Formulation and Evaluation of Coated Chitosan Microspheres of Prednisolone for Colon-Targeted Drug Delivery

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ABSTRACT

Prednisolone is a synthetic glucocorticoid, a derivative of cortisol, which is used to treat a variety of inflammatory and auto-immune conditions. The purpose of this research was to formulate and evaluate coated microspheres of prednisolone for colon targeting. Chitosan is used as polymer, which is a biodegradable polymer and undergo enzymatic degradation in the colon, so it is used as carrier to deliver the drug in the colon. Chitosan microspheres were prepared by ionotropic gelation method using different ratios of chitosan and sodium tripolyphosphate. Eudragit S100 coating of chitosan microspheres was performed by solvent evaporation method using coat: core ratio (5:1). The cumulative percentage drug release of chitosan microspheres and eudragit S100 coated nanoparticle (F2) after 12 hrs at different pH was found to be 89.12 and 89.45 respectively. Among the formulations F2 was found to be the best formulation. The particle size and zeta potential of optimized formulation F2 microspheres were found 166.2 nm and -40.5 mV respectively. The results showed that as the amount of the polymer increased the extent of drug release decreased. It was concluded from the present investigation that Eudragit coated chitosan microspheres are promising controlled release carriers for colon targeted delivery of prednisolone.

Introduction

Prednisone is a glucocorticoid that is extensively used in clinical practice [1]. Its therapeutic use is recommended for a variety of acute and chronic diseases, such as arthritis, hepatitis, allergic diseases, asthma, leprosy and numerous other autoimmune and inflammatory diseases [2]. Prednisone has the molecular formula $C_{21}H_{25}O_5$ and a molar mass of 358.43 g/mol [3]. It is an odourless white or almost white powder that melts at 233°C with decomposition and may exhibit polymorphism. At room temperature, this drug is poorly soluble in methanol, ethanol,

chloroform and dioxane with low solubility in water [3]. Prednisone is a neutral molecule and its pKa values are not reported in the literature. Its solubility in water is 0.133 mg/ ml at 25°C and three values for the partition coefficient (Log P) are reported in the literature: 1.46, 1.47 and 1.6 [4, 5]. Commercially active pharmaceutical ingredient prednisone is an anhydrous, monohydrate or prednisone acetate salt. According to parameters adopted by the International Pharmaceutical Federation (FIP), prednisone is classified as a class I drug of the biopharmaceutical classification system (BCS) and is highly soluble and permeable [6]. However, Vogt *et al*. (2007) [4] argued in a review study that in the absence of conclusive data on permeability, absorption and solubility under the conditions recommended by the BCS, this drug does not have a classification defined in relation to the BCS. It is mainly used to treat acute attacks in both ulcerative colitis and Crohn's disease. The past studies have shown that eight out of ten people with such attacks respond to treatment with steroids [7]. They can be given by injection, as tablets, or topically. On oral administration or parenteral administration, drug is distributed into whole body and very less concentration will reach to the colonic site. For the treatment of colon associated disease, hence to improve drug concentration at colon, large amount of drug is to be administered, either via oral or parenteral route. This may cause toxicity and side effects of the drug. Though steroids are naturally present in the body, still higher doses are needed to control inflammation which can have unwanted effects on the body [8]. Corticosteroids have several side effects such as weight gain, moon face, development of acne, and increase in blood sugar level, retention of salt which leads to increased blood pressure etc. These unwanted effects usually disappear when the dose is reduced. Therefore, it's a challenge to achieve greatest possible benefits with fewest side effects [9]. Hence to achieve this challenge coated microspheres drug delivery system was formulated which can considerably reduce the side effects of the drug prednisone. Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μm range in diameter having a core of drug and entirely outer layers of polymers as coating material. They are made up of polymeric, waxy or other protective materials i.e. biodegradable synthetic polymer and modified natural products such as starches, gums, proteins, fats and waxes. However, the success of these microspheres is limited due to their short residence time at site of absorption [10]. The presence of polymers in sustained release drug delivery systems is important, because almost all of the system using the polymer as a carrier. Some time ago, polymers are divided into three major groups that are soluble polymers, biodegradable polymers or bioerodible and

mucoadhesive polymer [11]. Over time, the presence of polymers today are quite varied, even leading to multifunctional polymers which can be as mucoadhesive, enzyme-inhibitor, permeation-enhancers and efflux pump-inhibitor [12]. Natural polysaccharide biodegradable polymers such as chitosan have been the focus of a large number of research studies, which reported its potential uses in biomedical research applications. Chitosan is an abundant hetero-polysaccharide which is structurally similar to glycosaminoglycans (main component of the bone extracellular matrix) and could be produced through deacetylation of chitin. It is mainly characterized for biocompatibility, biodegradability, cytocompatibility and hemocompatibility [13]. Chitosan depolymerisation in the acidic medium is the main disadvantage for using it in formulating controlled drug delivery system. Chemical crosslinking of chitosan with glutaraldehyde could form a pH-sensitive matrix that swells and releases a drug through its porous structure [14]. Eudragit RS100 is a synthetic methacrylate copolymer, which consists of 4.5 to 6.8 % of quaternary ammonium groups. These groups make the surface of the polymer positive leading to interactions with specific cells on targeted tissues. This property increases the cellular uptake of the drug-polymer conjugates to allow controlled and prolonged delivery of any drug substances to specific target sites [15]. Eudragit RS100 was chosen as a coating material because it can withstand the acidic pH 1-3.5 in lower GIT and pH 5-6.5 in the small intestine but at alkaline pH 7 in the colon it starts to dissolve [16]. Model drug candidates to be formulated as colon targeted drug delivery systems include drug substances that treat colonic disorders such as ulcerative colitis, Crohn’s syndrome, and irritable bowel syndrome.

Materials and methods

Materials

Prednisolone was given by Mankind Pharma Pvt. Ltd., Gurgaon (Haryana, India). Eudragit S100 (ES) and chitosan purchased from S.D Fine Chemical Limited, Mumbai. All other chemical were purchased from Himedia, Mumbai (India) and Sigma Aldrich. Sodium tripolyphosphate and glacial acetic acids were purchased from Merck Specialities pvt. Ltd., Mumbai, All other chemicals and reagent used were of analytical grade. Ultrapure water was used throughout the study.

Determination of absorption maxima

A solution of prednisolone containing the concentration 15µg/ml was prepared in 7.2 pH phosphate buffer. UV spectrum was taken using double beam UV/VIS spectrophotometer (Labindia-3000+). The solution was scanned in the range of 200-400nm.

Preparation calibration curve

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.2 pH phosphate buffer solution in 10 ml of volumetric flask separately. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.2 pH phosphate buffer solution. Prepare suitable dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). Linearity of standard curve was assessed from the square of correlation coefficient (r^2) which determined by least-square linear regression analysis.

Preparation of chitosan microsphere of prednisolone

Chitosan microsphere was prepared by ionotropic gelation method. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature using homogenizer (Remi motors, Mumbai) at 5000rpm for about 30 minutes, then drug (10 mg) was added to chitosan solution. Microspheres were formed by dropping the bubble-free dispersion of chitosan-drug solution through a disposable syringe (10 ml) onto a gently stirrer (100 rpm) at room temperature in 1% wt/vol (Sodium tripolyphosphate) STPP solution. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water and was air dried for twenty four hours followed by oven drying for six hours at 40˚C. The composition of formulations was given in Table 1.

Table 1 Formulations of the chitosan microsphere of prednisolone

Sr. No	Formulation Code	Prednisolone (mg)	Chitosan (mg)	STPP (mg)
1.	**F1**	10	250	500
2.	**F2**	10	250	750
3.	**F3**	10	250	1000
4.	**F4**	10	500	500
5.	**F5**	10	500	750
6.	**F6**	10	500	1000

Coating of chitosan microsphere

Chitosan microspheres were coated with Eudragil S-100 (ES) using solvent evaporation method. Microsphere (50 mg) were dispersed in 10 ml of coating solution prepared by dissolving 500 mg of ES-100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio). This organic phase was then poured in 70 ml of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours

to allow for the evaporation of solvent. Finally, the coated microsphere were filtered washed with n-hexane, and dried in desiccators over night.

Evaluation of microspheres

Percentage yield

The prepared microsphere F1-F6 were collected and weighed from each formulation.The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres. The percentage yield (%) was calculated using formula given below:

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

The various formulations of the chitosan microspheres were subjected for drug content. 10mg of chitosan microsphere from each batch were accurately weighed. The powder of chitosan microsphere were dissolved in 10 ml 7.2 pH phosphate buffer and centrifuge at 1000 rpm. This supernatant solution is than filtered through whatman filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.2 pH phosphate buffer. The supernant was analyzed for drug content by measuring the absorbance at 256nm.

Measurement of mean particle size

The mean size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement.

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Horiba Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

Shape and surface characterization of microspheres by scanning electron microscopy (SEM)

From the formulated batches of microsphere, formulations (F2) which showed an appropriate balance between the percentage drug releases was examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at

10KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.

In vitro drug release in gastrointestinal fluids of different pH

The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- *1st hour:* Simulated gastric fluid (SGF) of pH 1.2.
- *2nd and 3rd hour:* Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- *4th to 5th hour:* Simulated intestinal fluid (SIF) of pH 6.8.
- *6th hour and onward:* SIF pH 7.5

A weighed quantity of formulation (equivalent to 10mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 256.0 nm for percent of release Prednisolone using UV visible spectrophotometer. The release of prednisolone was calculated with the help of standard curve of prednisolone [17-19].

Stability studies for optimized formulation

Accelerated Testing, are the studies designed to amplify the rate of chemical degradation or physical change of a drug substance or drug product by exaggerated storage conditions as part of the formal stability studies. The optimized formulation F2 was taken and accelerated stability study was performed by taking suitable quantity of microspheres. The microspheres were placed in air-tight glass container at 40±2°C/75±5% RH. At suitable sampling interval the samples were withdrawn and evaluated for various parameters.

Results and discussion

Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 59.98±1.23-70.23±0.89%. The drug entrapment efficacies of different formulations were in range of 62.32±0.73-75.56±0.23% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during

preparation of prednisolone microspheres Table 2. On the basis of the maximum percentage yield and drug entrapment was found to be formulation F2 in prednisolone microspheres so formulation F2 was further studies. The results of measurement of mean particle size of optimized formulation F2 microspheres were found 166.2 nm Fig 1. Results of zeta potential of optimized formulation F2 microspheres were found to be -40.5 mV Fig. 2**.** Shape and surface characteristic of prednisolone microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of microspheres Fig. 3. The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C by using the simulated fluids at different time interval Table 3 & Fig. 4. According to ICH guidelines, 3 months accelerated stability study at 40±2°c and 75±5% RH optimized formulations (F2) was carried out. It showed negligible change over time for parameters like appearance, drug content, dissolution and assay etc., No significant difference observed in the drug content between initial and formulations stored at 40±2°c & 75±5% RH for 3 months.

Table 2 Percentage yield and drug entrapment for different formulation

Formulation	Percentage Yield	Drug entrapment (% w/w) of prepared microsphere
F1	65.56±1.56	69.32±0.45
F2	70.23±0.89	75.56±0.23
F3	60.32±1.23	65.45±0.65
F4	65.65±0.65	63.12±0.78
F5	62.23±0.48	68.85±0.85
F6	59.98±1.23	62.32±0.73

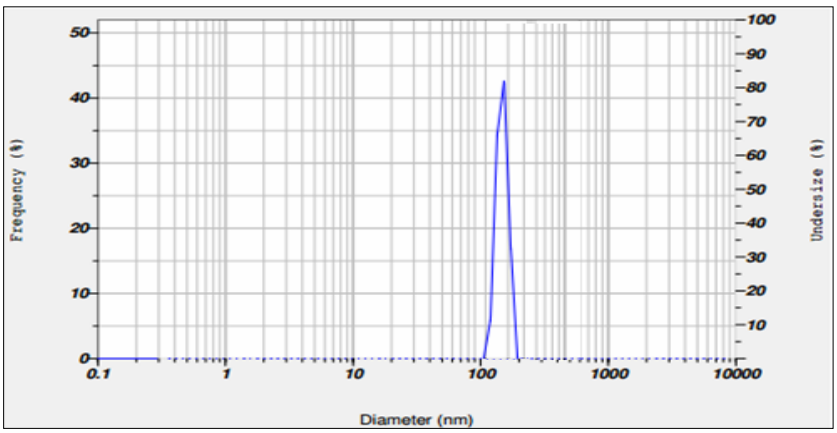

Fig.1 Particle size data of optimized microsphere formulation F2

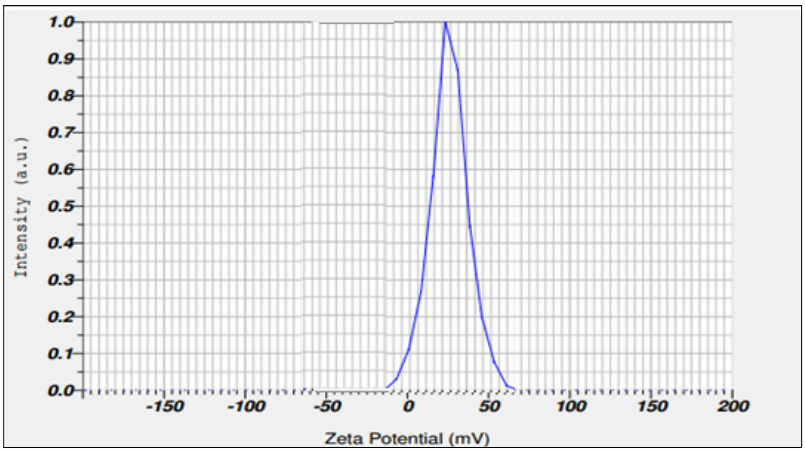

Fig. 2 Zeta potential data of mucoadhesive microsphere formulation F2

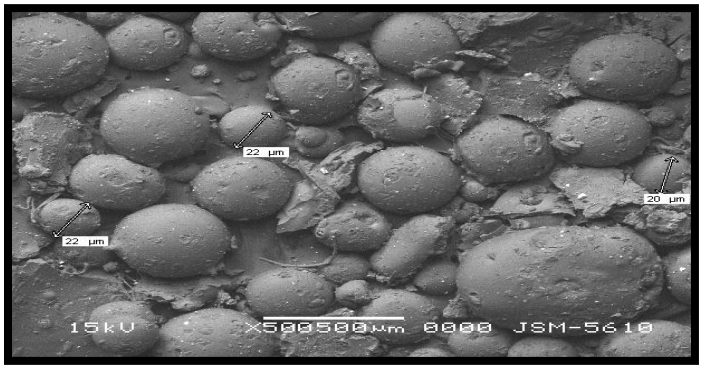

Fig. 3 SEM image of optimized mucoadhesive formulation F2

Table 3 Cumulative % drug release of prednisolone from plain and eudragit S100 coated microspheres at different pH

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release	
			Chitosan Nanoparticle	Eudragit S100 Coated Nanoparticle
1	SGF (pH 1.2)	1	15.65	1.23
2		2	22.23	1.89
3	SGF+SIF(pH 4.5)	3	36.65	2.23
4		4	45.56	4.56
5		5	52.23	11.12
6	SIF (pH 6.8)	6	62.12	16.65
7		7	69.98	33.32
8	SIF (pH 7.5)	8	73.32	45.56
9		9	79.98	69.98
10		10	85.56	73.32
11		12	89.12	89.45

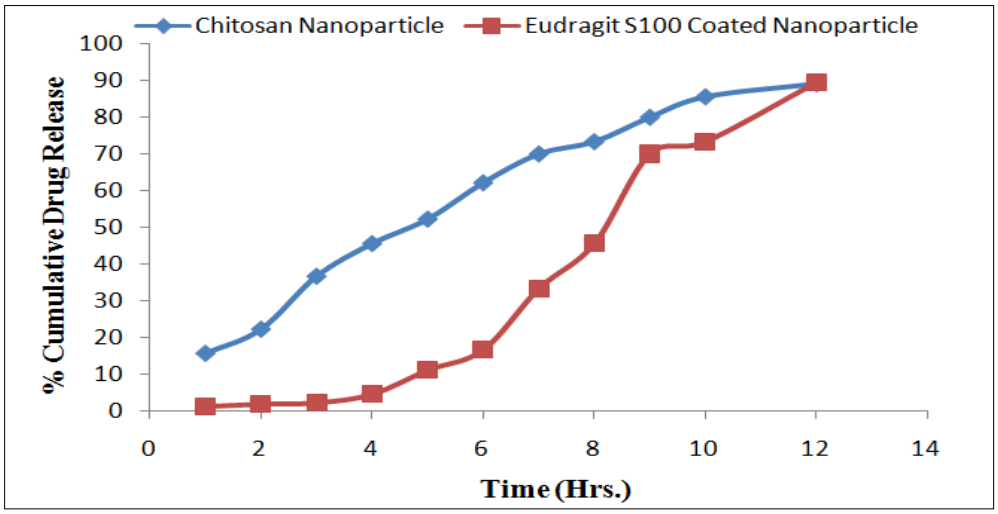

Fig. 4 Graph of cumulative % drug release of prednisolone from chitosan and eudragit S100 coated microspheres

Conclusion

Colon targeting microspheres of prednisolone can be successfully prepared using Chitosan as polymer by ionotropic gelation method and coated with Eudragit S100 by solvent evaporation method. The *in vitro* drug release study showed that the drug release decreased with increasing polymer concentration. The experimental results demonstrated that the prepared microspheres of prednisolone for colon targeting may reduce the side effects of the drug caused by its absorption from the upper part of GIT when given in conventional dosage forms. Thus the Eudragit S100 coated Chitosan microspheres have the potential to be used as a drug carrier for an effective colon targeted drug delivery system. Thus the developed formulations prove to be promising for the colon targeted drug delivery of prednisolone and thereby facilitating in the management of ulcerative colitis and inflammatory bowel disease.

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