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Kinetic study and *in vitro* drug release studies of nitrendipine loaded arylamide grafted chitosan blend microspheres

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Abstract

Hypertension continues to be a significant public health concern because of its associated morbidity, mortality, and economic impact on society. By the year 2025, 1.56 billion individuals will have hypertension. Nitrendipine, an antihypertensive drug, is used in this present study. Hydrophilic matrices are capable of controlling the release of the drug over an extended period. Microsphere based sustained drug delivery systems will provide a suitable alternative to reduce the side effects and increase the dissolution rate of the drug compared to oral drug delivery. We prepared drug encapsulated microspheres of acrylamide grafted chitosan (hydrophilic polymers) by the free radical mechanism. This mechanism involves the use of an initiator, potassium persulfate. Microspheres were synthesized from the grafted copolymers using water in oil emulsion technique with a cross-linking agent. Differential scanning calorimetry, scanning electron microscopy, and Fourier transform infrared spectroscopy were used to characterize the sample. The microspheres at stomach media showed low drug release characteristics, while at the intestinal organ, the drug release prolonged for 14 h. From the drug release kinetic models, Higuchi and zero-order were found to be the best model for the three ratios based on the correlation coefficient. The diffusion component is less than 0.5, which indicates quasi-fickian diffusion. From the kinetic study results, we concluded that the formed acrylamide grafted chitosan microspheres deliver the drug through the diffusion mechanism. Nitrendipine loaded microspheres prepared using water in oil emulsion technique showed favorable sustained drug release behavior.

1. Introduction

Hypertension is a significant risk factor for cardiovascular, cerebrovascular, and renal complications. The increasing prevalence of hypertension and the continually increasing expense of its treatment influence the prescribing patterns among physicians and compliance to the treatment by the patients. Many national and international guidelines for the management of hypertension have published. In the olden days, people used diuretics as first-line drugs for hypertension therapy. However, the recent guidelines by the Joint National Commission (JNC8 guidelines) recommend both calcium channel blockers as well as angiotensin-converting enzyme inhibitors as first-line drugs, in addition to diuretics.

Studies show that researchers have used various antihypertensive drug combinations for effective long-term management and to treat comorbid conditions [1].

Calcium channel blockers demonstrate efficacy in reducing cardiovascular disease in hypertension equal to the other recommended agents, including thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers (ARBs) and β-blockers, but appear to have added efficacy for stroke prevention [2]. Calcium channel blockers, when combined in low doses with these recommended agents, lower BP more than is observed when any of these agents were used in isolation at a doubled dose [3]. In the current situation, nitrendipine, a water-insoluble antihypertensive drug, is given to hypertensive individuals in 20 mg
We obtained Nitrendipine from Sigma Aldrich, India. We purchased acrylamide, glutaraldehyde, hydrochloric acid, chitosan, n-hexane, light liquid paraffin, span 80, potassium persulfate from Techcraft Solutions Pvt. Ltd, Coimbatore, India. We used Deionized double distilled water. The chemical used here was Analytical grade.

2. Experiments

2.1. Materials
We obtained Nitrendipine from Sigma Aldrich, India. We purchased acrylamide, glutaraldehyde, hydrochloric acid, chitosan, n-hexane, light liquid paraffin, span 80, potassium persulfate from Techcraft Solutions Pvt. Ltd, Coimbatore, India. We used Deionized double distilled water. The chemical used here was Analytical grade.

2.2. Methods

2.2.1. Graft copolymer preparation
We prepared CS-g-AAm in the ratio 1:7 by Free radical polymerization [11]. We dissolved 0.5 g of chitosan in 40 ml of 2% Acetic acid. Then, we added a solution containing 3.5 g of acrylamide in double-distilled water. We added Potassium persulfate as an initiator to start the reaction and we stirred the contents. After an hour, we took the solution in a reaction flask. It was heated in a thermostat water bath at 60 °C for 2 h. Then reduce the solution temperature to room temperature, followed by the addition of an excess of acetone for precipitation. The precipitate was vacuum dried at 40 °C for 24 h.

2.2.2. Removal of homopolymer
During the grafting process, the formation of the homopolymer (Polyacrylamide) may be possible. Soxhlet extractor was used to remove polyacrylamide. We kept the obtained precipitate in Acetone-Water mixture for 24 h, then the precipitate is filtered and vacuum dried at 40 °C [12, 13].

2.2.3. Preparation of Chitosan graft Acrylamide microsphere
Oil (w/o) emulsion cross-linking method was used to prepare the microspheres [10, 12, 13] in different ratios of grafted copolymer and drug. We made an appropriate volume of the polymer solution by mixing 1:7 of the...
grafted copolymer and polyvinyl alcohol in double-distilled deionized water. We added three samples (8 mg) of drug dissolved in alcoholic solutions to this mixture. We combined a suitable emulsifier under constant stirring. The matrix was cross-linked after 10 min by the addition of the cross-linking agent. Then we allowed to stirred for 2 h. After that, it was filtered and washed with a suitable solvent to remove the unreacted cross-linking agent and the emulsifier.

2.2.4. Fourier transform infrared spectroscopy (FTIR)
FT-IR was recorded using Thermo Nicolet is10 FTIR spectrometer using KBr pellets in the range of 400–4000 cm\(^{-1}\).

2.2.5. Scanning electron microscopy (SEM)
SEM images of empty microspheres and microspheres with the drug were absorbed using SIGMA HV—Carl Zeiss with Bruker Quantax 200—Z10 EDS Detector at different magnification. We coated the sample with gold and deposited it with brass hold. The images were taken at room temperature and scanned at an accelerating voltage of 5 kV.

2.2.6. Differential scanning calorimetry (DSC)
DSC Thermogram of grafted microspheres with and without drug was recorded on DSC Q20 V24.10 Build 122 make differential scanning calorimeter between 25 °C–400 °C temperature at 10 °C min\(^{-1}\) under nitrogen atmosphere.

2.2.7. Drug release characteristics
We used the USP Dissolution rate test apparatus Type II (Electro Lab model TDT-08 L) for drug-release studies for the drug encapsulated microspheres for 14 h. We encapsulated 25 mg of nitrendipine in the grafted copolymers of three different ratios, and we prepared the encapsulated microspheres. Then it was placed in 600 ml of phosphate buffer dissolution medium. The tests were carried out in acidic medium for the first four hours, and the rest of the hours in the base medium at an agitating speed of 100 rpm maintained at room temperature \[12\]. We removed 5 ml of the sample regularly at the time interval of one hour, and simultaneously we added a fresh medium to maintain the constant volume. We determined the drug concentration of the sample by using a 160—UV—visible Shimadzu spectrophotometer at a suitable wavelength. We adopted a similar procedure to measure the drug release characteristics in an acidic buffer solution.

2.2.8. Kinetic study
We used drug release kinetics to mainly determine the drug release mechanism and best-fit drug release model. In this study, five kinetic models were used \[14\].

The equation for zero-order release is

\[
C = K_0 t
\]

Where \(C\) is the concentration of drug in mg/ml, \(K_0\) is zero-order release constant, and 't' is the time in hr. We determine the zero-order release constant by plotting the graph between cumulative % of drug release versus time.

\[
\log C = \log C_0 - \frac{kt}{2.303}
\]

Similarly, by plotting the graph between the log of cumulative % of drug release versus time, we determine the first-order release constant.

Hixson Crowell release equation

\[
\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = K_{HC} t
\]

Where \(KHC\)—Hixson Crowell release constant.

We determine the Hixson Crowell release constant by the Plot \(Q_0 - Qt\) versus time.

Higuchi equation

\[
Q = Kt^{1/2}
\]

Where \(KH\)-Higuchi constant

Korsmeyer—Peppas equation

\[
F = \left(\frac{M_t}{M_0}\right) = K_m t^n
\]

were
\[ F = \text{Fraction of drug released at the time } 't'. \]
\[ M_t = \text{Amount of drug released at the time } 't'. \]
\[ M = \text{Total amount of drug in the dosage form in mg} \]
\[ K_m = \text{Kinetic constant} \]
\[ n = \text{Diffusion or release exponent} \]
\[ t = \text{Time in hours} \]

We determine the release component and kinetic constant value by plotting the graph between a log of cumulative % of drug release versus log time in hours.

3. Results and discussion

3.1. Preparation of microspheres

The addition of polyvinyl alcohol, the linking agent, forms microspheres, which helps in binding of the drug. The microspheres provide active sites for the chitosan (Chitosan backbone has undergone grafting with acrylamide). The formed colloidal particle was not stable. The addition of emulsifier stabilizes. We developed the interpenetrating polymer network by the addition of the cross-linking agent, glutaraldehyde. Then the formed polymer network was washed with hexane to remove the emulsifiers and then washed with water and glycine to remove the unreacted cross-linking agent. By increasing the ratio of polymer and PVA, the solubility increases. It will absorb the media, swell and prolong the release time of the drug. The microsphere formation is mainly dependent on the stirring, reaction time, and the washing. Increase the polymer concentration slows down the stirring process, increases the reaction time, and activates the extended drug release. But it requires more amount of solvent for washing. So the drug-polymer ratio 1:4 should be considered as the optimum condition for the microsphere formation in our study [12, 13].

3.2. Differential scanning calorimetry

Differential scanning calorimetry was used to detect the dispersion of the drug in the microspheres. On analyzing the active pharmaceutical ingredient sample, we observed an endothermic peak at a temperature of 158 °C, as shown in figure 1. We also examined the microspheres encapsulated with a drug and without drugs. We observed a broad peak for microspheres without the drug, and a narrow ridge is seen for microspheres with the drug. There was no peak at 158 °C. And this is shown in figure 1. We observed from the curves that the drug dispersed inside the microspheres. For the release of antihypertensive drug nifedipine, similar results were seen [13].

3.3. Fourier-transform infrared spectroscopy

FTIR spectral analyses were carried out to confirm the presence of grafted copolymer placebo microspheres and drug-loaded microspheres. The peak at 3261 cm\(^{-1}\) and 1647 cm\(^{-1}\) represents the presence of primary amides of –NH stretching and antisymmetric –N–H bending, respectively, as shown in figure 2. We observed an Aliphatic –C–H stretching at 2922.36 cm\(^{-1}\) leads to the conformation of grafting reaction, as shown in figure 2. We also found that Nitrendipine peak and encapsulated drug peak are the same as shown in figure 2. Kulkarni et al report a similar result for the grafting of the AAm–g-chitosan microspheres [15] and by Basu et al, for nitrendipine loaded Eudragit RL 100 microspheres [16].
3.4. Scanning electron microscopy

Figures 3(a) and (b) shows the SEM images of grafted microspheres and drug encapsulated microspheres. In figure 3(a), the SEM image is more or less spherical, and the surface of the grafted microsphere is smooth. In contrast, the surface of the drug encapsulated microsphere is rough in figure 3(b), which indicates the presence of the encapsulated drug. The addition of cross-linking agent gluteraldehyde plays a vital role in the size of microsphere formation. Some uncrosslinked PVA found as the debris on the surface of the microspheres, as shown in figures 3(c) and (d) along with drug encapsulated microspheres. Ms et al, Cui et al and Basu et al observed a similar image of SEM [16–18]. The size of the microsphere before drug loading is in the range of 300–350 micron. After drug loading, it reduces to 25–30 micron as shown in the SEM image, this may be due to electrostatic interaction between drug molecule and the grafted co-polymer.
3.5. Drug release characteristics

The grafted polymer concentration and drug concentration are the two main parameters that affect the drug release studies. We understood the drug release profiles of nitrendipine from the drug encapsulated microspheres by carrying out the in vitro drug release experiments in the acid and base buffer solutions. Figure 4 indicates the drug release to be at a maximum of 38% from nitrendipine-loaded microspheres at pH 1.2 for 240 min. Figure 5 shows the drug release to be at a maximum of 79% at a pH of 6.8. By increasing the polymer concentration, release of drug from the microspheres decreases, as shown in figures 4 and 5. From our results, we concluded that 1:4 drug: polymer ratio shows the sustained release behavior compared to the other ratios. The conventional tablets show the maximum drug release of almost 95% within 10 h at a pH of 6.8[19]. When compared with the commercial tablets, the prepared microspheres shows the maximum release percentage up to 24 h because of the nature of the polymer. Similar results were observed [18, 20–22] for the sustained release of nitrendipine, metformin HCl, Ciprofloxacin, and Theophylline drugs.

3.6. Drug release kinetics

We applied the equation form of the five kinetic models - zero-order release model, first-order release model, Korsmeyer-Peppas model, Higuchi, and Hixson Crowell model to analyze the in vitro drug release data. We used this to determine the best-fit release kinetic model. We have drawn the graphs using excel sheets, and we tabulated the calculated R2 values, as shown in tables 1 and 2.

From tables 1 and 2, we concluded that dissolution data were best fitted to the zero-order and Higuchi model for all the ratios based on the correlation coefficient. The Korsmeyer-Peppas equation determines the drug release mechanism. The calculated n value is less than 0.45. So it follows quasi –fickian diffusion. We observed similar results for the sustained release of metformin HCl, nitrendipine, Ibuprofen, and nifedipine drugs [13, 14, 16, 20].
4. Conclusion

We prepared the water in oil emulsion method for sustained-release microspheres for the adequate encapsulation of nitrendipine. We characterized microspheres with and without the drug. Drug release studies indicated the controlled release of drugs up to 14 h. From this study, we concluded that 1:4 showed the proper sustained release behavior when compared to other ratios. Zero-order and Higuchi kinetic model considered to be the best fit model. An increase in polymer concentration shows a prolonged release of the drug. Based on the diffusion component value, the diffusion mechanism was found to be Quasi-diffusion. However, in-vivo studies have been carried out for the appropriate nitrendipine sustained release formulation.

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Table 1. Release Kinetics model of microspheres.

| Drug:polymer ratio | Peppas | Zero | First |
|-------------------|--------|------|-------|
|                   | $R^2$  | $n$  | $R^2$ | $k$  | $R^2$ | $k$  |
| 01:04             | 0.89   | 0.31 | 0.98  | 5.131| 0.76  | 0.23 |
| 01:07             | 0.91   | 0.3  | 0.97  | 5    | 0.78  | 0.2  |
| 01:10             | 0.9    | 0.26 | 0.96  | 4.8  | 0.789 | 0.232|

Table 2. Release Kinetics model of microspheres.

| Drug:polymer ratio | Crowell | Higuchi |
|-------------------|---------|---------|
|                   | $R^2$   | $K$    | $R^2$ | $K$ |
| 01:04             | 0.81    | 0.1    | 0.98  | 5.13 |
| 01:07             | 0.82    | 0.14   | 0.97  | 5    |
| 01:10             | 0.829   | 0.16   | 0.96  | 4.8  |
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