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Development, *in-vitro* and *in-vivo* characterization of gelatin nanoparticles for delivery of an anti-inflammatory drug

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ABSTRACT

The present investigation involves the preparation of gelatin nanoparticles laden with naproxen, an anti-inflammatory drug. The nanoparticulate formulations were prepared by a modified two step desolvation technique upon varying the crosslinking ratio with drug and polymer and were assessed for their analgesic and anti-inflammatory activities. Particles of nanometer size range were formulated (177 ± 2.17 −142 ± 2.06 nm) and the size distribution was monodisperse in the entire prepared batches with low polydispersity index. Entrapment efficiency of the nanoformulations was from 65 to 42%. DSC thermograms reported molecular level dispersion of drug in the nanoparticles whilst FTIR studies exhibited no drug-polymer interaction. Batches (NGP1-NGP8) exhibited an initial burst release followed by controlled release of the drug, the Korsmeyer-Peppas showed better linearity and the formulations displayed non-Fickian drug release pattern. Investigations were executed to analyze the analgesic and anti-inflammatory effects of the optimized formulation. The drug loaded nanoparticles exhibited significant ($P<0.05$) analgesic activity (acetic acid induced abdominal constriction & tail flick test in mice) and anti-inflammatory activity (carrageenan induced rat paw edema) in comparison to marketed product.

1. Introduction

Polymeric nanoparticles made of natural or synthetic polymers are becoming an interesting area of research in the meadow of therapeutic agent's delivery due to their controlled release properties, also the safety they offer to the compound of interest [1]. In recent years, apposite nanoparticulate formulations based on natural polymers such as chitosan, gelatin, albumin, alginate [2,3], or synthetic for instance poly(lactic-co-glycolic acid), poly(glycolic acid), ploy(lactic acid), poly(cyanoacrylate) etc. have been efficaciously utilized for the effective delivery of the active pharmaceutical ingredients [4,5]. Utilizing their potential, polymeric carriers can enhance the solubility of the therapeutic agent, increase its stability and lessen the side effects associated with the drug [6].

Gelatin is a natural polymer available in two forms, Type A extracted by acidic or Type B extracted by alkaline pretreatment and thermal denaturation of collagen [7]. Gelatin being biodegradable [8], biocompatible, non-immunogenic [9–11] has been extensively employed as a carrier in drug delivery [12–14]. A lot of methods are available to prepare gelatin nanoparticles for example emulsification, coacervation but these methods generally forms particles in micrometer range and implicate high temperatures. To obtain particles of nanosize, desolvation process has proved to be a good method; also, treatment of polymer with high temperatures is bypassed [15].

Naproxen, a non-steroidal anti-inflammatory drug (NSAID), commonly used to treat moderate to severe pains and inflammation and is also used in osteoarthritis, rheumatoid arthritis, psoriatic etc. Generally, classical NSAIDs cause mutilation to the upper gastrointestinal tract leading to bleeding and life-threatening perforations. The reason for such side-effects might be due to the non-selective inhibition of cyclooxygenase enzyme [16]. Probably, such side effects can be abated by encapsulating the medication in

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nanoparticulate formulations with controlled release of the drug hence can be utilized in the dealing of inflammatory and painful *status quo* of the body like rheumatoid arthritis [17,18]. Nanoencapsulation inside polymeric carriers safeguards the drug in its active form [2] and by modifying quite a few characteristics of 'bare drugs' like their solubility, pharmacokinetics and pharmacodynamics [19–21].

On account of their controlled, prolonged and effective drug delivery to inflamed tissues (as lest of rheumatoid arthritis) by the nanoformulations [22], the current study focuses on the fabrication of a gelatin based nanoparticulate formulation of naproxen (NPX) with an objective of its physicochemical characterization and *in-vivo* performance.

2. Materials and methods

2.1. Materials

Naproxen was acquired from Yarrow Chem Products, Mumbai, India, Gelatin Type B (Bloom225), glutaraldehyde, acetone, potassium dihydrogen orthophosphate, acetic acid, carrageenan and dialysis membrane (molecular weight cut-off 12000–14000 Da) were purchased from Himedia, Mumbai, India. All other chemicals used were of highest analytical grade.

2.2. Animals

The research experiments were sanctioned by the Institutional Ethics Committee and were accomplished as per CPCSEA guidelines (approval no. BU/Pharm/IAEC/11/035). The trials were performed on Albino mice and Wistar rats of four months, of either sex weighing 140-180 gms. The animals were familiarized to the customary laboratory conditions in cross aerated animal house at temperatures 25 ± 2°C/75% relative humidity and light and dark cycles of 12:12 h, fed with regular pallet nutrition.

2.3. Fabrication of nanoparticles

Naproxen loaded gelatin nanoparticles were fabricated by a two-step desolvation technique following the method as defined by Coester et al., 2000 [23]. 1.25 g of gelatin type B (Bloom225) was dissolved in 25 ml of distilled water under gentle heating (40 °C). Desolvation and rapid sedimentation was achieved by adding a solution of 25 ml acetone (desolvating agent) and drug. The supernatant containing desolvated gelatin along was rejected following subsequent sedimentation of high molecular weight gelatin. The residue was dissolved again by adding distilled water under heating and the pH was accustomed to 2.5 by 0.1N HCl. Gelatin nanoparticles were formed *in-situ* when acetone was added dropwise during the second desolvation step under stirring (500 rpm). A 200 μl of glutaraldehyde (25% aqueous solution) was added after 10 min, to crosslink the nanoparticles. Gelatin nanoparticles were refined by a 3-fold centrifugation (16000 g for 20 min) and redispersion in acetone/water mix (30/70). Desolvating agent was removed by evaporating on a water bath at 50 °C after final redispersion.

2.4. Characterization of the nanoparticles

2.4.1. Size and surface morphology

The prepared naproxen loaded nanoparticles were dispersed in distilled water by sonication and vortex mixing for 30 s and the particle size & polydispersity index were determined using Zeta Sizer (Nano series, Malvern Instruments, England). Zeta potential was determined using Nanosizer ZS (Malvern Instruments, UK).

Surface morphology was studied using Scanning Electron Microscopy (SEM) (JSM-6500 F, Germany) at 5.0 kV and at a working distance of 9.7 mm. The samples for SEM were prepared by lightly scattering the lyophilized nanoparticles on a double adhesive tape, which was stuck on aluminium stubs coated with gold to a thickness of about 300 Å. Transmission Electron Microscopy was performed by suspending the lyophilized nanoparticles in phosphate buffer (pH 7.4) and examined under a transmission electron microscope (Philips Morgagni 268, Netherlands) at an acceleration voltage of 100 kV and photomicrographs were taken at suitable magnification.

2.4.2. %Entrapment efficiency

The %entrapment efficiency of naproxen loaded gelatin nanoparticulate preparation was determined by centrifuging the nanoparticles by using centrifuge (REMI, Gwalior, India) at 10000 rpm for 30 min and the supernatant containing free drug was analyzed by UV (Shimadzu-1700, Japan) at 230 nm. The amount of unentrapped drug was analyzed, now this amount is deducted from the total amount of drug added to the formulation, from which, the entrapped drug amount in the nanoparticles is calculated [24]. The %entrapment efficiency can be drawn out employing the formula; % Entrapment Efficiency = 100 × (Mass of drug in nanoparticles/Mass of drug used in formulation).

2.5. Physicochemical characterization

DSC thermograms of the samples were obtained employing Differential Scanning Calorimeter (DSC-60, Shimadzu, Japan) coupled with an electronic thermal analyzer. DSC was performed to analyze the endothermal transitions of the drug by assessing the thermogram acquired. DSC of the physical mixture of pure drug and the polymer was executed to ascertain the melting point of the drug and glass transition temperature of gelatin.

The thermal behavior of the drug in the nanoparticles was analyzed by heating the sample in a concealed sample pan under nitrogen gas flow over the temperature range of 50–300 °C with a heating flow rate of 10 °C/minutes.

FTIR spectra of the pure drug, gelatin, physical mixture of drug & polymer and nanoparticulate formulations were acquired by preparing a KBr pellet using FTIR (Perkin Elmer, BX-II Series, UK) and % Transmittance (%T) was recorded in the spectral region of 4000-400 cm^{-1}.

2.6. In-vitro drug release

A 2 ml naproxen loaded nanoparticulate formulation was enclosed in the dialysis bag (dialysis membrane, molecular weight cut off of 12000–14000 Da (Himedia, Mumbai, India) and was then incubated in 100 ml of simulated phosphate buffer (pH 7.4). The medium in the setup was agitated mildly and uninterruptedly at 100 rpm using a magnetic stirrer and the temperature was sustained at 37±2 °C. 1 ml sample from the release media was drawn at fixed time intervals and the sample withdrawn was replaced with 1 ml of fresh simulated phosphate buffer (pH 7.4) to sustain the sink condition. The extent of the drug released was determined spectrophotometrically (Shimadzu-1700, Japan) at 230 nm. All calculations were executed in triplicate and the (±SD) was calculated.

2.7. Drug release kinetics

To comprehend the kinetics and drug release mechanism, the data of *in-vitro* drug release from drug loaded gelatin nanoparticles were fitted with five primarily applied mathematical models [25] (stated below) for the assessment of release kinetics of naproxen