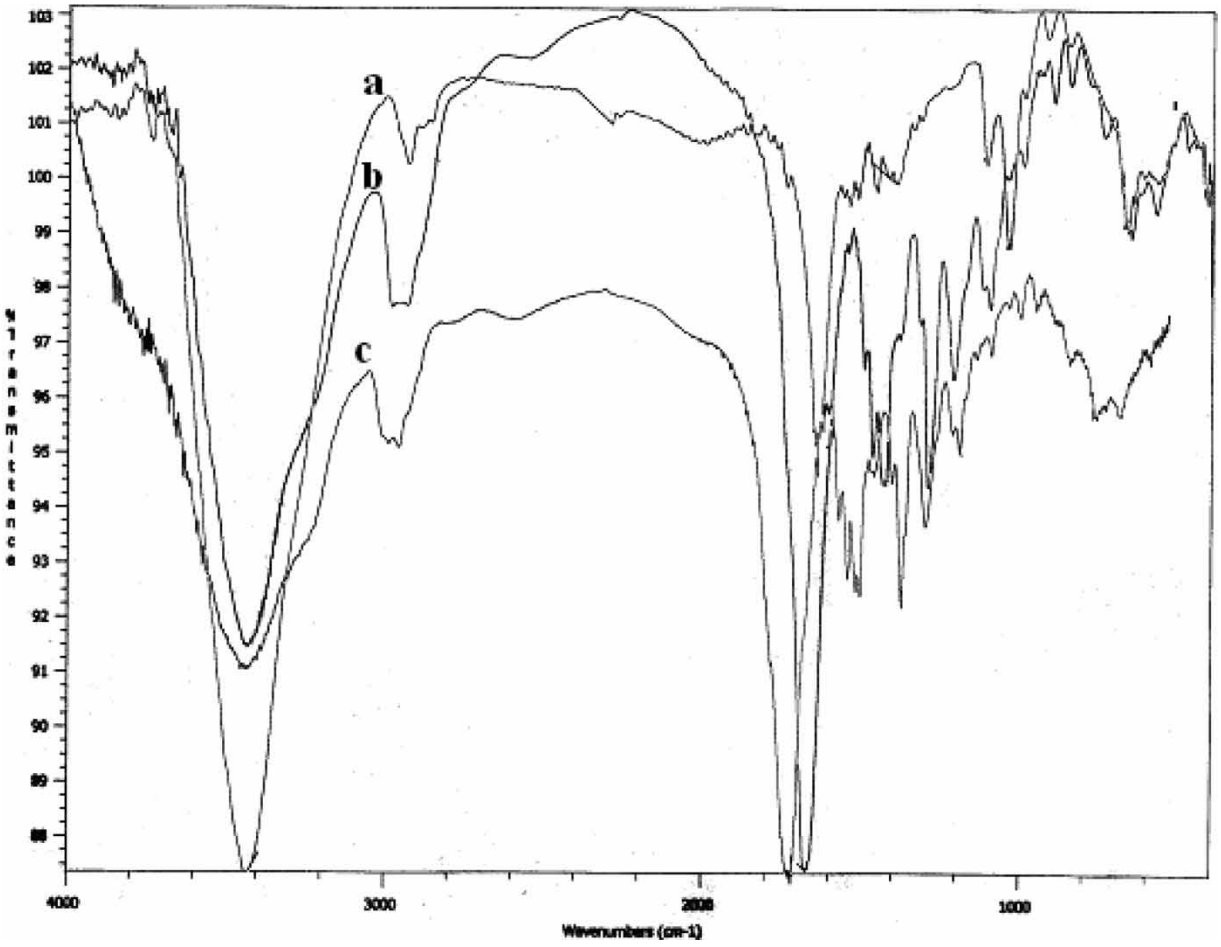


Fig. 1. FTIR spectra of (a) plain polymer sample, (b) insulin loaded polymer, (c) insulin.

human body. In order to investigate hydrogel-hemoprotein interactions, we synthesized samples with varying amounts of itaconic acid, in the range 1.3 mM to 3.2 mM and studied adsorption of hemoglobin solution onto them. It is clear from Figure 2 that the concentration of hemoglobin in solutions that contained hydrogel samples to contact remained the same and matched with the initial concentration. This indicates that the adsorption of hemoglobin onto hydrogel surfaces has been almost negligible, thus confirming fair compatibility of the system. This may probably be attributed to the fact that since the hemoglobin has been dissolved in alkaline medium (pH 8.0), its molecules are negatively charged due to presence of carboxylate groups. Moreover, at this pH, macromolecular chains present on the surface of hydrogels also contain $-COO^-$ groups. Therefore, the chances of electrostatic interactions between hemoglobin molecules in solutions and polymeric chains on the surface of hydrogels are almost nil. This accounts for almost negligible adsorption of hemoprotein onto surface of polymeric hydrogels, thus proving them as potential biomaterials.

We also determined percent hemolysis for the sample containing 2.3 mM of itaconic acid. The percent hemolysis was found to be nearly 22.1 percent.

3.3 Change in Mesh Size Upon Swelling

As stated in the Experimental section, the drug-loaded sample was put in SGF for 2 h and then transferred into SIF for the remaining time, so that its transition from mouth to colon could be mimicked. Figure 3 depicts the change in degree of swelling and mesh size of the hydrogel with time in the media of varying pH. It is clear that in the first 2 h, the gels swells to nearly 110% in SGF and later on, on transferring into SIF of pH 7.4, it undergoes a drastic increase in swelling which reaches to nearly 325% in the 7th hour. It is also clear that the mesh size of the swelling network is nearly10Å in SGF at the end of the second hour and then on transferring into SIF, it begins to increase and becomes

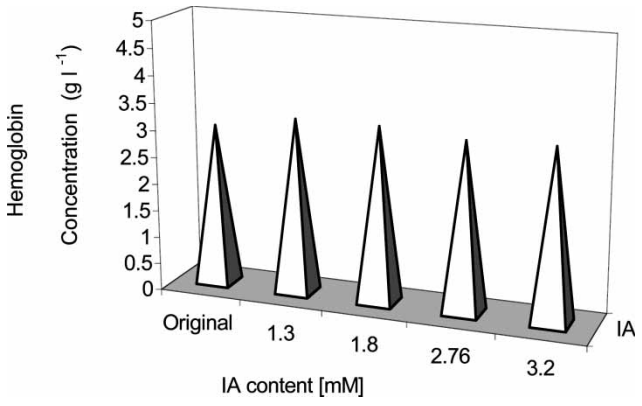


Fig. 2. Bar diagram showing adsorption of hemoglobin onto surface of hydrogels with varying concentrations of itaconic acid.

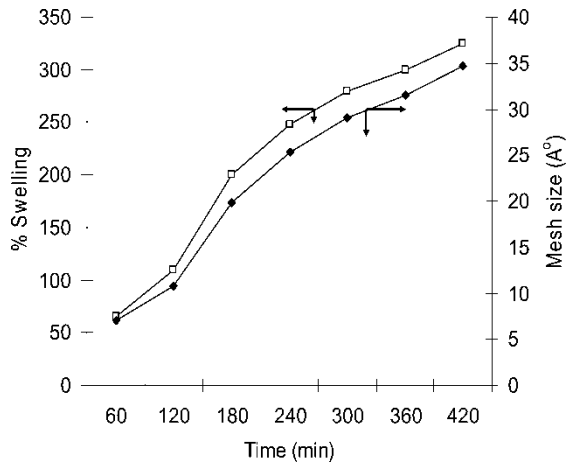
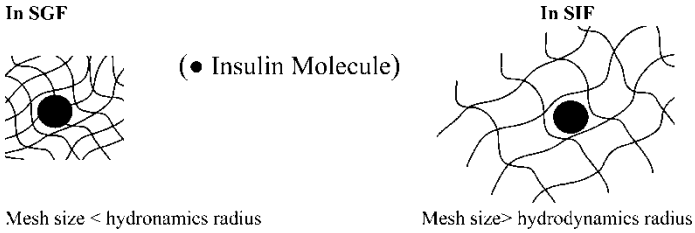


Fig. 3. Variation in percent water uptake and mesh size of the swelling network with time for the sample HG(0.324) in the media of varying pH at 37°C.

nearly 32Å at the end of the 7th hour. This variation in mesh size with time gives us some idea about the possible mode of release of insulin from the device in the media of varying pH. We know that hydrodynamic radius of insulin is nearly 11.1Å (11), therefore it may be expected that the molecules of insulin should remain within the hydrogel network in the first two hour period in artificial gastric fluid, since the mesh size of the network is slightly less than the hydrodynamic radius of the drug. However, when the gel is put in the SIF, its mesh size begins to increase and so the entrapped insulin molecules should emerge from the swelling network. This can be better understood from the following scheme:



3.4 Release in the Media of Varying pH

The dynamic release of insulin in the media of varying pH has been depicted in Figure 4. It is clear that nearly 12% insulin is released in the first 2 h in the medium of pH 1.2, which may be attributed to the fact that the smaller mesh size of the network does not permit drug molecules to emerge. However, the drug present on the surface of the device must have been released in this duration. When the gel is transferred into the simulating intestinal fluid, there is appreciable increase in amount of insulin released. This may simply be attributed to the increasing mesh size of the swelling network which permits the drug molecules to appear. In this way, it can be claimed that the

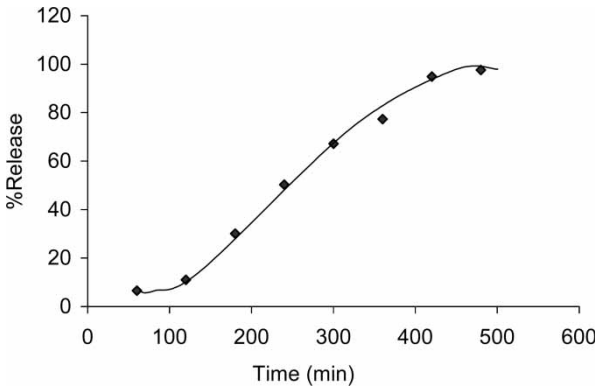


Fig. 4. Dynamic release of insulin as a function of time for the sample HG (0.324) in the media of varying pH at 37°C.

present hydrogel system is quite able to protect the insulin in the acidic environment and it releases most of the entrapped drug in intestinal fluid of pH 7.4 only.

3.5 Protection Efficiency of Hydrogel

From the above discussion it appears that the proposed hydrogel system has the potential to act as an oral delivery device because it does not permit much insulin to come out in artificial gastric fluid. However, as mentioned in the Experimental section, we also determined the percent protection efficiency of the hydrogel system crosslinked with 0.324 mM of crosslinker (i.e., sample HG (0.324). The PPE value of the sample was found to be 11.5, thus showing its extremely low efficiency to protect insulin from the enzyme containing acidic environment of the stomach. The observed low efficiency was really very embarrassing because the release profile displayed in Figure 3 indicates that when the gel was put in the acidic fluid (pH 1.2, no pepsin) for 2 h and then transferred into SIF of pH 7.4, the gel released only 10% of the total release in the first 2 h in SGF, proving its strong candidature as an oral insulin delivery system. However, when we treated the same drug-loaded sample HG (0.324) in the pepsin containing medium pH 1.2 for 2 h and then compared its release in the next 3 h in SIF (pH 7.4), with the release shown by dry samples in SIF for the same time period, (i.e., 3 h), the results obtained are very discouraging. This indicates that pretreatment of the sample in pepsin containing buffer of pH1.2 must have made the sample undergo appreciable loss in insulin content of the gel. Therefore, the observed extremely low efficiency can be attributed to the fact that when the insulin-loaded sample is put in pepsin containing buffer for 2 h, its swelling network (although only nearly 1.12 times) must have allowed the pepsin molecules to enter into the swelling network and hydrolyze the insulin present within the polymer network. That is why when the sample is transferred into SIF of pH 7.4, it demonstrates poor release as compared to the similar dry sample, which was directly put in the SIF for 3 h and its release subsequently measured. This suggests that swelling of a drug-loaded sample in pepsin containing SGF of pH 1.2 plays a significant role in governing the further release behavior of sample in the SIF of pH 7.4.

Finally, we also carried out a protection efficiency test for sample HG(0.454). The efficiency was found to be nearly 33.8, showing a slight improvement in protecting efficiency of the device. This could be simply attributed to the fact that the sample HG (0.454) demonstrates a very low degree of swelling (data not given) in pepsin containing SGF of pH1. Lack of swelling would not allow much pepsin to enter into the network to destroy the loaded drug.

4 Conclusions

From the above studies it can be concluded that the poly(N-vinyl-2-pyrrolidone-co-methacrylamide-co-itaconic acid) hydrogel system shows fair pH-dependent swelling. However, it does not prove to be highly efficient in protecting entrapped insulin in pepsin containing artificial gastric fluid of pH 1.2 in which it was put for 2 h followed by its transfer into SIF of pH 7.4. Samples HG(0.324) and HG (0.454) demonstrate nearly 10.5 and 33.8 percent protection efficiency, thus indicating that the amount of crosslinker may play a significant role in enhancing the efficiency of the device to protect entrapped insulin. Moreover, the release behavior of the device in the media of varying pH (i.e., 2 h in pepsin-free fluid of pH 1.2, followed by transfer into a medium of pH 7.4) cannot be considered as a basis for predicting *in vivo* performance until its protection-efficiency is tested in enzyme-containing gastric fluid.

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