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Eudragit RL 100-based nanoparticulate system of aceclofenac for ocular delivery

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ABSTRACT

The purpose of this study was to prepare Eudragit RL 100-based nanoparticles of aceclofenac by nanoprecipitation and evaluate the particle size, zeta potential, drug entrapment, particle morphology; *in vitro* drug release and *in vivo* efficacy. Change in drug–polymer ratio from 1:5 to 1:20 increased the particle size and entrapment efficiency. The particles showed sustained *in vitro* drug release which followed the Higuchi square-root kinetics. The results indicate that the nanoparticles release the drug by a combination of dissolution and diffusion. Based on the particle size (134.97 nm) and entrapment efficiency (95.73%), the formulation made with 1:10 drug–polymer ratio was selected for further studies. The particles were spherical with a polydispersity index of 0.186 and zeta potential of +30.5 mV. Powder X-ray diffraction and differential scanning calorimetry indicated decrease in crystallinity of drug in the nanoparticle formulation. In the *in vitro* permeation study, the nanoparticle formulation showed 2-fold higher permeation of drug through excised cornea compared to an aqueous solution of drug with no signs of corneal damage. The *in vivo* studies involving arachidonic acid-induced ocular inflammation in rabbits revealed significantly higher inhibition of polymorphonuclear leukocytes migration ($p < 0.05$) and lid closure scores by the nanoparticle formulation compared with the aqueous solution. The formulation was quite stable to ensure two year shelf life at room temperature.

1. Introduction

The most common disease affecting the eye is inflammation. Inflammation is manifested as a cellular and vascular response to the injury, infection, ischemia and excessive or inappropriate operation of immune mechanism. The response is amplified by activation of inflammatory cells and production of chemical mediators like acidic lipids *e.g.* prostaglandins, thromboxanes, leukotrienes, vasoactive amines, cytokines *etc.* The acidic lipids are produced through arachidonic acid metabolism. Arachidonic acid is released from the phospholipids component of cell membrane by the action of phospholipase A2. The arachidonic acid is fed into the cyclooxygenase and lipoxygenase pathways, resulting in production of pro-inflammatory prostaglandins and leukotrienes [1,2]. Topical therapy with corticosteroids is quite common in the treatment of ocular inflammatory disorders but their use is often associated with severe side effects such as increase in intraocular pressure, cataract formation and risk of infection [3]. Non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin [4], flurbiprofen [5], ketorolac [6] and diclofenac [7] which are devoid of these side effects have been found to be safer alternatives to steroids in treating ocular inflammation. Aceclofenac, 2-[[2-[2-[(2,6-dichloro phenyl)amino]phenyl]acetyl]oxy] acetic acid, is a NSAID of the phenyl acetic acid group which is structurally related to diclofenac. It possesses good anti-inflammatory and analgesic activities, while maintaining better gastric tolerance in comparison with other NSAIDs such as indomethacin and diclofenac. Aceclofenac acts as such by inhibiting the secretion of tumor necrosis factor (TNF-α) and interleukin-1 along with preferential selective cyclooxygenase-2 (COX-2) inhibition after conversion into active metabolite [8–10].

Mostly, all ocular therapeutics has been administered to the eye as aqueous solution. About 90% of the dose applied topically from such solutions is lost due to pre-corneal losses (lacrimation and drainage) which lead to poor ocular availability [11]. Accordingly, there is a need for an appropriate delivery system which could increase the contact time of the drug with the eye surface and facilitate the transport of drug molecules into the eye tissue. In this role, a controlled or sustained delivery of ophthalmic drugs would be beneficial.

A number of colloidal drug delivery systems such as liposomes [12], polymeric micelles [13], nanocapsules [14] and nanoparticles [15] have been evaluated for improved ocular bioavailability. Nanoparticles, because of their submicron size are well tolerated and have the tendency to deposit in the cul-de-sac for prolonged period. Nanoparticles of several synthetic polymers, *e.g.* poly(alkyl cyanoacrylate) [16], poly(lactic-co-glycolic acid) [17],

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Table 1
Effect of various drug–polymer ratios on particle size, zeta potential and entrapment efficiency of aceclofenac loaded Eudragit RL 100 polymeric nanoparticles.

Formulation code	Drug-to-polymer ratio	Particle size (nm ±SE)	PDI (±SE)	Zeta potential (mV ±SE)	Entrapment efficiency (% ±SE)	Viscosity of organic phase* (cPs ±SE)
A1	1:5	75.52 ± 6.7	0.380 ± 0.004	22.5 ± 0.62	58.33 ± 0.93	0.415 ± 0.009
A2	1:10	134.97 ± 10.3†	0.186 ± 0.01	30.5 ± 0.38	95.73 ± 0.28†	0.463 ± 0.004
A3	1:20	184.36 ± 20.2†	0.368 ± 0.07	32.6 ± 0.6	87.77 ± 0.88†	0.565 ± 0.001

* Viscosity of organic phase measured by Ostwald viscometer; values are mean ± SE ($n = 3$).
† Statistically significant ($p < 0.05$) compared with control (drug to polymer ratio – 1:5), as determined by one-way analysis of variance followed by Dunnett's test.

poly(epsilon-caprolactone) [18], as well as natural polymers such as chitosan [19] and gelatin [20] have demonstrated promising results for efficient drug delivery to the ocular tissues. Despite the positive results, these polymers have their own disadvantages like, poly(alkyl cyanoacrylate) causes disruption of corneal epithelium [21]. The higher cost and slow degradability of poly (lactic-co-glycolic acid) and poly (epsilon-caprolactone) limit their use.

Eudragit RL 100 polymer is a copolymer of poly (ethylacrylate, methyl-methacrylate, and chloro trimethyl-ammonioethyl methacrylate) containing an amount of quaternary ammonium groups between 8.8% and 12%. Eudragit RL 100 is insoluble at physiological pH and capable of limited swelling, thus appears to be a good polymeric carrier for the dispersion of drugs. The presence of quaternary ammonium group renders positive charge to the polymer by which it can interact with anionic drugs and mucin. The positive charge on the polymer may also impart mucoadhesion to the anionic cornea having isoelectric point (p*I*) of 3.2 and thereby increase its residence on corneal surface. Polymeric nanosuspensions prepared from Eudragit RL 100 and RS 100 have been investigated for the ocular delivery of flurbiprofen [22], cloriocromene [23], amphotericin B [24], methylprednisolone [25] and piroxicam [26]. It has already been stated that for treatment of ocular inflammation, NSAIDs are preferred over steroid like prednisolone due to lack of ocular side effects. Among the NSAIDs, one which selectively inhibits COX-2 could offer therapeutic advantage, as COX-2 is involved in prostaglandin production at the site of inflammation. Thus, aceclofenac being a COX-2 inhibitor appears to be an ideal candidate for ocular inflammation.

Hence, attempts were made to formulate and characterize Eudragit RL 100-based nanoparticles of aceclofenac and evaluate the anti-inflammatory activity of selected formulation against arachidonic acid-induced ocular inflammation in rabbits.

2. Materials and methods

2.1. Materials

Aceclofenac and Eudragit RL 100 (Evonik Degussa India Pvt. Ltd., Mumbai, India) were received as gifts from Ranbaxy Research Laboratories (Gurgaon, India) and Jubilant Organosys Ltd. (New Delhi, India), respectively. Acetone and methanol were purchased from S. D. Fine Chemical Limited (Mumbai, India). Mannitol molecular grade was supplied by S. D. Fine Chemical Ltd. Arachidonic acid was purchased from Merck chemical Ltd. (Darmstadt, Germany). All other chemicals purchased were of analytical grade and were used as received. Fresh eyeballs of goat were obtained from local butcher shop (Ambedkar Nagar, New Delhi, India). Rabbits were obtained from the disease-free small animal house of Delhi Institute of Pharmaceutical Sciences and Research, University of Delhi.

2.2. Methods

2.2.1. Preparation of nanoparticles

Polymeric nanoparticles (NPs) of aceclofenac were prepared with Eudragit RL 100 by nanoprecipitation technique [15]. In brief, accurately weighed quantity of Eudragit RL 100 (50, 100 or 200 mg) and aceclofenac (10 mg) were dissolved in 5 mL acetone. This solution was poured into 20 mL distilled water containing 0.02%, w/v Tween 80 as hydrophilic surfactant under constant stirring by mechanical stirrer at 2200 rpm (Remi, Mumbai, India). Nanoparticles were spontaneously formed and turned into a milky colloidal solution with a bluish opalescence. The resulting dispersion was stirred at room temperature for 16–18 h with a magnetic stirrer to allow evaporation of acetone. Subsequently, the solvent was evaporated under reduced pressure at 60 °C to 10 mL by Rota evaporator (Hiedolph, Germany). To this aqueous dispersion, 5%, w/v mannitol was dissolved as a cryoprotectant and the Eudragit NPs were lyophilized to get free flowing powder. The Freeze-Dryer (Allied Frost, New Delhi) was operated for 24 h at −60 °C, at a 0.02 mm Hg pressure. The process variables involved in NPs preparation are presented in Table 1. Replicate batches of different formulations in varying drug: polymer ratios were prepared for experimentation. One batch of Eudragit NPs having drug to polymer ratio of 1:10, was also prepared without the addition of mannitol.

2.2.2. Nanoparticles- size, zeta potential and surface morphology

Freeze-dried nanoparticles were dispersed in distilled water after treatment in an ultrasonicator for 30 s. The mean particle size (z average), zeta potential, and polydispersity index (PDI) of the aqueous dispersion of aceclofenac-loaded nanoparticles were measured by a dynamic light scattering method using a Zetasizer Nano ZS-90 (Malvern Instruments, Worcestershire, UK) equipped with the DTS software. Each value quoted was the average of determinations of three independent samples.

Morphological evaluation of the freeze-dried nanoparticles was performed using transmission electron microscopy (268D, FEI, Holland). Samples of the nanoparticle suspension (5–10 μL) were dropped onto copper grids coated with colladion in amyl acetate (Plano GmbH, Wetzlar, Germany). After complete drying, the samples were stained using 2% w/v phosphotungstic acid. Digital micrograph and soft imaging viewer software (Olympus, Singapore) were used to perform the image capture and analysis.

2.2.3. Entrapment efficiency of nanoparticles

It is the percentage of the actual mass of drug entrapped in the polymeric carrier, relative to the initial amount of loaded drug and was calculated using the following equation:

$$\text{Entrapment efficiency}\,\% = \frac{\text{Actual loading}}{\text{Theoretical loading}} \times 100 \quad (1)$$

Theoretical drug loading was calculated from the amount of drug taken relative to the amount of total drug and excipients used in the preparation of nanosuspension as follows:

$$\text{Theoretical loading}\,(\%) = \frac{\text{Total drug}}{\text{Total drug} + \text{Total excipients}} \quad (2)$$

For actual drug loading, the nanosuspension prepared by dispersing 25 mg of the lyophilized powder in 2 mL of distilled water was centrifuged at 13,000 rpm (Superspin, Mumbai) for 20 min. The clear supernatant was analyzed for free aceclofenac content