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Carbopol gel containing chitosan-egg albumin nanoparticles for transdermal aceclofenac delivery

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ABSTRACT

In the present work, various aceclofenac-loaded chitosan-egg albumin nanoparticles were prepared through heat coagulation method. These aceclofenac-loaded nanoparticles were characterized by FE-SEM, FTIR, DSC and P-XRD analyses. The *in vitro* drug release from nanoparticles showed sustained drug release over 8 h. Aceclofenac-loaded nanoparticles (prepared using 200 mg chitosan, 500 mg egg albumin and 2% (w/v) NaTPP) showed highest drug entrapment (96.32 ± 1.52%), 352.90 nm average particle diameter and −22.10 mV zeta potential, which was used for further preparation of Carbopol 940 gel for transdermal application. The prepared gel exhibited sustained *ex vivo* permeation of aceclofenac over 8 h through excised mouse skin. The *in vivo* anti-inflammatory activity in carrageenean-induced rats demonstrated comparative higher inhibition of swelling of rat paw edema by the prepared gel compared with that of the marketed aceclofenac gel over 4 h.

1. Introduction

Transdermal drug delivery systems are prepared to deliver drugs through skin at predetermined rate escaping the first-pass effect by liver [1]. The most difficult aspect of transdermal drug delivery system is to overcome the skin barrier. There is evidence that the rate-limiting step in transdermal transport occurred at the outermost layer of the skin, stratum corneum [2]. Many approaches have been investigated to enhance the drug permeation through the barrier of skin for the use in transdermal drug delivery [1,3–7].

Currently, nanoparticles have shown great potential as novel drug carriers for transdermal drug delivery [7–9]. The smaller size of nanoparticles could ensure close contact with the stratum corneum and increases the encapsulated drug amount penetrating into the skin. The advantages of the use of these kinds of colloidal carriers are protection of unstable drugs from degradation and control of drug release rate from these colloidal carriers [9,10]. Nanoparticles are solid particles ranging in size, 1–1000 nm [11,12]. Presently, polymeric nanoparticles have received lots of attention due to their stability and ease of surface modification specificity [13].

Chitosan is a cationic biocompatible and biodegradable natural polysaccharide obtained by alkaline deacetylation of chitin, which is composed of α-1,4-linked 2-amino 2-deoxy α-D-glucose (N-acetyl glucosamine) [14,15]. Although chitosan is used in drug delivery, it has a limited capacity for controlling drug release. So researchers investigated various chemical modifications of chitosan to develop chitosan-based formulations for controlled drug release applications [16–18].

Egg albumin has recently received attention for its use in food and pharmaceutical applications [19,20]. It behaves as anionic polymer above its isoelectric point (pH 4.8) [21]. Therefore, it is hypothesized that anionic egg albumin and cationic chitosan might form a polyelectrolyte complex, if processed with each other. Additionally, this approach could be beneficial to control the drug release. Carbopols are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol [22]. Because of their hydrophilic nature, the cross-linked structures of Carbopols make them potential candidates for the use as gel-type formulations for topical use [23,24]. In the previous literature, Carbopol gels containing drug-loaded nanoparticles were investigated for transdermal delivery [7,22]. In the present investigation, we attempted to develop a transdermal Carbopol gel containing aceclofenac-loaded chitosan-egg albumin nanoparticles.

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) with short half-life (4 h) indicated for the symptomatic treatment of pain and inflammation [15,25]. It is reported to produce side-effects

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like gastric irritation, ulcer, abdominal pain and flatulence, as a result of prolonged treatment [26]. In view of the side-effects associated with the oral administration of aceclofenac, it is increasingly administered through topical route [27]. Furthermore, the transdermal route of administration eliminates side-effects, increases patient compliances, avoids first-pass metabolism and maintains the plasma-drug level for a long period. In the current study, we prepared aceclofenac-loaded chitosan-egg albumin nanoparticles by heat coagulation method, incorporated these nanoparticles within Carbopol 940 gel and evaluated this newly formulated gel for transdermal delivery of acecalofenac, *in vitro* and *in vivo*.

2. Materials and method

2.1. Materials

Aceclofenac was received as a gift sample from Drakt Pharmaceutical Pvt. Ltd., India. Chitosan (85% deacetylated) was commercially purchased from Indian Sea Foods, Cochin, India. Egg albumin, Carbopol 940, crospovidone and sodium tripolyphosphate (NaTPP) were purchased from Loba Chemie Pvt. Ltd., India. All other chemicals, and reagents used were of analytical grade.

2.2. Preparation of aceclofenac-loaded chitosan-egg albumin nanoparticles

Aceclofenac-loaded chitosan-egg albumin nanoparticles were prepared by heat coagulation method [28]. Different strength of chitosan solutions were prepared by dissolving required amount of chitosan in 1% acetic acid solution and egg albumin solutions were prepared dissolving them in distilled water. Then, accurately weighed amount of aceclofenac (100 mg) was dispersed into the chitosan solutions and mixed thoroughly using a homogenizer (Remi Motors, India). Chitosan-aceclofenac mixtures were added drop-wise into egg albumin solutions under continuous stirring (500 rpm) for 30 min. The resultant pH of these aceclofenac-polymeric disperpersions was observed in between 4.00 and 4.30, and was finally adjusted to 5.4 with the help of 0.1 N sodium hydroxide. These adjusted dispersions were heated at 80 °C for 30 min to aid in developing nanoparticles due to heat coagulation. The prepared nanoparticles were collected by centrifugation and were lyophilized (Eyela FDU 1200) to obtain dried samples. Different chitosan-egg albumin nanoparticles formulations containing aceclofenac along with amounts of chitosan, egg albumin, and NaTPP were enlisted in Table 1.

2.3. Characterization of aceclofenac-loaded chitosan-egg albumin nanoparticles

2.3.1. Estimation of drug entrapment efficiency

10 mg of lyophilized nanoparticles containing aceclofenac from each formulation batch were accurately weighed and were taken separately in 50 ml phosphate buffer, pH 7.4 to keep for 48 h under continuous stirring followed by centrifugation at 2000 rpm for 10 min. Then, the supernatant fraction was further filtered through Whatman® filter paper (No. 40). The aceclofeanc 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 nanoparticles was calculated using the following formula:

$$\text{Drug entrapment efficiency}\,(\%) = \frac{\text{theortical drug content in nanoparticles} - \text{amount of drug present in the filtrate}}{\text{theoretical drug content in nanoparticles}} (1)$$

2.3.2. Particle size and zeta potential determination

The nanoparticles were dispersed into 10 ml phosphate buffer, pH 7.4 and sonicated for 5 min before size measurement. The obtained homogeneous suspensions were examined for particle size and zeta potential using a laser scattering particle size analyzer (MAL500999, UK).

2.3.3. Field emission-scanning electron microscopy (FE-SEM)

The lyophilized particles were spread onto metal stubs and platinum coating was applied by using an ion-sputtering device. The coated particles were then examined under FE-SEM (JSM 6701; JEOL, Japan).

2.3.4. Fourier transform-infrared (FTIR) spectroscopy

Samples were powdered and analyzed as KBr pellets by using a FTIR spectrophotometer (Perkin Elmer Spectrum RX I, USA). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 4000 and 600 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 2 mm/s.

2.3.5. Differential scanning calorimetry (DSC)

Moisture free nanoparticles (7 mg) were placed into a platinum crucible 40-μl aluminum pan in hermetically sealed condition, where alumina powder was used as a reference. Thermograms were recorded from 29.80 to 351.90 °C at the heating rate of 5 °C/min under a constant flow of an inert nitrogen gas atmosphere with the flow rate of 20 ml/min. These analyses were done using a Differential Scanning Calorimeter (Perkin Elmer® Instrument, Japan).

2.3.6. Powder X-ray diffraction (P-XRD)

Samples were exposed to CuKα radiation (40 kV × 20 mA) in a wide-angle X-ray diffractometer (Siemens D5000, Germany). The instrument was operated in the step-scan mode in increments of 0.050° 2θ. The angular range was 10°–60° 2θ, and counts were accumulated for 1 s at each step.

2.4. In vitro release study of aceclofenac-loaded chitosan-egg albumin nanoparticles

In vitro release of aceclofenac from these prepared nanoparticles was measured using dialysis bag diffusion technique. Accurately weighed quantities of nanoparticles containing drug equivalent to 50 mg aceclofenac were placed in one end of the dialysis bag (Cellophane membrane, molecular cut off 10,000–12,000 Da, Hi-Media, India) containing 5 ml of phosphate buffer, pH 7.4. After that, other side of the dialysis bag was tied and immersed 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 Spectronic UV-1, USA) by measuring absorbance at λ_{Max} of 274 nm.