

PREPARATION AND CHARACTERIZATION OF NITRENDIPINE LOADED GRAFTED COPOLYMER MICROSPHERES: A PRELIMINARY STUDY

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ABSTRACT

Objectives: Developing countries like India suffers mostly from cardiovascular problems and kidney failure. An antihypertensive drug is mainly used to avoid the cardiovascular problems and kidney failure. Nitrendipine is a calcium antagonist widely used in the treatment of hypertension. The bioavailability of drug, when administered orally, is low. Sustained drug delivery systems are using microspheres emerging as an effective method to increase the bioavailability of the drug. The current work involves the encapsulation of nitrendipine - acrylamide grafted chitosan which helps to overcome poor solubility and hydrophobic nature of the nitrendipine. This will enhance the drug dissolution and reduce the side effects of the antihypertensive drug.

Methods: By emulsion cross-linking method, grafted copolymers were prepared. The physicochemical interactions between the drug and grafted copolymer were analyzed by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC).

Results: Encapsulated drug and grafted copolymer were characterized by FTIR to understand the chemical interactions and to identify the functional groups present. Morphology and particle size of the grafted copolymer with drug and without drug were assessed by SEM. DSC was used to identify the melting endotherms of a drug, copolymer cross-linked microspheres, and drug encapsulated microspheres.

Conclusion: Thus, the drug encapsulated grafted copolymer increases the hydrophilic character and thereby making the polymers feasible for functioning as drug carriers for sustained drug delivery system.

Keywords: Nitrendipine, Chitosan, Microspheres, Graft copolymerization.

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INTRODUCTION

In most of the countries, hypertension considers being an important public health problem due to its impact on the society. Cardiovascular, cerebrovascular, and renal complications mainly occur due to pressure variation. For managing the hypertension problem, a large number of national and international guidelines have been published. In addition to diuretics, recently, the Joint National Commission guidelines suggested that both calcium channel blockers as well as angiotensin-converting enzyme inhibitors considered to be the best drugs [1].

To reduce the cardiovascular disease related to hypertension, calcium channel blockers considered to be an effective antihypertensive drug compared to the other antihypertensive drugs. Calcium channel blockers also used to prevent stroke [2]. Combination of calcium channel blockers with other antihypertensive drugs reduces the blood pressure efficiently [3].

The drug delivery system plays an important role for the proper release of active pharmaceutical ingredient inside the human body to obtain a good treatment effect. To treat the diseases, sustained drug delivery systems are gaining importance nowadays. These delivery systems provide an alternative to parenteral drug delivery system. These include liposomes, proliposomes, microspheres, gels, prodrugs, cyclodextrins, and among others. Microsphere-based sustained drug delivery system will provide a suitable alternative to reduce the side effects and increase the dissolution rate of the drug. Angadi *et al.* 2010 observed the controlled release (CR) of an isoniazid drug with the grafted copolymer microspheres cross-linked with glutaraldehyde (GA). Polymers blend microspheres were prepared by the water-in-oil

emulsion technique for the sustained release of drug 5-fluorouracil [4]. Phadke *et al.* 2015 prepared the novel pH-sensitive microspheres with the grafted copolymer for the sustained release of drugs.

In the current situation, daily intake of nitrendipine, a water-insoluble antihypertensive drug in the oral tablet form is 20 mg [5]. This amount reduces the blood pressure by 15–20% within 1–2 h of administration [5]. Nitrendipine has oral bioavailability between 10% and 20% because of high first-pass metabolism. CR of drugs using polymers is a well-established technology. This technology is mainly used to encapsulate drug with the polymers and the release of drug from the polymer matrix takes place in a predetermined manner [6–8]. Recently, many researchers considered micro/nanoparticles derived from the biopolymers as the potential drug delivery devices compared to the conventional type dosage forms [9,10].

Hydrophilic matrices have the capability to control the release of drug over an extended period. Hydrophilic polymers are frequently used for the sustained release of drugs. Recently, natural polymers, because of its low cost and extensive sources, have been receiving considerable attention. Chitosan (CS), a natural polymer obtained through alkaline deacetylation of chitin. Due to the hydrophilicity and biocompatibility of the polymer, it finds application in various fields [8,11]. High water retention property of acrylamide (AAM) made it suitable for its usage as biomaterials. They have been extensively used for biomedical applications, such as drug delivery systems [12]. Here, we considered two hydrophilic biodegradable polymers CS and AAM.

Therefore, the objective of the work is to discuss the encapsulation of the drug with the hydrophilic grafted copolymer for the sustained

release of nitrendipine. This is the preliminary study attempted first on the usage of CS grafted AAM copolymer for the nitrendipine, an antihypertensive drug.

METHODS

Nitrendipine was purchased from Sigma Aldrich, India. CS, AAM, acetic acid, potassium persulphate, acetone, polyvinyl alcohol, light liquid paraffin, hydrochloric acid (HCl), n-hexane, and glycine were purchased from Techcraft solutions, India. Span 80 and GA used were purchased from Modern Scientific Company, India. Water used was deionized and double distilled. All the chemicals used were of AR grade.

Preparation of copolymer

Graft copolymerization of CS with AAM was carried out at different ratios 1:3, 1:5, and 1:7. In 160 ml of 4% acetic acid solution, 2 g of CS was dissolved [13]. Then, a solution of 14 g of AAM in double-distilled and deionized water was added, where the ratio of CS and AAM is 1:7. The initiator potassium persulfate was added to the mixture and the mixture was stirred at room temperature for 1 h. Then, the solution was taken in a reaction flask and it was further heated in thermostat water bath at 60°C for 2 h. After that, it was cooled to the ambient temperature followed by the addition of excess amount of acetone for precipitation. The insoluble product was filtered. Then, the filtered product was vacuum dried at 40°C. The grafting % was calculated using the following equation,

$$\text{Grafting \%} = \frac{W_g - W_o}{W_o} * 100$$

where w_o and w_g denote the mass of the CS and grafted CS with AAM.

Extraction of homopolymer of AAM

During the grafting process, the formation of homopolymer may be possible. Soxhlet Extractor was used to remove the homopolymer formed during the reaction. The solution mixture of acetone-water was used in the extractor for 30 h to remove the homopolymer from the precipitate.

Preparation of blend microspheres

Equal amounts of grafted copolymer and polyvinyl alcohol (PVA) were added with double distilled deionized water to form the 20 ml of 6% (w/v) polymer solution [13]. A small volume of alcohol was added to this mixture and stirred it to get the homogeneous solution. Light liquid paraffin containing 1% (w/w) Span 80 was slowly added to emulsify the solution under constant stirring. To crosslink the matrix, GA was added along with HCl after 10 min. For the complete formation of microspheres, the mixture was stirred continuously for 2 h and rigid microspheres were filtered. Suitable solvent was used to remove light liquid paraffin and Span 80 from the formed microspheres. Microspheres were again

washed with glycine and water simultaneously to remove the inactive GA. Then, they were dried at low pressure for 24 h at 40°C.

Fourier-transform infrared spectroscopy (FTIR)

FTIR of chitosan-grafted-acrylamide (CS-g-AAM) grafted copolymer microspheres and drug-encapsulated CS-g-AAM microspheres were measured using Thermo Nicolet is 10 FTIR spectrometer. FTIR spectra of samples were analyzed between the wavelength of 500 and 4000 cm^{-1} using KBr pellets at room temperature.

Scanning electron microscopy (SEM)

Images of SEM were recorded using a scanning electron microscope to examine the surface morphology of the empty microspheres of grafted copolymer cross-linked with GA and drug-loaded microspheres of grafted copolymer cross-linked with GA. The microspheres of grafted copolymer and drug encapsulated grafted copolymer microspheres were deposited on a brass holder and sputtered with a thin coating of gold, under vacuum. At room temperature with appropriate magnification, the SEM image was observed.

Differential scanning calorimetry (DSC)

DSC was used to measure the amount of heat absorbed or released during physical transformation such as phase transitions. Nitrendipine, CS grafted AAM cross-linked microspheres, and drug encapsulated grafted copolymer microspheres were analyzed by DSC Q20 V24.10 Build 122 at a heating rate of 10°C/min from 0 to 400°C under a constant flow of nitrogen gas.

RESULTS AND DISCUSSION

Grafting %

Free-radical polymerization reaction was obtained in the presence of potassium persulfate for graft copolymerization of CS-g-AAM. The complex formation between $-\text{NH}_2$ and $-\text{OH}$ groups of CS decomposed to generate the free radical sites at 60°C. This facilitates the reaction site on the CS backbone for AAM monomer. At three different monomer concentration used in this study as shown in Table 1, grafting percentage of 90.96 % was obtained for the 1:7 ratio of CS and AAM. This ratio was used for further preparation.

Preparation of microspheres

PVA was blended with the grafted product of 1:7 and microspheres were prepared. To stimulate the active groups of grafted CS, PVA mixed with grafted copolymer. Then, only crosslinking with GA should be effective. Sodium alginate coating was used to improve the hydrophilic nature of microspheres. Many researchers [4,13–19] obtained the similar results and the formed microspheres were used as the controlled drug release devices.

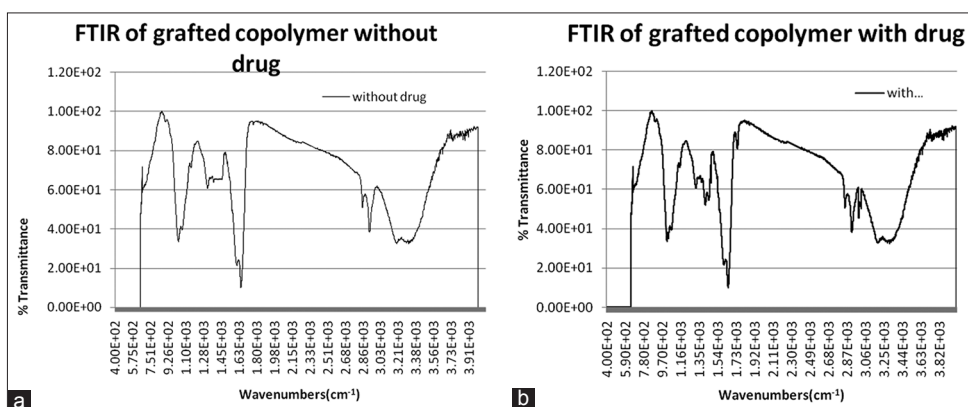


Fig. 1: (a) Fourier-transform infrared spectroscopy (FTIR) of grafted copolymer microspheres. (b) FTIR of drug encapsulated grafted copolymer microspheres

FTIR

From the IR spectra, the characteristic peak around 3312 and 2922 cm^{-1} corresponds to the stretching of O-H and aliphatic C-H group of CS. CS grafted AAM shows the additional peak at 3193 cm^{-1} denotes the bonded NH stretching vibrations and antisymmetric NH bonding at 1653 cm^{-1} due to the primary amides. From these spectra, we conclude that AAM grafted properly on the CS as shown in the Fig. 1a. In the IR spectra of the nitrendipine encapsulated CS grafted AAM copolymer as shown in the Fig. 1b. The peak at 2970 cm^{-1} specifies the CH stretching and the peak formed between 1700 and 1720 cm^{-1} denotes the carbonyl ester vibration. The principal peaks for nitrendipine appeared between 1020 and 1329.56 cm^{-1} indicate the presence of carboxyl and carboxylate groups as well as NO group between 1349.56 and 1500.34 cm^{-1} . Similar results were obtained for the drug encapsulated microspheres with different polymers [15].

Table 1: % Grafting of different ratios of grafted copolymer

Type of CS-g-AAM	Mass of CS (g)	Mass of AAm (g)	% Grafting
P1	1	3	68.9
P2	1	5	52.6
P3	1	7	90.96

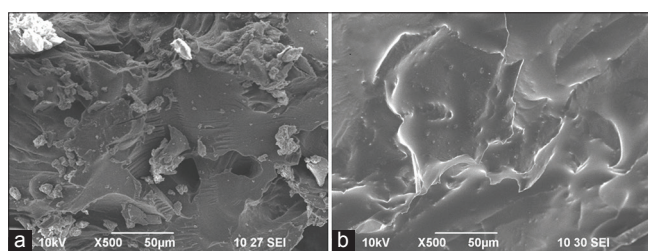


Fig. 2: Scanning electron microscopy image of the (a) surface of the empty microspheres and (b) surface of the drug-loaded microspheres

SEM

Shape and surface morphology of the microsphere formation were conformed from the SEM images. SEM image of the surface of the empty microspheres and surface of the drug-loaded microspheres is shown in the Fig. 2a and 2b. Smooth surface was observed in the empty grafted copolymer cross-linked microspheres as shown in the Fig. 2a, whereas nitrendipine loaded microspheres showed an irregularity in the surface as shown in the Fig. 2b. For the controlled release of Nitrendipine microspheres loaded with different polymers and additives, Nifedipine interpenetrating network microsphere blended with grafted copolymers, Theophylline and isoniazid IPN microspheres encapsulated with hydrophilic polymers and chitosan sodium alginate carbamazepine microspheres shows a smooth surface and rough surface for the unloaded and drug loaded microspheres [13-20]. From the result, we observed that the formed microsphere is suitable for the sustained release studies.

DSC

As shown in the Fig. 3a, the sharp endothermic peak at 158.61°C indicates the presence of a crystalline form of the drug. The glass transition temperature of CS grafted AAM increased to 219°C with respect to the pure CS due to the increase in the AAM chain length of the polymer as shown in the Fig. 3b [13]. No peak was observed at the endothermic peak of nitrendipine as shown in the Fig. 3c of nitrendipine loaded microspheres. From this, we observed that amorphous form of drug exist in the microspheres and it was dispersed in the grafted copolymer. Rokhade *et al.* observed a sharp peak at 277°C from the DSC of theophylline which indicates the melting of the drug. For the drug-loaded microspheres, due to endothermic transitions, three peaks were observed at 41°C, 74°C, and 181°C from the DSC. No peak was observed at 277°C which represents the amorphous dispersion of theophylline into matrix [19]. Similarly, Angadi *et al.* obtained the DSC thermogram for placebo, drug, and drug-loaded microspheres [18]. Fude *et al.* and Basu *et al.* obtained the sharp peak from DSC for the pure drug, drug loaded with polymer showed the peak at various temperature which shows the dispersion of the drug [15,20]. From this preliminary study, we observed that grafted copolymer encapsulated with drug will be suitable for the sustained release of drugs compared with the existing literature [15-17,20].

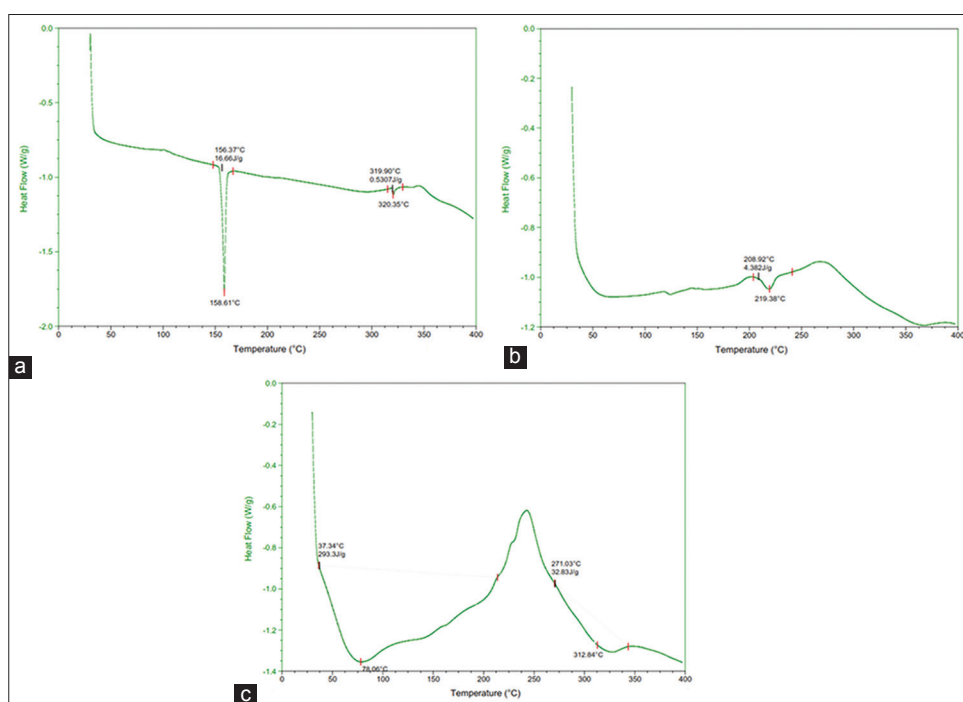


Fig. 3: (a) Differential scanning calorimetry (DSC) curve of pure nitrendipine drug. (b) DSC curve of grafted copolymer microspheres. (c) DSC curve of drug encapsulated grafted copolymer microspheres

CONCLUSION

Emulsion cross-linking method was used to prepare microspheres from grafted copolymer mixed with PVA and cross-linked with GA. From the characterization, we conclude that drug properly encapsulated with grafted copolymers in the form of microspheres. This drug encapsulated copolymer should be used for the sustained release of drugs. Drug release studies have to be carried out for the obtained microspheres by varying the amount of polymer, cross-linking agent, and drug.

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CONFLICTS OF INTEREST

All authors have none to declare.

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