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Research Article

Preparation and Characterization of Chitosan Based Polyelectrolyte Complex as a Carrier of Aceclofenac

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Abstract

The influence of polyelectrolyte complexes composed of chitosan and pectin on the release behaviour of aceclofenac has been investigated. Polyelectrolyte complexes between chitosan and pectin were prepared at different ratios by mixing solutions of chitosan and pectin with same ionic strength. The drug entrapment efficiency of these polyelectrolyte complex microparticles was found 30.29±1.82% to 77.64±1.85% and their average particle sizes were ranged from 440.75 ± 28.54 to 548.73 ± 41.34 µm. FT-IR spectra were analysed to study the degree of interactive strength between polyions. The *in vitro* drug release from these aceclofenac-loaded chitosan-pectin polyelectrolyte complex microparticles showed sustained release of aceclofenac over 8 h and followed the Korsmeyer-Peppas model (R^2 = 0.9832–0.9856) with anomalous (non-Fickian) diffusion drug release mechanism.

Keywords: Chitosan, Pectin, Aceclofenac, Polyelectrolyte complex, Sustained release

1.Introduction

Polyelectrolyte complexes are generally formed by electrostatic interaction between two oppositely charged polyelectrolyte solutions.[1, 2] These complexes exhibit unique physical and chemical properties, as the electrostatic interactions within the polyelectrolyte complex gels are considerably stronger than most secondary binding interactions. Many studies have been done to use polyelectrolyte complex of chitosan and polyanions for pharmaceutical applications.[3, 4]

Chitosan is a cationic biocompatible and biodegradable natural polysaccharide obtained by alkaline deacetylation of chitin.[5] It is composed of α-1,4-linked 2 - amino- 2-deoxy- α- D-glucose (N-acetyl glucosamine). It is a FDA GRAS (Generally Recognized as Safe) material and has been widely utilized in many applications including drug delivery, tissue engineering and food technology. Chitosan have been used in the development of various pharmaceutical formulations.[6] Although chitosan is a very promising biopolymer for use as carrier material in drug delivery systems, it has a limited capacity for controlling drug release from oral dosage forms due to its fast dissolution in the stomach. To overcome this disadvantage chemical modification of chitosan has been employed in the development of drug delivery systems.[7] Among them, chitosan-based polyelectrolyte complexes were reported in very few literatures for the use in drug delivery applications.

Pectin is a natural, non-toxic and anionic polysaccharide extracted from cell walls of most plants. Pectin consists mainly of linearly connected α-(1-4)-D-galacturonic acid residues partially esterified with methanol. Functional properties of pectin are derived from their molecular weight distribution and from the degree of methoxylation of carboxyl groups. [8, 9]

To achieve better drug delivery, Polyelectrolyte complex (PEC) between pectin as an anionic polyelectrolyte and chitosan as a cationic species was prepared using aceclofenac as a model drug. Aceclofenac is chemically 2-[(2´,6´-dichlorophenyl) amino] phenyl acetoxyacetic acid, as a non-steroidal anti-inflammatory drug (NSAID) with short half-life (4 h) indicated for the symptomatic treatment of pain and inflammation.[10] It is also used in the treatment of arthritis, osteoarthritis and rheumatoid arthritis.[11] Aceclofenac is reported to produce side effects like gastric irritation, ulcer, particularly diarrhoea, nausea, abdominal pain and flatulence, etc. as result of prolong treatment.[11, 12] Due to its short half-life, its recommended dose is considered as 200 mg daily in divided doses. To reduce dosing frequency and adverse effects during prolong aceclofenac treatment, sustained aceclofenac release at a slow rate over an extended period is essential. Therefore, in this present study, the attempt to design aceclofenac-loaded chitosan-pectin polyelectrolyte complex for prolonged aceclofenac release was investigated.

2. Materials and Methods

2.1. Materials

Aceclofenac was received as a gift sample from Drakt Pharmaceutical Pvt. Ltd., India. Chitosan was commercially purchased from Everest Edward, India. Pectin was purchased from Sisco Research Laboratories Pvt. Ltd., India. Glutaraldehyde was procured from Loba Chemie, India. All chemicals, and reagents used were of analytical grade.

2.2. Preparation of aceclofenac-loaded chitosan-pectin complex

1% (w/v) pectin solutions were prepared using distilled water and 0.8% (w/v) chitosan solutions were prepared by dissolving in 1% (v/v) aqueous glacial acetic acid solution. Then, pectin solutions were added in the prepared chitosan solution with continuous stirring until a homogeneous solution was obtained. The required amount of aceclofenac was added to the chitosan-pectin blend solution and allowed to stir for 1 h. After that, the pH of the chitosan-pectin solution containing aceclofenac was adjusted to pH 5.5 using 0.2 M sodium hydroxide solution. Then glutaraldehyde was added to the mixture solution and stirred for 1 h. The solution is centrifuged at 5000 rpm for 20 min. The precipitate were then separated by filtration process and washed with glycine and double distilled water to remove unreacted glutaraldehyde. The complete removal of unreacted glutaraldehyde was confirmed by the aldehyde negative test of the washings with Fehling's reagent. Then the precipitate were dried at 40°C for overnight and stored in desiccators until further use. The similar method was adopted for the preparation of blank complex without the addition of aceclofenac. Different aceclofenac-loaded chitosan-pectin complex formulations along with amounts of chitosan, pectin, aceclofenac and glutaraldehyde are enlisted in Table 1.

2.3.Characterization of aceclofenac-loaded chitosan-pectin complex microparticles

2.3.1. Particle size measurements

The size of the prepared complex microparticles was measured by using digital slide callipers (CD-6"CS, Mitutoyo Corporation, Japan).

100 particles were taken and inserted in between the space of two metallic plates. Diameters of resultant particles were displayed in the digital screen of the previously calibrated equipment.

2.3.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of chitosan, pectin, pure aceclofenac and aceclofenac-loaded chitosan-pectin polyelectrolyte complex were obtained using FTIR spectrophotometer (Perkin Elmer Spectrum RX I, USA). Each sample was grounded and mixed with dry potassium bromide (KBr). The mixture was compressed into pellets in a hydraulic press at a pressure of 100 kg/cm^2 for 15 min. Each pellet was scanned at 2mm/s speed over the wave number range of 400–4000 cm^{-1}. The characteristic peaks were recorded for each of the sample.

2.3.3. Estimation of drug entrapment efficiency

Accurately weighed 10 mg of complex microparticles containing aceclofenac from each formulation batch were taken separately and kept suspended in 50 ml phosphate buffer, pH 7.4 for 48 h under continuous stirring and sonicated for 20 min using a sonicator (Frontline Sonicator, FS-600, Frontline Electronics and Machinery Pvt. Ltd., India) followed by centrifugation at 2000 rpm for10 min. Then, the supernatant fraction was further filtered through Whatman® filter paper (No. 40). The drug (aceclofenac) content in the filtrate was determined using a UV–VIS spectrophotometer (Shimadzu, Japan) by measuring absorbance at λ_{max} of 274 nm. The drug entrapment efficiency of microparticles was calculated using this following formula: Drug entrapment efficiency (%) = (Actual drug content/Theoretical drug content) × 100.

2.3.4. In vitro release study

In vitro drug release from these prepared complex microparticles was evaluated using dialysis bag diffusion technique. Accurately weighed quantities of complex microparticles containing aceclofenac equivalent to 20 mg aceclofenac were placed in one end of the dialysis bag (Cellophane membrane, molecular cut off 10-12kDa, Hi-Media, India). Drug release study was evaluated in simulated gastric pH 1.2 for 2h. After that, drug released study was performed in phosphate buffer (pH 7.4) contained in the USP type II dissolution apparatus (Veego VDA-6D, Veego Instruments Co-operation, India). The system was maintained at 37 ± 1°C under 100 rpm speed. The dialysis bag acts as a donor compartment, and the vessel of dissolution apparatus acts as the receptor compartment. 5 ml of aliquots was collected at regular time intervals, and the same amount of fresh dissolution medium was replaced into dissolution vessel to maintain the sink condition throughout the experiment. The collected aliquots were filtered, and suitably diluted to determine the absorbance using a UV–VIS spectrophotometer (Thermo SpectronicUV-1, USA) by measuring absorbance at λ_{max} of 274 nm.

2.3.5. Analysis of in vitro drug release kinetics and mechanism

In order to predict and correlate the *in vitro* release behaviour of drug from formulated chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac, it is necessary to fit into a mathematical model.The *in vitro* release data were evaluated kinetically using various important mathematical models like zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models.[13,14]

Zero-order model: $Q = kt + Q_0$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

First-order model: $Q = Q_0 e^{kt}$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant.

Higuchi model: $Q = kt^{0.5}$; where Q represents the drug released amount in time t, and k is the rate constant.

Hixson-Crowell model: $Q^{1/3} = kt + Q_0^{1/3}$; where Q represents the drug released amount in time t, and Q_0 is the start value of Q; k is the rate constant. Korsmeyer-Peppas model: $Q = kt^n$; where Q represents the drug released amount in time t, k is the rate constant and n is the diffusional exponent, indicative of drug release mechanism.

Again, the Korsmeyer-Peppas model was employed in the *in vitro* drug release behaviour analysis of these formulations to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous transport), and case-II transport (relaxation-controlled release).[15]

2.3.6. Statistical analysis

All measured data are expressed as mean ± standard deviation (S.D.). The simple statistical analyses were conducted using Med-Calc software version 11.6.1.0.

3.Results and Discussion

In the present study, efforts have been made to prepare aceclofenac loaded chitosan-pectin polyelectrolyte complex microparticles. The formulations were prepared by using different ratios of pectin and Chitosan. Polyelectrolyte complex (PEC) was formed between anionic pectin and polycation chitosan. The reaction is:

$$P\text{-}COOH + C\text{-}NH_3^+ \xrightarrow{pH\ 5.4} \underset{\text{polyelectrolyte complex}}{P\text{-}COO^-\ {}^+NH_3\text{-}C} + H^+$$

Table 1 indicates Entrapment efficiency data of all the formulations of aceclofenac loaded chitosan-pectin polyelectrolyte complex. Entrapment efficiency was expressed as the percentage of aceclofenac entrapped in these prepared chitosan-pectin polyelectrolyte complex compared to the initial amount of aceclofenac included in the formulation. The drug entrapment efficiency of these particles was achieved 30.29±1.82% to 77.64±1.85%. Highest drug entrapment efficiency was found in case of formulation F-4, prepared using chitosan and pectin in the ratio of 2:2.

Table 1. Formulation chart of chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac with entrapment efficiency and particle size

Formulation code (ml)	Chitosan (mg)	Pectin (mg)	Aceclofenac (mg)	Glutaraldehyde (ml)	Entrapment efficiency (%)[a]	Particle size (µm)[b]
F-1	400	-	100	1	30.29±1.82	548.73 ± 41.34
F-2	300	100	100	1	38.45±1.44	511.18 ± 31.44
F-3	100	300	100	1	54.76±1.68	468.42 ± 36.69
F-4	200	200	100	1	77.64±1.85	440.75 ± 28.54

[a]*Mean ± S.D.; n = 3;* [b]*Mean ± S.D.; n = 100*

The particle size of aceclofenac-loaded chitosan-pectin polyelectrolyte complex microparticles were measured and found within the range, 440.75 ± 28.54 to 548.73 ± 41.34 µm (Table 1). Fig.1 indicates Comparative particle size distribution of various aceclofenac loaded chitosan-pectin polyelectrolyte complex microparticles. From Fig. 1 it is clearly observed that microparticles, which are prepared by using chitosan: pectin in the ratio of 2:2 (F-4) having the particles in smaller size range than the formulation prepared using the chitosan: pectin in the ratio of 3:1(F-2), chitosan: pectin in the ratio of 1:3 (F-3) and chitosan (F-1). This may be due to the formation of more rigid polymer network in formulation containing chitosan: pectin in the ratio of 2:2.

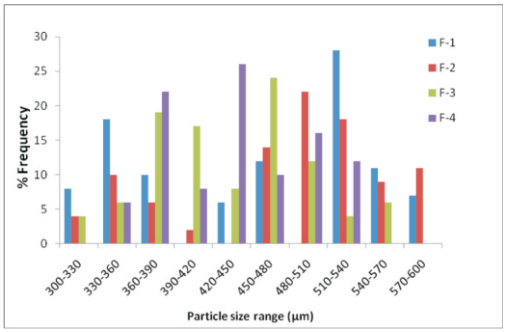

Fig. 1. Comparative particle size distribution of various aceclofenac loaded chitosan-pectin polyelectrolyte complex microparticles

FTIR spectra of aceclofenac, chitosan, pectin and aceclofenac-loaded chitosan-pectin polyelectrolyte complex are shown in Fig. 2. The FTIR spectra of aceclofenac showed principal characteristic peaks at 3028 cm^{-1} due to aromatic -C-H stretching vibrations and 2937 cm^{-1} due to aliphatic C-H stretching vibrations, a band at 1717 cm^{-1} due to C=O stretching, a sharp band at 1772 cm^{-1} due to C=O stretching of carboxylic acid, band at 3320 cm^{-1} due to secondary N-H rocking vibrations, and two sharp peaks at 716 cm^{-1} due to 1, 2 di-substituted C-Cl stretching. In case of chitosan, a broad band was observed at 3433 cm^{-1} due to N-H stretching. Two characteristics bands were observed at 2923 and 2812 cm^{-1} represent the presence of C-H aliphatic stretching vibrations. Three characteristic bands also were appeared at 1650, 1592, and 1381 cm^{-1} due to amide- I, amide-II, and amide-III, respectively. Pectin showed the typical C=O band of methyl ester group at 1752 cm^{-1} and C=O band of carboxylate at 1610 cm^{-1}. The shift in amine band of chitosan to 1565 cm^{-1} in the spectrum of complexes indicates a change in the environment of amino group through its interaction with pectin. The intensity of esterified carboxyl group at 1752 cm^{-1} in the pectin decreased and shifted to a lower frequency, i.e. 1740 cm^{-1} following chitosan -pectin complexation. A strong peak at 1617 cm^{-1} (asymmetric stretching of carboxylate) appeared and indicated the formation of intermolecular ionic bonds, *i.e.,* the carboxyl group of pectin and amino group of chitosan. In fact the complexes were stabilised by electrostatic interaction between positively charged chitosan (NH_3^+) and negatively charged pectin (COO^-).

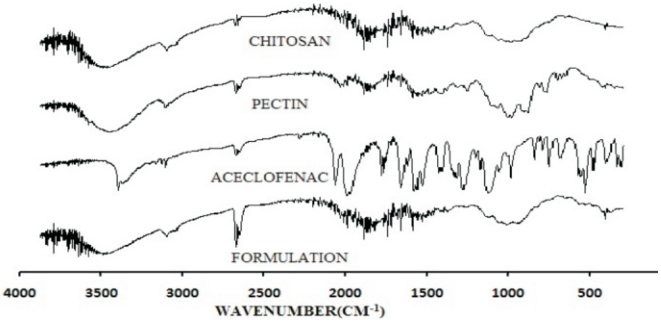

Fig. 2. FTIR spectra analysis of chitosan, pectin, pure aceclofenac and aceclofenac-loaded chitosan-pectin polyelectrolyte complex

In vitro drug release from these prepared aceclofenac-loaded chitosan-pectin polyelectrolyte complex was evaluated using dialysis bag diffusion technique in gastric pH 1.2 for 2h (Fig. 3a) and phosphate buffer, pH 7.4. The cumulative percentage drug release from aceclofenac-loaded chitosan-pectin polyelectrolyte complex was found sustained over a period of 8h (Fig. 3b). The percentage drug released from aceclofenac-loaded chitosan-pectin polyelectrolyte complex in 8h was within the range of 70.54±2.15 (F-4) to 91.44±2.32% (F-1). The lowest drug release was obtained with chitosan: pectin 2:2 in F-4. The higher degree of interaction between chitosan and pectin was responsible for sustained release of aceclofenac.

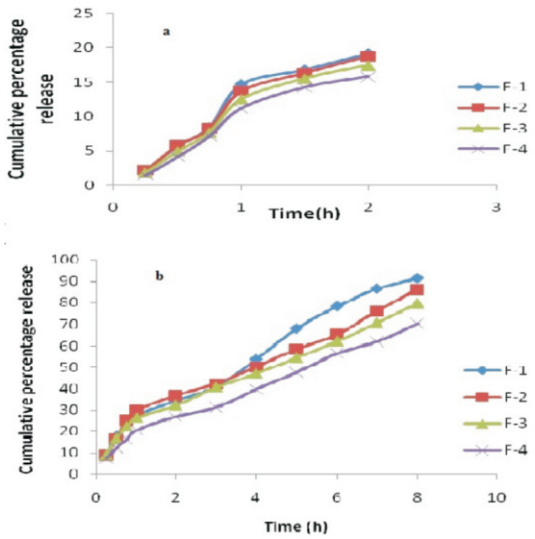

Fig. 3. The *in vitro* drug release from various chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac in (a) gastric pH1.2 and (b) phosphate buffer pH7.4

Table 2. Results of curve fitting of the *in vitro* release profile of chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac

Formulation code (ml)	Zero order model (R^2)	First order model (R^2)	Higuchi model (R^2)	Hixson–Crowell model (R^2)	Korsmeyer-Peppas model	
					R^2	*n*
F-1	0.8512	0.9136	0.9476	0.8468	0.9856	0.64
F-2	0.8846	0.8878	0.9254	0.9694	0.9832	0.53
F-3	0.9362	0.9448	0.8672	0.9674	0.9848	0.61
F-4	0.8534	0.9488	0.9278	0.9588	0.9836	0.76

The *in vitro* drug release data from various aceclofenac-loaded chitosan-pectin polyelectrolyte complex microparticles were evaluated kinetically using various important mathematical models like zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models. The R^2 values of these models were determined for evaluation of accuracy. The result of the curve fitting into various mathematical models is given in Table 2. When the respective R^2 of these aceclofenac-loaded chitosan-pectin polyelectrolyte complex microparticles were compared, it was found to follow the Korsmeyer-Peppas model (R^2 = 0.9832-0.9856) over a period of 8h. The value of release exponent (n) determined from in vitro aceclofenac release data of various aceclofenac-loaded chitosan-pectin polyelectrolyte complex microparticles ranged from 0.53 to 0.76, indicating anomalous (non-Fickian) diffusion mechanism for drug release. The anomalous diffusion mechanism of drug release demonstrates both diffusion controlled, and swelling controlled drug release.

4. Conclusion

This investigation verified the formation of polyelectrolyte complexes between chitosan and pectin for prolonged aceclofenac release. These complex microparticles had average particle size of 440.75±28.54 to 548.73±41.34µm. The drug entrapment efficiency of these chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac was found within the range of 30.29±1.82% to 77.64±1.85%. The electrostatic interaction between positively charged chitosan (NH_3^+) and negatively charged pectin (COO^-) following complexation and stability of aceclofenac in the complex were confirmed using FTIR analysis. The *in vitro* dissolution of chitosan-pectin polyelectrolyte complex microparticles containing aceclofenac showed sustained release of aceclofenac over 8h. The kinetics of in vitro drug release profiles has been studied by computing R^2 values of various important mathematical models that suggested to follow the Korsmeyer-Peppas model (R^2= 0.9832-0.9856) with anomalous (non-Fickian) diffusion drug release mechanism. These results clearly confirmed the ability of these newly developed chitosan-pectin polyelectrolyte complex containing aceclofenac for the maintenance of prolonged activity through sustained release of aceclofenac.

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Conflicts of Interest

The authors report no conflict of interest.

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