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Review Article

**NANOPARTICULATE FORMULATION IN OPHTHALMIC  
DRUG DELIVERY SYSTEM**

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*The major challenge faced by today's pharmacologist and formulation scientist is ocular drug delivery. Topical eye drop is the most convenient and patient compliant route of drug administration, especially for the treatment of anterior segment diseases. Delivery of drugs to the targeted ocular tissues is restricted by various precorneal, dynamic and static ocular barriers. Also, therapeutic drug levels are not maintained for longer duration in target tissues. So, the present work on ocular drug delivery research make advanced towards developing a novel, safe and patient compliant formulation and drug delivery devices/techniques, which may surpass these barriers and maintain drug levels in tissues. Anterior segment drug delivery advances are witnessed by modulation of conventional topical solutions with permeation and viscosity enhancers. Also, it includes development of nano formulations have also been introduced for anterior segment ocular drug delivery. these novel devices and/or formulations are easy to formulate, no/negligibly irritating, possess high precorneal residence time, sustain the drug release, and enhance ocular bioavailability of therapeutics. An update of current research advancement in ocular drug delivery necessitates and helps drug delivery scientists to modulate their process and develop novel and safe drug delivery strategies followed by current nanotechnology-based formulation developments and also, recent developments with other ocular drug delivery strategies employing in situ formulation.*

**Keywords:** Nanoparticles, Clecoxib, Mucoadhesive polymers**Corresponding author:****Suchita G**

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**INTRODUCTION:**

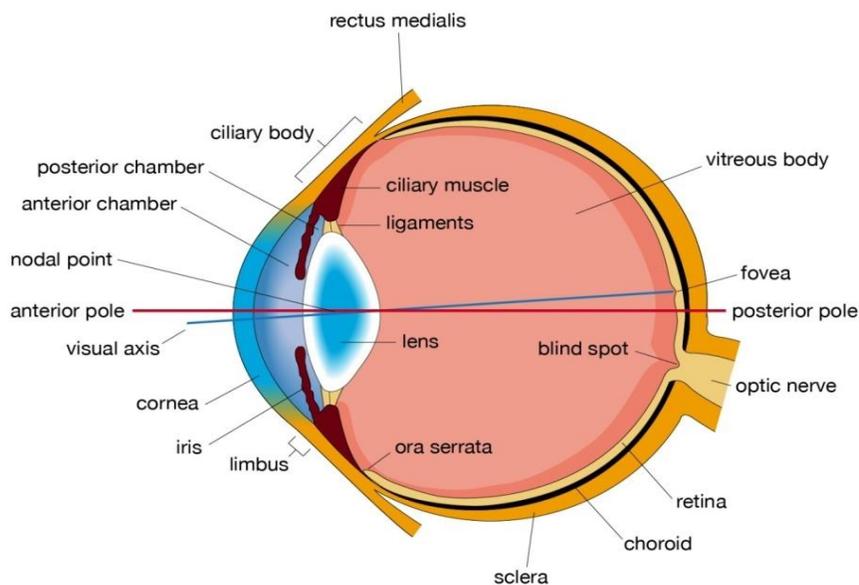
Eye is the most interesting organ due to its drug disposition characteristics. Generally, topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy [1]. A significant challenge to the formulator is to circumvent (bypass) the physiological barriers of the eye without causing permanent tissue destruction. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity. The aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration. [2] Ocular disposition and elimination of a drug depends upon its physicochemical properties as well as the relevant ocular anatomy and physiology. An effective and successful design of a drug delivery system, therefore,

requires an integrated knowledge of the drug molecule and the constraints offered by the ocular route of administration. [3]

These novel devices and/or formulations are easy to formulate, no/negligibly irritating, possess high precorneal residence time, sustain the drug release, and enhance ocular bioavailability of therapeutics. An update of current research advancement in ocular drug delivery necessitates and helps drug delivery scientists to modulate their process and develop novel and safe drug delivery strategies followed by current nanotechnology-based formulation developments and also, recent developments with other ocular drug delivery strategies employing in situ formulation.

**Anatomy of human eye:**

The human eye can be usually divided into the anterior and the posterior chambers. The anterior segment includes the cornea, conjunctiva, iris, ciliary body, aqueous humor and lens while the posterior segment constitutes sclera, choroid, retina and vitreous humor.



**Figure 1.1:** Anatomy of human eye

The cornea is the outermost transparent multi layered membrane of the eye which lacks blood supply and acquires its nourishment from the aqueous humor and limbal blood capillaries. The human cornea is composed of five layers i.e. corneal epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. A fluid present in the anterior segment of the eye is called as aqueous humor. It provides the major nutrients to the crystalline lens and cornea. The iris is the colored portion of the eye

comprising tanned epithelial cells and circular muscles (constrictor radial sphincter muscles). The aperture in the midpoint of the iris is called the pupil. The iris sphincter and dilator muscles help in regulating the pupil size which regulates the amount of light entering the eye. The ciliary body, a ring-shaped muscle attached to the iris, comprises ciliary muscles. The shape of the lens is controlled by contraction and relaxation of the ciliary muscle. The lens is a crystalline and flexible component consisting of layers

of tissue enclosed in a capsule. It is suspended from the ciliary muscles by very thin fibres called the zonules. The conjunctiva is a clear mucous membrane that edges the inner part of the eyelids and spreads from the anterior surface of the sclera up to the limbus. It aids lubrication in the eye by generating mucus and helps attachment of the tear film. The sclera is a white cover surrounding the eyeball and is called “white of the eye”. It acts as a main safeguard to protect the internal organs. The sclera is connected by a highly vascularized tissue known as the choroid, which is sandwiched between the retina and the sclera. The choroid nourishes the photoreceptor cells in the retina. The retina is made up of a multi-layered sensory, light sensitive tissue that covers the back of the eye. It contains millions of photoreceptors or photosensitive constituents. These constituents capture light rays and

convert them into electrical impulses. <sup>4</sup> These impulses travel along the optic nerve to the brain, where they are converted into an image. The vitreous humour is a jelly-like substance or a hydrogel matrix, distributed between retina and lens. [5,6]

#### Routes of ocular drug delivery:

There are several possible routes of drug delivery into the ocular tissues. The selection of the route of administration depends primarily on the target tissue. Compared with drug delivery to other parts of the body, ocular drug delivery must overcome important challenges posed by various ocular barriers. Many of these barriers are inherent and unique to ocular anatomy and physiology making it a challenge to deliver the appropriate dose at the appropriate place.

Sr.No	Route	Special Utility	Limitations & Precautions
1.	<b>Topical</b>	--Convenient -- Economical --Relatively safe	--Compliance --Corneal & conjunctival toxicity --Nasal mucosal toxicity --Systemic side effects from nasolacrimal absorption
2.	<b>Subconjunctival, sub-Tenon's &amp; Retrobulbar injections</b>	-Anterior segment infections -Posterior uveitis -Cystoid Macular Edema (CME)	-Local Toxicity -Globe perforation -Optic nerve trauma -Central retinal artery or vein occlusion
3.	<b>Intraocular Injections</b>	Anterior segment surgery or infections	-Corneal toxicity -Relatively short duration of action
4.	<b>Intravitreal Injection</b>	Immediate local effect	Retinal toxicity

Figure 1: Ocular Routes of Administration (Conventional)

#### Method of Preparation of Nanoparticles:

Nanoparticles (NPs) were prepared using emulsification solvent diffusion method [129] with some modifications. Table 1 shows the formulations for preparation of NPs for preliminary study and optimization. The polymer (either Sodium alginate or Chitosan) was dissolved in Dichloromethane (DCM) then Lecithin was dissolved in the polymer solution in a concentration of 2% (w/v), and finally acetone was added to the previous organic solution. Poloxamer 188 or Polyvinyl alcohol (PVA) as stabilizers were dissolved in different concentrations ranging from

0.2% to 1% (w/v) in deionized water (DIW). The organic solution was injected into the aqueous solution (under magnetic stirring, 700 rpm) with an injection flow rate of 0.8 mL/min using an infusion pump that is connected to a syringe attached to a 25 gauge needle. The obtained emulsion was sonicated at 100% amplitude for 10 min in an ice bath. The obtained NPs dispersion was stirred overnight at a moderate stirring rate (200 rpm) to allow complete evaporation of DCM. The NPs dispersion was then filtered through a Puradisc syringe filter to remove any agglomerates from the system. Fifty milliliters of DIW was added to

the filtrate to allow for complete diffusion of acetone to the aqueous phase. It was then centrifuged for 2 h at 60,000 rpm to separate the NPs from the preparation medium. The NPs pellets were washed by resuspending in 20 mL of DIW and recentrifuged for 2 h at 60,000 rpm to remove excess emulsifiers and the trace acetone. The washing process was repeated thrice after which NPs pellets were resuspended in 10

mL of DIW with 0.5 g of trehalose (i.e., 5%, w/v) dissolved to serve as a cryoprotectant in a preweighed 20 mL screw-capped glass vial. The glass vials were then kept overnight in a deep freezer at  $-80^{\circ}\text{C}$  and was subjected for lyophilization for 2 days at a temperature of  $-50^{\circ}\text{C}$  and reduced pressure of 0.002 mbar. The freeze-dried NPs vials were sealed and kept at  $4^{\circ}\text{C}$  until subjected for further analysis.

**Table 1: Formulation of Sodium alginate -plain nanoparticles (SA-NPs)**

Formulation	Ingredients (%w/v)						
	Sodium alginate	Lecithin	Poloxamer	PVA	Acetone	DCM	Distilled water to
F1	1	1	0.2	-	4	8	100
F2	1	1	0.5	-	4	8	
F3	1	1	1	-	4	8	
F4	1	1	-	0.2	4	8	
F5	1	1	-	0.5	4	8	
F6	1	1	-	1	4	8	

#### Preparation of Celecoxib loaded nanoparticles:

Celecoxib-loaded NPs were prepared by the same method with Celecoxib dissolved in the organic phase at a concentration of 0.05%. The optimized Celecoxib-loaded NPs were incorporated in the above mentioned plain ophthalmic formulations to obtain a final concentration of Celecoxib equivalent to 0.1% (w/v). The final formulation was stirred well using a magnetic stirrer (600 rpm) and then placed in clean, dry, and sterile glass containers and kept at  $4^{\circ}\text{C}$  until subjected for further analyses.

#### Evaluation of Ophthalmic Formulations Containing Celecoxib-Loaded NPs:

##### Measurement of pH:

One gram of each formulation was suspended in 20 mL of distilled water and the pH was determined using a calibrated pH meter.

##### Determination of the Formulations Viscosity:

Viscosity of the formulations was measured by Brookfield viscometer at 10 rpm using spindle 61 for the purpose of comparative evaluation of viscosity before and after formation of *in situ* gel. 133

##### Drug Content Uniformity:

One gram of each formulation was placed in a volumetric 100 mL measuring flask. Five milliliters of DMSO was added, and the flask was shaken for 30 min. Subsequently, 50 mL of ethyl alcohol was added and the flask was shaken for additional 30 min after which it was brought to volume by ethyl alcohol. The

solution was centrifuged for 15min at 10,000 rpm and passed through a syringe filter of pore size 0.22  $\mu\text{m}$ . The obtained clear solution was assayed spectrophotometrically at 252nm for its drug content with appropriate blanks.

##### In Vitro Release Study:

The *in vitro* release study of Celecoxib from the formulation was carried out using the dialysis membrane. The freshly prepared simulated tear fluid (pH 7.4) was used as diffusion medium. A dialysis membrane soaked overnight in the diffusion medium was tied from both ends and 2ml volume of the formulation was accurately pipetted into this assembly. The dialysis membrane was dispersed in a beaker containing 100 ml of diffusion medium at  $(37 \pm 0.5)^{\circ}\text{C}$ . This assembly was kept on magnetic stirrer at 50 rpm. The samples were diluted with dissolution medium and analyzed by UV Spectrophotometer at 252nm. 13

##### In vitro Cytotoxicity:

MTT assay is one of the most commonly used colorimetric assays to evaluate cytotoxicity or cell viability 134. This assay determines principally cell viability through determination of mitochondrial function of cells by measuring activity of mitochondrial enzymes such as succinates dehydrogenase. In this assay, MTT is reduced to purple formazan by NADH, This product can be quantifies by light absorbance at a specific wavelength.

**Stability studies:**

The purpose of work was to study the effect of different storage temperatures on physical and chemical stability of the ophthalmic formulations containing Celecoxib-loaded nanoparticles.

The optimized ophthalmic formulations were packed in air tight glass vials and stored at different temperatures (30, 35 and 45°C) for six months. Initial samples were analysed then samples were withdrawn at predetermined time intervals 1, 2, 3, 4, 5 and 6 months. The samples were examined for their drug content, pH and viscosity.

**Celecoxib-loaded SA, CS-NPs were evaluated as follows:**

**Formulations pH**

The pH of lacrimal fluid is 7.4 and because of their natural buffering capacity, the eye can tolerate ophthalmic formulations with a wide pH range (i.e., from pH 3.5 to 8.5). Because the typical ophthalmic dose is only 1 or 2 drops, the tear film can rapidly restore neutral pH, if necessary.<sup>153</sup> The results given in Table 7.4 showed that all the ophthalmic formulations have pH values ranging from 7.18 to 7.86, which are ideal values that can be easily tolerated by the eye without any itching or discomfort.

**Table 2:** Formulations Containing Optimized Celecoxib-Loaded NPs pH

Formulation			pH
SA-NPs	F1	Eye drops	7.21±0.20
	F2	<i>In situ</i> gel	7.18±0.12
	F3	Preformed eye gel	7.37±0.46
Chitosan-NPs	F4	Eye drops	7.18±0.73
	F5	<i>In situ</i> gel	7.86±0.06
	F6	Preformed eye gel	7.77±0.02

**Formulations Viscosity and rheological behaviour:**

The viscosity of ophthalmic preparations is given in Table 2 and the rheological profiles of ophthalmic formulations containing Celecoxib loaded SA, CS-NPs are given in Figure 2. The rheological profiles of eye drops showed that the eye drop formulations followed Newtonian flow behaviour, as they exhibited constant viscosities at various shear rates. These results are in agreement with those obtained by Wu et

al.<sup>14</sup> who observed that formulations containing HPMC alone exhibited Newtonian flow behaviour under both physiological and non physiological conditions. While the rheological profiles of *in situ* gelling systems (Figure 2) showed that they followed non-Newtonian pseudoplastic (i.e., shear thinning) flow behaviors, as they exhibited high viscosities at lower shear rates and the viscosity decreased as the shear rate increased.<sup>155</sup>

**Table 3:** Formulations Containing Optimized Celecoxib-Loaded NPs Viscosity

Formulation			Viscosity (cp)
SA-NPs	F1	Eye drops	59.75
	F2	<i>In situ</i> gel	1275.58
	F3	Preformed eye gel	4283.00
CS-NPs	F4	Eye drops	70.28
	F5	<i>In situ</i> gel	1278.07
	F6	Preformed eye gel	5781.57

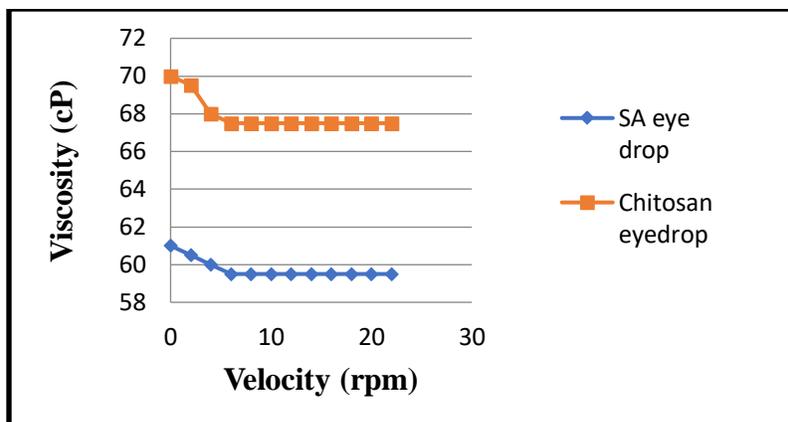


Figure 2: Rheological profiles of Celecoxib-loaded SA, CS-NPs eye drops

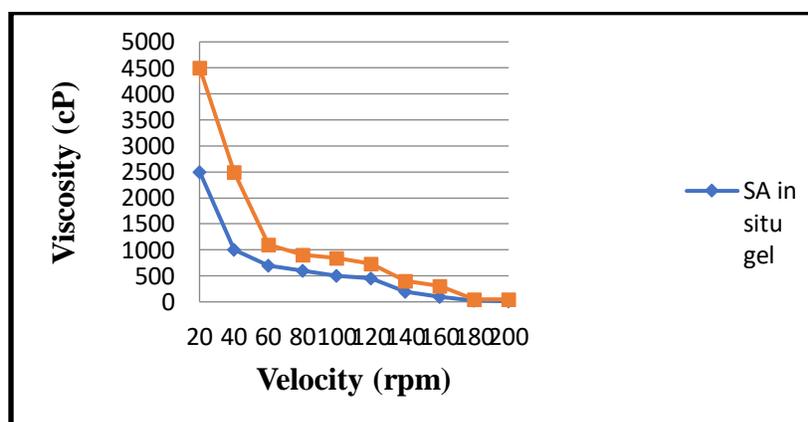


Figure 3: Rheological profiles of Celecoxib-loaded SA, CS-NPs *in situ* gel

### Drug Content

As shown in Table No 4, the actual Celecoxib content of the ophthalmic formulations ranges from 97.52% to 102.87% of the actually incorporated Celecoxib-loaded NPs. These results showed that the deviation of the drug content from the actually added active

constituents is less than  $\pm 3\%$ , which complies with USP official standards<sup>155</sup>. The small values of the standard deviation indicate a uniform distribution of the Celecoxib-loaded NPs in the ophthalmic formulations.

Table 4: Formulations Containing Optimized Celecoxib-Loaded NPs drug content

Formulation			Drug content (%)
SA-NPs	F1	Eye drops	97.52 $\pm$ 1.2
	F2	<i>In situ</i> gel	101 $\pm$ 1.1
	F3	Preformed eye gel	100.07 $\pm$ 1.04
CS-NPs	F4	Eye drops	100.89 $\pm$ 1.2
	F5	<i>In situ</i> gel	101.27 $\pm$ 1.32
	F6	Preformed eye gel	102.87 $\pm$ 2.7

**In Vitro Release Study:**

The *in vitro* release study was conducted for 24 h, as the prepared formulations are proposed for ocular use. The results are plotted as release profile (Figure 3). From the results it was confirmed that it is possible to prepare sustained release ophthalmic formulations containing Celecoxib-loaded Sodium alginate, Chitosan nanoparticles. It was observed that all the formulations exhibited a sustained drug release rate that is free from any burst release that may cause a toxic effect. It is known that the burst release is a characteristic of drugs released from rigid NP systems, which is due to the rapid dissolution of the drug molecules located at or near the NPs surface upon dilution under the sink conditions of the release experiment. 156

The percentage of the cumulative amount of Celecoxib released after 24 h from eye drops, gel, and the *in situ* gelling system, respectively, are 49.5%, 41.3%, and 37.1% for SA-NPs preparations; 37.9%, 31.8%, and 33.3% for CS -NPs preparations.

**CONCLUSION:**

NPs offer several advantages such as longer shelf life, being prepared from intact and safe materials and have the ability to permeate through principal mucosal barriers, such as the intestinal, nasal, and ocular barriers.<sup>43</sup> The optimized NP formulations of Celecoxib and Moxifloxacin have desirable particle sizes, zeta potential, and surface morphology. The prepared formulations also possess pH and viscosity values that are compatible with the eye. Moreover, they have uniform drug content that complies with the USP official requirement. *In vitro* release data of ophthalmic formulations demonstrate a sustained release free from any burst effect, and the formulations follow a Higuchi non-Fickian diffusion mechanism. The *in vitro* cytotoxicity studies reveal that all the prepared formulations are nontoxic. NPs as a novel drug delivery system are good source for drug targeting and sustained release, especially when prepared from bio-adhesive materials. Unfortunately, NPs usually suffer from the lack of stability, especially in aqueous systems. Also, topical route is considered to be the best method for drug delivery to the eye due to the ease of application and patient acceptance. However, this route suffers from poor bioavailability due to the rapid drainage of the formulation from the eye, as only about 5% of the topically applied dose is absorbed. Therefore, the production of stable NP-based ophthalmic formulations that possess a bio adhesive effect and can sustain the drug effect for prolonged period is remedy to this disadvantage. All the prepared formulations possessed desirable shelf

lives, especially the SA and CS-NPs in situ gel and PCL and PLA-NPs in situ gel.

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