ENZYMATICALLY SYNTHESIZED pH-RESPONSIVE IPN FOR IN-SITU RELEASE OF PANTOPRAZOLE SODIUM

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ABSTRACT

Objective: This study involves the synthesis of Gum tragacanth (gt) based interpenetrating polymer network (ipn) and its utilization for sustained release of anti-ulcerative drug i.e. pantoprazole sodium.

Methods: IPN was synthesized from Gum tragacanth, polyacrylic acid (gt-cl-paa) hydrogel. gt-cl-paa was kept in distilled water. Further, acryamide (aam) and methylmethacrylate (mma) was added and then kept for overnight. Later on, lipase and glutaraldehyde were added. Homopolymers and the unreacted monomers were removed using acetone. Synthesized IPN was dried at 50 °C for further study.

Results: Gt, paa and mma based crosslinked IPN were synthesized using lipase-glutaraldehyde as initiator-crosslinker system. The synthesized IPN was pH sensitive and possessed the desired swelling capacity required for the controlled and systematic liberation of pantoprazole sodium at 37 °C. The kinetic of drug release was studied and found that lateral diffusion $(D_L)$ of drug was higher as compared to the initial diffusion $(D_0)$. The prepared IPN can be used as prospective carrier for prolonged drug delivery.

Conclusion: A novel pH sensitive and colon targeted IPN was synthesized. It acts as an effective device for the controlled release of drug pantoprazole sodium.

Keywords: Interpenetrating polymer network (ipn), Drug delivery, Methylmethacrylate and pantoprazole sodium

INTRODUCTION

Colon-specific drug delivery is considered as one of the essential sectors nowadays across the globe. Cure of colonic ailments, like ulcerative colitis etc. requires colon targeted drug delivery device. Colonic drug delivery system needed a time-consuming release of the drug in the stomach and in the intestine faster release of the drug. Best options for this is to use pH responding smart materials, timed specific release and utilization of pro-drug [1, 2].

Today research study has been centered on the preparation of ipn system as they have superior characteristics than that of the individual monomers from which it is formed. Today’s demands cannot be achieved with homopolymer and grafted product [3-6]. As the requirements have increased both in the case of properties and performances, interpenetrating polymeric network (ipn) fulfill all the above demands. ipn hydrogels comprised of two or more than two polymer chains that are made by the proximity of each other. These have been found to be resourceful for different applications. The characteristics of ipn-system such as porous behavior, flexibility and reaction to stimuli can be exploited in the different sectors like pharmaceutical, agriculture, biomedical etc. [7-10].

The ability to hold a significant quantity of water content makes IPN flexible and resembles the biological tissues [11-14]. The diffusion of the molecules is specific from the IPN matrix. IPN matrix responds to the change in temperature, pH, electric and magnetic fields. Its spongy characteristics permit easy loading of drugs into the ipn and subsequent discharge of the drug at a determined pace and at a particular interval of time [15-17]. Such characteristics of the ipn beneficial to use them as a device for regulated liberation of the drugs. The gastrointestinal (gt) liberation of sodium diclofenac has carried out through chitosan/polyethylene glycol hydrogel beads. Researchers are working on the pH sensitive and glucose-sensitive insulin release through hydrogels both in vitro and in vivo [18-21].

Gum tragacanth (gt) is an anionic polysaccharide that contains two main parts: one is H₂O soluble and other is H₂O swellable. Both the portions can be easily separated. Baeosorin and tragacanth composition differ notably regarding their contents of uronic acid and methoxy[22-24].

The current study comprised of synthesis of new IPN based on gt, aa, aam and mma, crosslinked with glutaraldehyde and catalyzed by enzyme lipase and studied as the possible GI drug delivery device for the model drug employing, pantoprazole sodium.

The novelty of the present work lies on that a green method i.e. enzyme was used instead of chemical initiator for the synthesis of ipn. Natural polysaccharide gt was modified for the synthesis of ipn. gt based product was already in used in food industry, thus no harsh effect for synthesizing IPN based drug delivery device. The synthesized ipn respond to the external pH thus effective for the controlled release of pantoprazole sodium in the gi tract.

The synthesized matrix contains both hydrophilic and hydrophobic chains. Moreover, hydrophobic part helps in the controlled liberation of the drug from the matrix. Thus, the ipn matrix is the promising approach for the in vitro release of the drug. It improves the assimilation of pantoprazole sodium in the gi tract for a prolonged time. Pantoprazole sodium is an acid-labile drug and is effective when absorbed in the gi tract. Therefore, drug liberation was studied at different pH.

MATERIALS AND METHODS

Chemicals

gt, glutaraldehyde, aa, aam and mma (MERCK), India, Lipase (MP Biomedical). FTIR of the gt and IPN were recorded on Perkin spectrophotometer by KBr pellet method. SEM was taken on LEO-435VF. uv-vis spectrophotometer (Systronics, 2201) was used to
study the pantoprazole sodium liberation rate through the IPN. Thermal analysis of the samples were studied through TG/DTA 6300, in nitrogen with 10 °C/min heating rate.

Enzymatic synthesis of IPN
Initially, the gt and aa based hydrogel was synthesized by following the method reported in our previous publication [25]. For the preparation of ipn, the already prepared hydrogel of gt and AA i.e. gt-cl-paa was swelled in the distilled water. Further, aam and mma were added and kept for overnight. Later on, lipase and glutaraldehyde were added. Homopolymers and the unreacted monomers were removed using acetone. Synthesized ipn was dried at 50 °C until stable weight (wt.) was obtained. Percentage swelling \( P_s \) is the criteria for optimizing the reaction parameters. The \( P_s \) was determined with the help of the mathematical equation given below [25].

\[
P_s = \frac{W_s - W_d}{W_d} \times 100
\]

Where, \( W_s \) is the wt. of swollen ipn and \( W_d \) is the weight of dry IPN.

Drug release behavior of ipn
The drug loading i.e. pantoprazole sodium drug molecules into the IPN and investigation of the drug liberation mechanism from the ipn polymer matrix was studied by the method reported in the literature [26-29].

RESULTS AND DISCUSSION

Enzymatic synthesis of the ipn

Synthesis of the gt-cl-paa
Different reaction parameters viz. reaction time, treatment temperature, solvent, pH, concentration of lipase, aam, mma and glutaraldehyde were optimized to get the product with maximum \( P_s \) [25]. The effect of these parameters on \( P_s \) was studied one by one. The optimized reaction parameter to get the maximum \( P_s \) are reaction time = 48h, reaction temperature = 40 °C, amount of water = 4 ml, pH = 5.0, concentration of lipase =0.03 x 10^{-6} molL^{-1}, aa concentration = 1.459 x 10^{-4} mol/l, glutaraldehyde concentration = 10.61 x 10^{-6} mol/l, aam concentration 1.352 x 10^{-3} mol/l [25].

Optimization of MMA
The optimum concentration of mma for maximum \( P_s \) (258.9%) was found to be 2.45 x 10^{-3} mol/l (fig. 1). However, beyond the optimum monomer concentration, the further rise in the monomer content leads to a reduction in \( P_s \). The obtained behavior is ascribed to predominance of cross-linking and compactness of the candidate polymer over \( P_s \).

Fourier transforms infrared spectroscopy
FTIR spectrum of gt showed peaks at3426.92 cm\(^{-1}\)is due to the stretching o-H group of polysaccharides, 2892.89 cm\(^{-1}\)due to asymmetric stretching of-CH\(_2\), 2500-3000 cm\(^{-1}\)due to OH of COOH, 1038.96 cm\(^{-1}\) due to stretching of C-O from C-O-C vibrational stretching and peak at 642 cm\(^{-1}\)is due to pyranose (fig. 3a).

The additional peaks were observed in case of ipn at 1718.18 cm\(^{-1}\) and 1150.93 cm\(^{-1}\), which was due to C=O group of mma and C-O stretch of ester group of monomer moiety, respectively (fig. 3b). The

Characterization

Morphological analysis
The morphological study of the backbone and IPN was carried out using SEM. SEM images of both the samples displayed changes in the surface morphology. SEM images of gt revealed homogenous surface, while gt-cl-poly (aa-ipn-aam+mma) has shown morphological variation due to cross-linking present in the gt-cl-poly (aa-ip-aam mma)+(fig. 2a,b). The SEM images certainly revealed morphological differences in the characteristics of gt and functionalized gt. The variation in surface morphologies taken place due to grafting of paa chains onto gt and further interpenetrating of paam and pmma chains in the gt-cl-paa matrix resulting in the formation of ipn hydrogels. The IPN hydrogel was more compact and stable through cross-linking with glutaraldehyde [29-32].
appearance of extra peaks and change in the intensity of the peaks confirmed the grafting of monomers onto gt.

Fig. 3(a, b): FT-IR spectrum of (a) gt and (b) gt-cl-poly(aa-ipn-aam+mma)

Thermal analysis
Two stages breakdown was found in the TGA of gt. The first stage breakdown occurred due to the subtraction of H₂O and impulsive molecules, whereas the second phase of breakdown was due to the polymer chains due to the de-polymerization reactions. The first phase breakdown appeared at a temperature from 196.9 °C-281 °C with 22.5% wt. loss 22.5%. The second phase breakdown occurred at 479.0 °C (fig. 4a). DTG of Gt showed breakdown at 75.9 °C (wt. loss 253.9 µg/min) and 285.3 °C (wt. loss 437.9 µg/min). DTA result gave exothermic peak at 292.1 °C (energy loss 11.2 µV) (fig. 4a).

It has been observed from the fig. 4b that TGA result of IPN showed early decomposition from 217.8 °C to 281.6 °C with 25.4% wt. loss. However, final decomposition occurred at 589.1 °C. The DTA results showed an exothermic peak at 586.1 °C (53.5µV) and this is in accordance with the TGA results. The DTG results showed decomposition at 269.8 °C and 586.2 °C with 356.3µg/min and 397.7µg/min weight loss, respectively. It is clearly indicated from the results that the thermal stability of synthesized IPN was higher as compared to gt[31, 32].

Fig. 4(a, b): TGA/DTA/DTG of (a) gt and (b) gt-cl-poly(aa-ipn-aam+mma)

Pantoprazole sodium liberation through gt-cl-poly(aa-ipn-aam+mma)
The result from one-way ANOVA and post hoc tests (table 1 and table 2) pointed that a less diffusion was observed in the drug release in the neutral medium. Similarly, no significant difference was found in neutral and acidic mediums. However, the drug release was higher in amount in basic medium. The drug entangled in the ipn was found to be released after the dissemination of the water from the ipn, which resulted in the distension of the ipn device and liberation of the imbibed drug.
molecules. The drug diffusion started at the IPN surface, after its swelling. So, drug release was linked to the swelling characteristics of the IPN matrix [22, 28]. IPN was observed to work as a useful device for systematic liberation of the drug. It is apparent from the Fig. 5a, that IPN showed the initial release 66.08 ppm after 30 min. with initial diffusion coefficient i.e. $D_I = 7.4$, in alkaline medium followed by 49.98 ppm at acidic medium i.e. (2.0 pH) $D_I = 5.3$ and least release 37.96 ppm at neutral pH ($D_I = 1.78$). Equilibrium was attained after 8 h with maximum release 1033.21 ppm followed by 959.65 ppm and 757.89 ppm at alkaline, neutral and acidic pH, respectively. The drug liberation kinetics showed that the drug liberation behavior increased with increased in pH. This behavior can be discussed on the fact that at acidic pH, –COOH groups present in unionized form, this leads to the distortion of the IPN matrix. As the pH increases –COOH ionize to –COO– and thus, keep away from one another leads to increase the pantoprazole sodium liberation from IPN device. Drug liberation showed non-fickian diffusion mechanism at alkaline pH, Case II diffusion at acidic and neutral pH (Table 3). This clearly indicated that in alkaline medium drug liberation rate is similar to the relaxation time of IPN device. Whereas, in acidic and neutral media, drug liberation was faster as compared to the relaxation time of the IPN. The value of gel characteristics constant i.e. ‘K’ was greater at alkaline pH, which revealed that highest drug liberation occurred at pH 9.2. $D_I$ has lesser value as compared to $D_L$, this showed slower drug liberation in the initial stage than the lateral stage [32]. All the experiments were carried-out in triplicate, so as to maintain the accuracy and reproducibility.

![Fig. 5(a-d): Effect of pH onto pantoprazole sodium release behavior through gt-cl-poly(aa-ipn-aam+mma), (a) conc. vs time, (b) ln(1-$M_t$/$M_\infty$) vs time, (c) $M_t$/$M_\infty$ vs t$^{1/2}$ and (d) ln$M_t$/$M_\infty$ vs ln t](image)

**Table 1: Oneway ANOVA model of the drug release**

| Source                   | Sum of squares | Df  | Mean square | F       | Sig.  |
|--------------------------|----------------|-----|-------------|---------|-------|
| Between Groups           | 544610.5       | 2   | 272305.8    | 4.755   | .012  |
| Within Groups            | 2355461        | 42  | 56082.426   |         |       |
| Total Groups             | 2900073        | 44  |             |         |       |

Values are expressed as mean (n=3). The drug release significantly varies with the pH level ($F = 4.76, P = 0.012$).

**Table 2: Post Hoc Test for the drug release behavior**

| (I)VAR00002 | (J)VAR00002 | Mean Difference(I-J) | Std. Error | Sig.  |
|-------------|-------------|----------------------|------------|-------|
| 1.0         | 2.00        | -60.421              | 86.43      | .76   |
| 1.0         | 3.00        | -257.680°            | 86.43      | .012  |
| 2.0         | 1.00        | 60.421               | 86.43      | .76   |
| 2.0         | 3.00        | -197.210             | 86.47      | .070  |
| 3.0         | 1.00        | 257.680°             | 86.47      | .013  |
| 3.0         | 2.00        | 197.210              | 86.47      | .070  |

Values are expressed as mean (n=3).
CONCLUSION

gt, paa and mma based crosslinked IPN were synthesized using lipase-glutaraldehyde as initiator-crosslinker system. FTIR analysis proved the successful graft co-polymerization of vinyl monomers onto gt. Moreover, thermal analysis showed that ipn were more stable than the pristine gt. The synthesized ipn possessed the desired swelling capacity required for the controlled drug delivery and was pH sensitive, when used in the systematic liberation of pantoprazole sodium at 37 °C. The result revealed that the Dl was higher as compared to D2, demonstrating that pantoprazole sodium liberation rate was larger in the lateral stages. The foregone discussion showed that the prepared ipn could be used as prospective carrier for prolonged drug delivery.

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AUTHORS CONTRIBUTIONS

Synthesis work was done by Dr Saruchi and Drug kinetics was done by Dr Vaneet Kumar

CONFlict OF INTERESTS

All authors have none to declare

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**Table 3: Diffusion exponent, Gel characteristic constant and diffusion coefficient of pantoprazole sodium release behavior through loaded gt-cl-poly (aa-ipn-aam+mma)**

| Sample                        | pH | Diffusion exponent ‘n’ | Gel characteristic constant ‘k’×10^-2 | Diffusion coefficient (m^2/hr)×10^-7 |
|-------------------------------|----|------------------------|--------------------------------------|--------------------------------------|
| gt-cl-poly(AA-ipn-AAm+MMA)    | 2.0| 0.886                  | 0.049                                | 5.350                                |
|                              | 7.0| 0.863                  | 0.064                                | 1.784                                |
|                              | 9.2| 0.868                  | 0.056                                | 7.400                                |

Values are expressed as mean (n=3). Where, D1, D2, and D3 are the initial, average and later diffusion coefficient respectively.
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