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FORMULATION AND CHARACTERIZATION OF EUDRAGIT RS 100 NANOSUSPENSION FOR OCULAR DELIVERY OF INDOMETHACIN

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ABSTRACT

Several colloidal drug delivery systems were designed to obtain a control release for ophthalmic applications were investigated. These are composed of a polymeric support with or without drug. The drug was normally incorporated as a dispersion or solution in the polymeric support. The main objective of the nano suspension is to increase the contact time of the preparation with conjunctiva tissues so as to ensure a Sustained / Controlled release suited for the topical or systemic treatment. However the details about the preparations and their model of construction are given in many literatures but no specific guideline are mentioned. In the present work, a revival attempts has made us to investigate Indomethacin ocular nano suspension utilizing Eudragit RS 100 as drug reservoir membrane and rate controlling component and its possible utilization in designing and evaluating it for ophthalmic use. All formulations were subjected to various evaluation parameters like identification and compatibility studies of drug and polymer using IR spectrometer and were evaluated for parameters like pH, particle size analysis, drug entrapment efficiency, rheological studies; rabbit eye irritation, *in vitro* release studies, *in vivo* drug studies and stability studies. The uniformity of the nano suspension revealed that the drug was uniformly distributed throughout the nano suspension. Increase in the polymer ratio increases the entrapment efficiency of drug which observed by entrapment efficiency studies. Further future work can be progressed to establish the therapeutic utility of these systems by pharmacokinetic and pharmacodynamic studies in human beings. The different Novel Technology can be adopted in the formulation for ophthalmic nano suspensions and thereby investigate their effect on release pattern.

KEYWORD: Introduction, Drug Profile, Experimental Investigation, Drug and Polymer Profile, Summary.

INTRODUCTION

Topical application of drugs to the eye is the most popular and well- accepted route of administration for the treatment of various eye disorder. The bioavailability of ophthalmic drug is however very poor due to efficient protective mechanisms of the eye. Blinking, base line and the reflex lachrymation and drainage remove rapidly foreign substances including drug from the surface of the eye. Frequent instillation of the eye drops is necessary to maintain a therapeutic drug level in the tear film or at the site of action. But the frequent use of highly concentrated solutions may induce toxic side effects and cellular damage at the ocular surface[1]. Drug delivery in ocular therapeutics is a challenging problem and is a subject of interest to scientists working in the multi-disciplinary areas pertaining to the eye, including chemical, and biochemical, pharmaceutical, medical, clinical, and toxicological sciences. Recently, increased attention has been focused on two main objectives.

(A) To find or tailor make newer, effective, and safe drug molecules for various ocular conditions and diseases that are poorly controlled.

(B) To improve existing ocular dosage forms and exploits newer delivery systems for improving the ocular bioavailability of existing molecules. Current trends in ocular therapeutics and drug delivery suggest that the existing dosage forms will be replaced by novel drug delivery systems. That offer improved biopharmaceutical properties with the capability to deliver therapeutic agents more precisely to targeted receptors in the eye in a predictable manner. Drugs are commonly applied to the eye for a localized action on the surface or in

the interior of the eye. A major problem in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Poor bioavailability of drugs from ocular dosage form is mainly due to the precorneal loss factors which include tear dynamics, on-productive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane. Due to these physiological and anatomical constraints, only a small fraction of the administered drug, effectively 1% or even less of the instilled dose is ocularly absorbed .This forces the clinician to recommend a frequent dosing at an extremely high concentration and pulse type dosing results in several side effects of ophthalmic products. In order to overcome the problems of conventional ocular therapy such as short residence time, drug drainage, and frequent instillation; newer delivery systems are being explored, in general, to improve the ocular bioavailability of the drug. Various approaches, like viscosity enhancement, use of muco adhesive, particulate drug delivery, vesicular drug delivery, prodrugs, and other controlled systems, like ocuserts are being explored since conventional dosages suffers from a serious disadvantage of poor bioavailability due to several biological factors (Fig. 1), which exist to protect the eye and consequently limit the entry of ocular drugs.

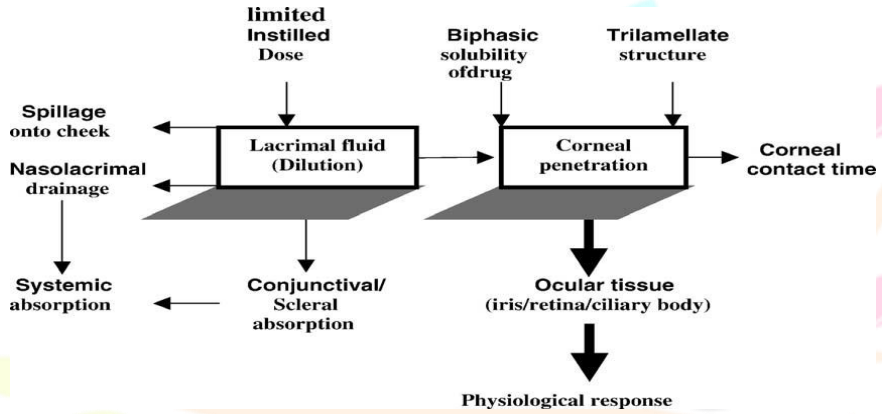


Fig No: 1. Factors attributing to poor bioavailability of ophthalmic formulation

To design effective drug delivery approaches, it is first necessary to understand the relevant anatomical and physiological constraints that impede or modify ocular drug and vehicle.[2]

1.2. OCULAR PHARMACOKINETIC & PHARMACODYNAMIC[4]:

S.No.	Pharmacokinetic parameter	Rabbit	Human
1	Tear volume (µl)	5-10	7-30
2	Tear turn over rate(µl/min)	0.5-0.8	0.5-2.2
3	Spontaneous blinking rate	4-5 times/hr	6-15time/hr
4	Bowman's membrane	Partially absent	Present
5	Nictating membrane	Present	Absent
6	pH of lacrimal fluids	7.3-7.7	7.3-7.7
7	Lacrimal fluid turn over rate	7	16
8	Buffering capacity of lacrimal fluids (% min^{-1})	Poor	Poor
10	Corneal thickness(mm)	0.35-0.45	0.52-0.54
11	Corneal diameter(mm)	15	11-12
12	Corneal surface area(cm^2)	1.5-2	11-12
13	pH of aqueous humor	8.2	7.1-7.3
14	Aqueous humor volume(ml)	0.25-0.3	0.1-0.25
15	Aqueous humor turn over rate (µl/min)	3-4.7	2-3

Table NO: 1. Anatomical and Physiological difference between New Zealand Rabbit and Human eye pertinent to ophthalmic pharmacokinetic

a) Absorption: Absorption is somewhat more complex to estimate in the eye, since lag time and drainage lengthen and shorten respectively, over the length of time that the absorption process is operative. For example: The first order absorption rate constant for phenylephrine is 4.5×10^{-5} min^{-1}, which gives a half life of 128.3 hr, however, drainage permits drug to remain at the corneal absorption site for approximately 3-6min only, depending on the volume and viscosity of the instilled solution. Consequently the absorption process is abruptly terminated from theoretical expectations. The short residence time of drug at the absorption site results in

exceptionally poor bioavailability.

b) Distribution Phase: This distribution phase can be identified visually as the concave portion of the log concentration time curve immediately following the time to peak. The latter log linear phase is the elimination or post distributive phase. The distribution phase, which is expected to be shorter than the elimination phase, cannot be visually identified as easily as absorption and elimination.

1.3. MECHANISM OF OCULAR ABSORPTION[2, 5]:

Topical delivery into the cul-de-sac is by far, the most common route of ocular drug delivery. Absorption from this site may be

(i) Corneal
(ii) Non-Corneal

The non-corneal route of absorption involves penetration across the sclera and conjunctiva into the intraocular tissues. This mechanism of absorption is usually not productive, as drug penetrating the surface of the eye beyond the corneal-scleral limbs is picked up by local capillary beds and removed to the general circulation. This non-corneal absorption in general precludes entry into the aqueous humor. The non-corneal route of administration may be significant for drug molecules with poor corneal permeability. Studies with insulin, timolol maleate, gentamicin, suggests that these drugs gain intraocular access by diffusion across the conjunctiva and sclera.

Corneal absorption: Represents the major mechanism of absorption for most therapeutic entities. Topical absorption of these agents is considered to be rate limited by the cornea. The anatomical structures of the cornea exert unique differential solubility requirements for drug candidates. Cornea can be viewed as a trilaminate structure consisting of these major diffusional barriers.

(i) Epithelium
(ii) Stroma
(iii) Endothelium

Out of three, the epithelium and endothelium contains on the order of 100 fold the most of lipid material than stroma. Depending on the physicochemical properties of the drug entity, the diffusional resistance offered by the tissues varies greatly. The outermost layer, the epithelium, represents the rate-limiting barrier for transcorneal diffusion of most hydrophilic drugs. The flattened epithelial cells preclude paracellular transport of most ophthalmic drugs and limits lateral movement within the anterior epithelium. Corneal surface epithelial intracellular pore size has been estimated to be about $60A^{0.}$ Hence small ionic and hydrophilic molecules appear the gain access to the anterior chamber through these pores. However for most drugs paracellular transport is precluded by the interjunctional complexes. The stroma comprises 85-90% of the total corneal mass and is composed mainly of hydrated collagen. The stroma exerts a diffusional barrier to highly lipophilic drugs owing to its hydrophilic nature. There are no tight junction complexes in the stroma and paracellular transport through this tissue is possible. The innermost endothelium is lipoidal in nature. However it does not offer a significant barrier to the transcorneal diffusional of most drugs. Studies have shown that endothelial permeability depends solely on molecular weight and not on the charge or hydrophilic nature of the compound. Transcellular transport across the corneal epithelium and stroma is the major mechanism of ocular absorption of topically applied ophthalmic pharmaceuticals. This type of Fickian diffusion is dependent upon many factors. i.e., surface area, diffusivity, the concentration gradient established and the period over which the concentration gradient can be maintained. The productive absorption of most ophthalmic drugs results from diffusional process across the corneal membrane

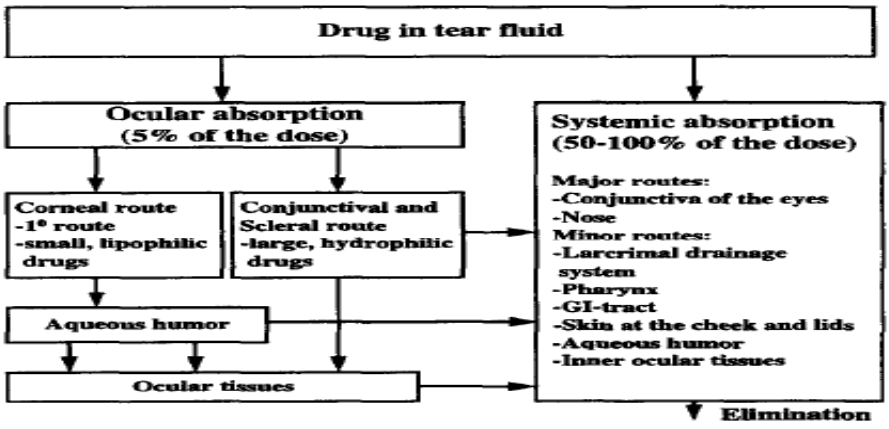


Fig. no.-03

Factors affecting corneal transport:

1. The physiological properties of the drug substance like ionization constants, aqueous, oil/water partition coefficients.
2. The formulation in which the drug is prepared e.g. pH of the solution, types, and concentrations of buffers, viscosity inducing agents and stabilizers.

1.4 OCULAR BIOAVAILABILITY[5]:

The topical application of opthalmically active drugs to the eye is the most prescribed route of administration for the treatment of various ocular disorders. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. Upon instillation of an ophthalmic solution most of the instilled volume is eliminated from the pre-corneal area, this loss mainly due to

drainage of the excess fluid by the nasolacrimal duct and dilution and elimination of the solution by tear turnover and results in poor ocular bioavailability.

Factors affecting intraocular bioavailability:

1. The presence of lacrimal fluid in the cul-de-sac dilutes the drug solution instilled into the pre-corneal area of the eye, and the continual inflow and outflow of lacrimal fluid can also cause a significant loss of applied drug.
2. Drug kinetics in the conjuctival cul-de-sac that is pre-corneal.
3. The efficient nasolacrimal drainage, acts as a conduit through which an instilled drug solution may be drained away from the pre-corneal area.
4. Spillage of drug by over flow.
5. Dilution of drug by tears turns over.
6. Enzymatic metabolism.

1.6. NANOSUSPENSIONS:

Controlled drug delivery technology represents one of frontier areas of science, which involves multidisciplinary scientific approach contributing to human health care. These delivery systems offer numerous advantages compared to conventional dosage forms, which improved efficacy, reduced toxicity and improved patient compliance and convenience. Such systems often use nanoparticles as carrier for the drug. This field of pharmaceutical technology has grown and diversified rapidly in recent years. Understanding the derivation of the methods of controlled release and the range of new polymers can be a barrier to involvement from the nanospecialist of the different dosage forms reported, nanoparticles attained much importance due to a tendency to accumulative in inflamed areas of the body. Nanoparticles for their attractive properties occupy unique position in drug delivery technology.

1.6.2: Definition of Nanosuspension[7, 8]:

Nanosuspension can be defined as colloidal dispersions of nano-sized drug particles that produced by suitable method and stabilized by a suitable stabilizer. These can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. Nanosuspensions have some outstanding features which include…

- ✓ Increased saturation solubility
- ✓ Increased dissolution velocity
- ✓ Prolong residence time in cul-de-sac
- ✓ Avoidance of high tonicity created by water soluble drugs.

The macromolecular material from which Nanosuspension are made can be of synthetic or natural origin.

Two different types of nanoparticles can be obtained:-

1) Nanospheres- consist of dense polymeric matrix in which the drug can be dispersed.
2) Nanocapsule- they are constituted of a liquid core surrounded by a polymeric shell.

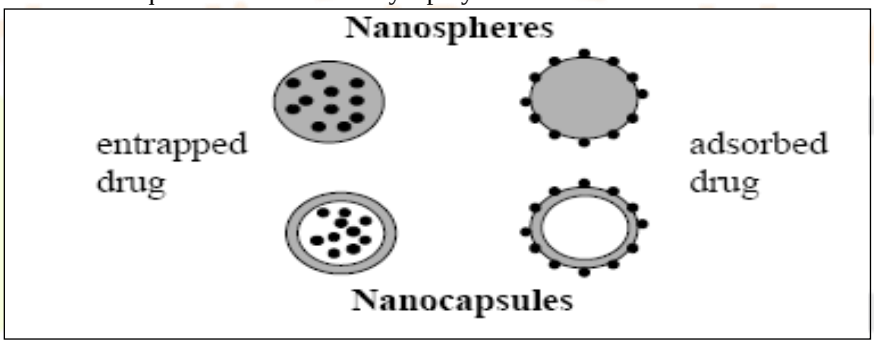


Fig no.-04 Nanospheres & Nanocapsule

1.6.3: Advantage of Nanosuspension:

1) They are biodegradable, non-toxic, site- specific and capable of being stored for at least one year.
2) They are capable of targeting a drug to specific site in body.
3) They offer controlled rate of drug release.
4) They offer better therapeutic effectiveness and overall pharmacological response/ unit dose.
5) Possess better stability and high drug entrapment efficiency as compared to liposomes.
6) They can be freeze- dried so obtained in dry power form.
7) Inexpensive alternative to liposomes.

1.6.4: Limitations:

1) Presents biocompatibility restrictions.
2) Difficult to manufacture in large quantities.

1.7.2. Homogenization in Water (Dissocubes)

R.H.Muller developed Dissocubes technology in 1999. The instrument can be operated at pressure varying from 100 – 1500 bars (2800 –21300psi) and up to 2000 bars with volume capacity of 40ml (for laboratory scale). For preparation of nano suspension, we have to start with the micronized drug particle size less than 25µm to prevent blocking of homogenization gap hence it is essential to prepare a presuspension of the micronized drug in a surfactant solution using high speed stirrer.

1.7.3. Homogenization in Nonaqueous Media (Nanopure)

The drugs that are chemically labile can be processed in such nonaqueous media or water-miscible liquids like polyethyleneglycol-400 (PEG), PEG1000 etc. The homogenization can be done at room temperature, 0° C and below freezing point (-20°C)

1.7.4. Combined Precipitation and Homogenization (Nanoedege)

The precipitated drug nanoparticles have tendency to continue crystal growth to the size of microcrystals. They need to be processed with high-energy forces (Homogenization). The are in completely amorphous, partially amorphous or completely crystalline which create problems in long term stability as well as in bioavailability, so the precipitated particle suspension is subsequently homogenized which preserve the particle size obtained after the precipitation step.

1.8. DRUG RELEASE FROM NANOSUSPENSION:

Drug from nanosuspension is released by following mechanism:-

❖Desorption of surface bound drug
❖Diffusion through nanoparticle matrix
❖Diffusion through polymer wall of nanoparticles
❖Nanoparticle matrix erosion
❖Combined erosion diffusion process.

Factors which govern drug release rate:

❖Release mechanism
❖Diffusion coefficient
❖Bio- degradation rate

1.9. CHARACTERIZATION OF NANOSUSPENSION: [8]

- Particle size and size distribution
- Zeta potential
- Drug loading efficiency
- Dissolution/ diffusion velocity
- Adhesion properties
- Stability studies.

EXPERIMENTAL INVESTIGATION

7.1 PREFORMULATION STUDIES: Preformulation testing is the first step in the rational development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms which can be mass produced.

The FT-IR spectrum of pure Indomethacin, Eudragit RS 100 and physical mixture of Indomethacin, Eudragit RS 100 were analyzed for compatibility study.

7.2. PREPARATION OF STANDARD CURVE

Preparation of Phosphate Buffer pH 7.4[45]: 50.0 ml of 0.2 M potassium di-hydrogen phosphate was placed in a 200 ml volumetric flask, added the specified volume of 39.1 ml of 0.2 M sodium hydroxide and then made up to the volume by water.

Potassium di-hydrogen phosphate, 0.2 M: 27.218 g of potassium di-hydrogen phosphate was dissolved in distilled water and diluted to 1000 ml.

Sodium hydroxide solution 0.2 M: 8 g of sodium hydroxide was dissolved in distilled water and diluted to 1000ml.

7.2.1 Preparation of Standard Curve of Indomethacin with Phosphate Buffer pH7.4: 100 mg of Indomethacin was accurately weighed and dissolved in a small portion of phosphate buffer pH 7.4 in a 100 ml volumetric flask then the volume was made up to 100 ml with phosphate buffer pH 7.4. This was primary stock solution, contained 1000 µg/ml. From this primary stock solution 10 ml was pipetted out and transferred in to a 100 ml volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4 which contained the concentration of 100 µg/ml. From the second stock solution again 10 ml was pipetted out and diluted up to 100 ml with phosphate buffer pH 7.4 to get concentration of 10 µg /ml. From third stock solution aliquots equivalent to 1-10 µg (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml) were pipette out in to a series of 10 ml volumetric flask and volume was made up to 10 ml with phosphate buffer pH 7.4.

The absorbance of these solutions was measured against the phosphate buffer pH 7.4 as blank at 320 nm using UV- Visible double beam spectrophotometer. Then a calibration curve was plotted taking concentration in µg/ml on X-axis and absorbance on Y-axis.

7.3. PREPARATION OF NANOSUSPENSION: [46, 26, 47] Nanosuspensions were prepared by the quassi- emulsion solvent diffusion technique. Nanosuspension was prepared by using different drug to polymer ratio. Quantity of drug in all formulation was kept constant i.e. 100 mg.The different ratio of drug and polymer is as given in table no.4. The drug and polymer were co- dissolved at room temperature in ethanol (5 ml) and sonicated for 10 minutes. The solution was slowly injected with syringe into 45 ml water containing Tween 80 (0.02 %w/v) and benzalkonium chloride (0.1 % w/v) and kept at low temperature in an ice water bath. During injection the mixture was mixed by mechanical stirring (propeller 4000 rpm) for one hour. The solution immediately turned into a pseudo-emulsion of the drug and polymer-ethanol solution in the external aqueous phase. The counter diffusion of ethanol and water out of and into the micro droplets. After completion of stirring the solution dispersion was subjected to ultra sonication for a period of 10 minutes. The gradual evaporation of the organic solvent determined the *in situ* preparation of the polymer and the drug with the formation of matrix type nanoparticles. Ethanol residues were left to evaporate off under slow magnetic stirring of the nanosuspensions at the room temperature for 8-12 hours. Using this above method 5 formulations of nanosuspension FN-1, FN-2, FN-3, FN-4 and FN-5 were prepared by varying polymer concentration.

S.No.	Ingredients	FN-1	FN-2	FN-3	FN-4	FN-5
1.	Indomethacin (% w/w), mg	100	100	100	100	100
2.	Eudragit RS 100 (%w/w), mg	500	400	300	200	100
3.	Tween 80 (% w/v)	0.02	0.02	0.02	0.02	0.02
4.	Benzalkonium chloride (% w/v)	0.1	0.1	0.1	0.1	0.1
5.	Ethanol (ml)	5.0	5.0	5.0	5.0	5.0
6.	Water q.s. to (ml)	50	50	50	50	50

Table no.04: Formulation of different batches of Indomethacin Nanosuspension.

7.4. EVALUATION OF NANOSUSPENSION:

7.4.1. pH:[26] pH is one of the important factors involved in the formulation process. Two zones of basic significance are the impacts of pH on solvency and security. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and same time there would be no irritation to the patient upon administration of the formulation. The pH of the prepared formulations was checked by using pH meter.

7.4.2. Particle Size and surface morphology:[26,46] Particle size analysis was done by Scanning Electron Microscopy (SEM).SEM is the most commonly used method for characterizing drug delivery systems, due to simplicity in sample preparation and ease of operation. The three dimensional information about macro – (0.1- 10 nm) micro (1- 100 nm) & nanostructure (10-1,000 nm), is often found within the same micrograph. SEM has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface.

Cleaned brass specimen studs were used for mounting the samples. Wet solvent paint was applied on these studs and while the paint was wet, the pellets were placed on each studs and allowed to dry. Then the sample was observed in scanning electron microscope and photographs were taken.

7.4.3. Determination of Drug Entrapment Efficiency:[46,48] The percentage of incorporated indomethacin (entrapment efficiency) was determined spectro photometrically at 320 nm. After centrifugation (5000rpm, for 5 min.) of the aqueous suspension, amount of the free drug was detected in the supernatant and the amount of incorporated drug was determined as the result of the initial drug minus the free drug.

The percentage efficiency (EE %) could be achieved by the following equation.

$$\text{Entrapment efficiency (\%)} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

7.4.4. Rheological studies:[49] Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The viscosity determinations of prepared formulations were carried out using Brookfield DV-111 + Rheometer with spindle cp-40. The prepared suspensions were allowed to measure the viscosity. Viscosities of samples were measured at different angular velocities. Atypical run comprised changing angular velocity from 10-100 rpm with equal wait for each rpm. The hierarchy of angular velocity was reversed (100-10 rpm) with similar wait. The average of two readings was used to calculate the viscosity.

7.4.5. Zeta potential:[50, 51] The particle charge is one of the factors determining the physical stability of emulsions and suspensions. The higher particles are equally charged, the higher is the electrostatic repulsion between the particles and the higher is the physical stability. Typically the particle charge is quantified as called zeta potential, which is measured e.g. via the electrophoretic mobility of the particles in an electrical field. On the other hand the molecule charge can be evaluated in surface charge per surface unit, dictated by colloid titration. Zeta potential is a condensing for electrokinetic potential in colloidal frameworks. In the colloidal science writing, it is normally meant utilizing the Greek letter zeta, thus ζ-potential. From a hypothetical perspective, zeta potential is electric potential in the interfacial twofold layer (DL) at the area of the slipping plane versus a point in the mass liquid away from the interface.

S.No.	Zeta Potential [mV]	Stability behavior of the colloid
1.	from 0 to ±5,	Rapid coagulation or flocculation
2.	from ±10 to ±30	Incipient instability
3.	from ±30 to ±40	Moderate stability
4.	from ±40 to ±60	Good stability
5.	more than ±61	Excellent stability

Table no.6: Zeta potential & Stability behavior of Nanosuspension

7.4.6. Differential scanning calorimetry*:* [14,52] DSC is an important evaluation technique to find any possible interaction between drug and polymer. Any such cooperation prompts diminish entanglement effectiveness of polymer and furthermore adequacy of medication. Differential filtering calorimetric investigation was performed utilizing Shimadzu DSC-60 framework. Polymeric sample of nanosuspension was sealed in aluminum cells and set in a shimadzu DSC-60 apparatus between 30ºC - 300ºC. Thermal analysis was performed at a heating rate maintained at a 10ºC per minute in a nitrogen atmosphere. Alumina was used as the reference substance.Enthalpy changes (ΔH) were calculated from peak areas of samples and to study the polymeric changes in formulations.

7.4.7. *In vitro* Drug Release Studies*:* [15, 47] The *in vitro* release of indomethacin from a formulation was studied through Dialysis membrane-100 (cut of: 350 Da) using modified apparatus. The disintegration medium utilized was newly arranged 0.14 M phosphate support arrangement (pH 7.4).Dialysis layer - 100, recently absorbed for the time being the disintegration medium was attached to one finish of an explicitly planned glass chamber (open at both end). 5 ml of detailing was precisely positioned into this get together. The cylinder was attached to a stand and suspended in 50 ml of dissolution medium maintained at 37± 1º C so that the membrane just touched the receptor medium surface. The disintegration medium was blended at low speed utilizing attractive stirrer. Aliquots every one of 1 ml volume were pulled back at hourly stretches and supplanted by an equivalent volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV- VIS spectrophotometery at 320 nm To check the eventual limiting effects of the dialysis membrane on drug dissolution, separate experiments were run in duplicate with a solution of saline, freshly prepared 0.14 M phosphate buffer solution (pH 7.4) in pure indomethacin of the drug concentration in the nanosuspension.

7.4.8. Rabbit eye irritation*:* [53 ,15] Ocular irritation studies were performed on five male albino rabbits each weighing 1.5-2.2 kg The possible visual bothering or potentially harming impacts of the nanosuspension under test were assessed by watching them for any redness, irritation (or) expanded tear creation. Formulation was tested on four rabbits by dispensing nanosuspension in the cul-de-sac of the left eye. Both eyes of the rabbits under test were examined for any signs of irritation before treatment and observed up to 24 hours.

7.4.9. Kinetic modeling [54, 55``]

a) Zero order kinetics*:* Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation –

$$Q_t = Q_o + K_o t$$

Where, Q_t = Amount of drug dissolved in time t,
Q_o = Initial amount of drug in the solution and
K_o = Zero order release constant.

b) Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs incorporated in semisolids and or solid matrices. Numerical articulations were gotten for drug particles scattered in a uniform network acting as the dissemination media. And the equation is

$$Q_t = K_H . t^{1/2}$$

Where, Q_t = Amount of drug released in time t,
K_H = Higuchi dissolution constant.

c) Krosmeyer and Peppas release model: To study this model the release rate data are fitted to the following equation

$M_t / M_\infty = K.t^n$

Where M_t / M_∞ is the fraction of drug release, K is the release constant, t is the release time and n is the Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form. If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion.

7.4.10. Short term stability*:* [56]

Information on the stability of drug substance is an integral part of the systemic approach to stability evaluation. The purpose of stability testing is to provide evidence on how the quantity of a drug substance or drug product varies with time under influence of variety of environmental factors, for example, temperature, mugginess, and light and to build up a re-trial for drug substance or a time span of usability for the medication item and suggested stockpiling conditions.

Stability is defined as the extent to which a product remains within specified limit throughout its period of storage and use. A drug formulation is said to be stable if it fulfils the following requirements:

- It should contain at least 90% of the stated active ingredient.
- It should contain effective concentration of the added preservatives, if any
- It should not exhibit discoloration or precipitation nor develops foul odor.

- It should not develop irritation or toxicity.

- **Procedure*:***

From the 5 batches of Indomethacin loaded nanosuspension formulation FM-3 was tested for stability studies. Formulation was divided into 3 sample set and stored at:

- 4º C in refrigerator
- 27º C ± 2º C / 65% RH in humidity control oven
- 40º C ± 2º C / 65% RH in humidity control oven.

After 3 months drug content of all the samples were determined by the method discussed previously in entrapment efficiency section.

7.4.11. *In Vivo* Studies: [46, 47, 57] Indomethacin is well known anti-inflammatory, reducing prostaglandin synthesis, which found to be most effective against ocular infection. Hence here also an attempt made to determine its anti-inflammatory activity with the help of best formulation (FN_3) in comparison standard preparation. The study protocol was approved by institutional Animal Ethical Committee for the use of animal in research (proposal no.688/2/C/CPCSEA). *In vivo* were performed on groups of six male New Zealand albino rabbits weighing 1.8-2.2 kg, and with no signs of ocular inflammation or gross abnormalities. Animals were divided into two groups one for standard indomethacin as controlled preparation and another for formulation FN-3.The 50 µl of preparations were instilled into the conjunctival sac (left eye control preparation and right eye nanosuspension.) at 60,120,240 and 360 minute durations. To perform the paracentesis (The removal of fluid from a body cavity using a needle/puncture of the wall of a fluid filled cavity by means of a hallow needle to draw off the contents), animals were lightly anaesthetized with an intramuscular injections of ketamine hydrochloride 50 mg/kg, Xylazine 10mg/kg was instilled into the conjunctival sac. Aqueous humor samples from each animal were collected with a 26 gauge needle attached to tuberculine syringe. The needle was introduced into the anterior chamber through the cornea, tacking care not damage the iris, the lens and the anterior uvea. Eye conditions were examined using slit lamp every hour after paracentesis and Cyclopentolate hydrochloride (Cyclogik) eye drops were put to avoid inflammation.50 µl of aqueous humor were collected and analyzed by HPLC for drug concentration. For analysis the sample were mixed with an equal volume of methanol containing 6% v/v perchloric acid. After centrifugation (3 min at 12000 r.p.m), 20 µl of the supernatant was analyzed by HPLC at detection wavelength 254 nm.

Mobile phase = acetonitrile –water –acetic acid (65:35: 1 v/v)
Flow rate = 1.0 ml /min
Detection = 254 nm
Column = Phenomenex C18 column (Luna 5 µm, 250 × 4.6 mm).
The statistical significance of the differences between means of Indomethacin concentration values in aqueous humor was evaluated using an ANOVA test.

7.4.12. Sterility Testing[58]: One of the requirements of an ophthalmic preparation is its sterility. The tests for sterility are intended for detecting the presence of viable forms of microorganisms in ophthalmic preparations. These tests were carried out under conditions designed to avoid accidental contamination of the product during the test. The tests were based upon the principle that if micro-organisms are placed in a medium which contains nutritive material and water, and kept at a favorable temperature, the creatures will develop and their essence can be demonstrated by turbidity in the initially clear medium. In the present study, three media namely, Alternate thioglycolate medium (ATGM), Fluid thioglycolate medium (FTGM) and Soyabean casein digest medium (SCDM) were used to investigate the presence of aerobic, anaerobic organisms and fungi.

Alternate Thioglycolate Medium (ATGM) ATGM was used in order to detect the growth of anaerobic organism. 2.9 g of readymade ATGM, which contains L-cysteine, sodium chloride, dextrose, yeast extract (water soluble), pancreatic digest of casein and sodium thioglylcoate was dissolved in 100ml of distilled water and adjust with 1M NaOH when necessary. So that the medium will have a pH 7.1 ± 0.2 after sterilization at 121°c for 15 minutes and at 15 pounds pressure in an autoclave.

Soyabean Casein Digest Medium (SCDM) This media is useful for the detection of aerobic microorganisms and fungi. 3g of readymade SCDM, which contains pancreatic digest of casein, papaic digest of soyabean meal, sodium chloride, dibasic potassium phosphate and dextrose dissolved in 100ml of distilled water and adjust with 1M NaOH when necessary. So that the medium will have a pH 7.1 ± 0.2 after sterilization at 121°C for 15 minutes and at 15 pounds pressure in an autoclave. The medium was freshly prepared or heated in steam bath and was allowed to cool just prior to use.

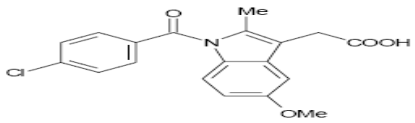
Fluid Thioglycolate Medium (FTGM) FTGM was used in order to detect the growth of aerobic micro organism. 3gm of readymade FTGM, which contains cystine, agar, sodium chloride, glucose, yeast extract, casein, thioglycollic acid, resazurin dissolved in 100ml of distilled water and adjust with 1M NaOH when necessary. So that the medium will have a pH of 7.1 ± 0.2 after sterilization at 121°C for 15 minutes and at 15 pounds pressure in an autoclave. The medium was freshly prepared or heated in steam bath and was allowed to cool just prior to use.

The ideal batch of nanosuspension was surface sterilized by exposing to ultraviolet radiation for 10 minutes and inoculated into the above mentioned test mediums and incubated at 37°C for 7-14 days to investigate the presence of Aerobic and Anaerobic organisms. And to investigate the presence of fungi the SCDM medium is incubated at 25°C for 7-14 days. Observations were taken from 1-7 days and at the end of 14 days.

DRUG PROFILE [40,41]

Drug: Indomethacin

Structure:



IUPAC name: [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl] acetic acid

Empirical formula: $C_{19}H_{16}ClNO_4$

Molecular weight: 357.8

Synonym: Indomethacin

Melting point: 158°C to 162°C

Description: A white or yellow, crystalline powder.

Solubility: Practically insoluble in water. It is soluble in ethanol, dichloromethane, ether, acetone and castor oil.

Stability/Storage: Stable at room temperature, Store in a well- closed container, protected from light.

Dosage Forms: Capsule (Indocin®): 25 mg, 50 mg Capsule, sustained release (Indocin® SR): 75 mg Injection, powder for reconstitution, as sodium trihydrate (Indocin® I.V.): 1 mg Suspension, oral (Indocin®): 25 mg/5 mL (237 mL) [contains alcohol 1%; pineapple-coconut- mint flavor.

Dose: Ophthalmic 1% w/v one drop three times daily.

Mechanism of Action: Inhibits prostaglandin synthesis by decreasing the activity of the enzyme, cyclooxygenase, which results in decreased formation of Prostaglandin precursors.

Pharmacodynamics/kinetics:

Onset of action : ~30 minutes
Duration : 4-6 hours
Absorption : Prompt and extensive
Distribution : V_d: 0.34-1.57 L/kg; crosses placenta; enters breast milk
Protein binding : 90%
Metabolism : Hepatic; significant enterohepatic recalculation
Plasma half-life : 2-5 hours
Time to peak : Oral: ~3-4 hours
Excretion : Urine (primarily as glucuronide conjugates)

Adverse effects: A high incidence (upto 50 %) of gastrointestinal and CNS side effects is produced. Gastric irritation, nausea, anorexia, gastric bleeding and diarrhea are prominent. Frontal headache (very common), dizziness, ataxia, mental confusion, hallucination, depression and psychosis.

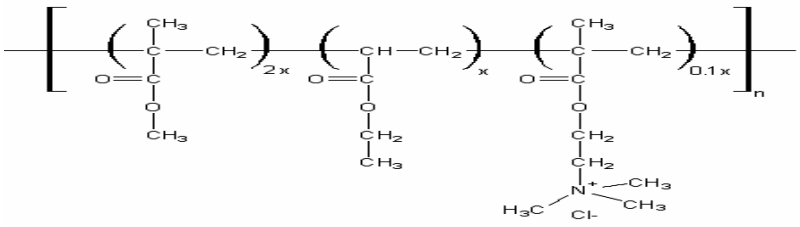
Applications: Indomethacin is an anti-inflammatory, non-steroid agent that's blocks prostaglandin Biosynthesis by inhibiting cyclooxygenase. This inhibitory activity is relevant to cancer chemoprevention because cyclooxygenase catalyzes the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandins which can stimulate tumor cell growth and suppress immune surveillance. Indomethacin eye drop is available in the market for the treatment of various eye diseases such as Uveitis, cystoid macular oedema, prevention of miosis during ocular surgery and other ocular inflammatory conditions.

POLYMER PROFILE [42, 43]

EUDRAGIT RS 100

Chemical name: Poly (ethyl acrylate, methyl methacrylate) trimethylammonioethyl methacrylate chloride.

Structural Formula:



Molecular weight: 1, 50,000

Functional category: Film former, Tablet binders, Tablet diluents.

Behavior in Digestive Juices: Insoluble film of low permeability.

Bulk Density: 0.390 g/cm^3

True Density: 0.816 - 0.836 g/cm^3

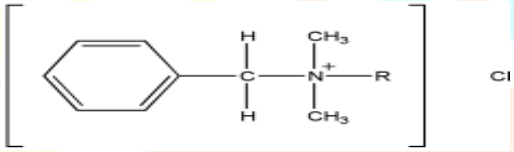
Solubility: Soluble in acetone, alcohol, dichloromethane, ethyl acetate, insoluble in water and petroleum ether.

Marketed forms: Granules
Storage: Protect from warm temperature and against moisture.
Flash point: Not inflammable.

Description: Polymethacrylates are film coating and matrix structure based on polymeric methacrylates. Eudragit RS and RL are biocompatible pH independent, cationic polymers synthesized from acrylic and methacrylic acid esters. The structure of Eudragit RS and RL differ only in the extent of quaternary ammonium substitution with being much lower than RL.Eudragit RS is not a water soluble polymer, and it does not show pH dependency. At pH 1, self-buffering does not take place.

Applications: Eudragit is employed as a coating material, usually for coating pallets or microparticles that are filled in to capsules or compressed into tablets. Eudragit RS has been used as a sustained release coating material. Water can penetrate in the Eudragit RS material and dissolve the encapsulated material, which then diffuses in the aqueous phase and finally into bulk solution. Eudragit serves as a matrix in which the active is embedded. The matrix structure is obtained by direct compression and wet granulation. Eudragit may additionally be used to form the matrix layers of transdermal delivery system. They have also been used to prepare novel gel formulation for rectal administration.

BENZALKONIUM CHLORIDE [44]
Nonproprietary Name:
F: Benzalkonium chloride
Functional Categories:
NF: Antimicrobial preservatives wetting & solubilizing agent.
Others: Antiseptic detergent
Chemical Names: Ammonium- alkyldimethyl (phenyl methyl)-chloride
Alkylbenzyldimethylammonium chloride
Empirical Formula: $[C_6H_5CH_2N(CH_3)_2R]CL$
Molecular Weight: 360
Structural Formula:



R = mixture of alkyls: *n*-C_8H_{17} to *n*-$C_{18}H_{37}$; mainly *n*-$C_{12}H_{25}$ (dodecyl), n-$C_{14}H_{29}$ (tetradecyl), and *n*-$C_{16}H_{33}$ (hexadecyl).

Method of Manufacture: Benzalkonium chloride if formed by the reaction of a solution of N-alkyl-N-methyl-benzylamine with methyl chloride in an organic solvent suitable for precipitating the quaternary compounds as it is formed.

Description: A white or yellowish-white, thick gel or gelatinous flakes with a mild aromatic odor. It is very better to taste, its aqueous solution foams when shaken. It is hygroscopic and affected by light and air.

Typical properties: Solubility: practically insoluble in ether; very soluble in acetone, ethanol (95%), methanol, propanol, and water. Aqueous solutions of benzalkonium chloride foam when shaken have a low surface tension and possess detergent and emulsifying properties.
Chemical: cation –active quaternary ammonium compound.
Antimicrobial: Active against Gram positive organisms. Not active against all the Gram negative organisms.Ineffctive against some Pseudomonas *aeruginosa* strains, *Mycobactrium tuberculosis*, *Trichophyton interdigitale* and *T. rubrum.* Combined with disodium edetate (0.01 - 0.1%), the activity against Pseudomonas *aeruginosa* is increased. In the presence of citrate and phosphate buffers (but not borate), activity against Pseudomonas can be reduced It is relatively inactive against spores and molds, but active against some viruses, fungi, and protozoa. Its activity increased with increasing pH, optimal activity occurring in the pH range of 4-10.

SUMMARY

Eye is unique organ and drug administration into eye is a challenging task. Eye is prone to number of diseases and one of them is ocular inflammation. Indomethacin is a drug of choice in treatment of ocular inflammation. The drawbacks associated with the current formulation of Indomethacin like poor bioavailability, blurring of vision and frequent instillation made to us consider for detailed survey on nanoparticulate systems.
Several colloidal drug delivery systems were designed to obtain a control release for ophthalmic applications were investigated. These are composed of a polymeric support with or without drug. The drug was normally incorporated as a dispersion or solution in the polymeric support. The main objective of the nanosuspension is to increase the contact time of the preparation with conjunctival tissues so as to ensure a Sustained / Controlled release suited for the topical or systemic treatment. However the details about the preparations and their model of construction are given in many literatures but no specific guideline are mentioned. In the present work, a revival attempts has made us to investigate Indomethacin ocular nanosuspension utilizing Eudragit RS 100 as drug reservoir membrane and rate controlling component and its possible utilization in designing and evaluating it for ophthalmic use.
In the present attempt polymers like Eudragit RS 100 were utilized for the development of Indomethacin nanosuspension. Tween 80 was incorporated as surfactant and Benzalkonium chloride as preservative.. All formulations were subjected to various evaluation parameters like identification and compatibility studies of drug and polymer using IR spectrometer and were evaluated for parameters like pH, particle size analysis, drug entrapment efficiency, rheological studies; rabbit eye irritation, *in vitro* release studies, *in vivo* drug studies and stability studies. The uniformity of the nanosuspension revealed that the drug was uniformly distributed throughout the nanosuspension. Increase in the polymer ratio increases the entrapment efficiency of drug which observed by entrapment efficiency

studies. There was no effect of the extremely high humidity and dry conditions on the integrity of the drug in nanosuspension, which was observed by entrapment efficiency.

In vitro dissolution studies revealed that the release rate of Indomethacin was in order FN-3> FN2->FN-1>FN-5>FN-4. The plot of cumulative percentage drug are retained v/s time for formulations FM-3 & FM-2 was found linear this indicated that drug diffusion from these inserts followed zero order kinetic. These formulations showed maximum release and fulfilled many requirements once a day delivery system. Hence these were considered as the formulations of choice for *in vivo* studies and stability studies.

Scope of the future studies:

As the present formulations proved the release for once a day therapy, the work can be prolonged to formulate the system for once day to once a weak therapy releasing the drug in a preprogrammed manner thereby improving the therapeutic efficacy of the drug and thus improved patient compliance.

Further future work can be progressed to establish the therapeutic utility of these systems by pharmacokinetic and pharmacodynamic studies in human beings.

The different Novel Technology can be adopted in the formulation for ophthalmic nanosuspensions and thereby investigate their effect on release pattern.

CONCLUSION

In the present study an attempt was made to develop the ophthalmic nanosuspension of Indomethacin with improved bioavailability, avoidance of repeated administration and dose reduction.

From the experimental finding, it is concluded that:

- Eudragit RS 100 is a good film forming biodegradable polymer and is a promising agent for ocular delivery.
- The pH of all definitions was discovered to be acceptable accordingly there would be no aggravation to the endless supply of the plan.
- The particle size analysis revealed that the nanoparticles were in nanometer range and all the formulations showed ideal surface morphology. Particle size of formulation FN-3 was smallest and discrete.
- The *in vitro* release studies showed biphasic release pattern for all formulation, with an initial burst effect, which may be attributed to the drug loaded on the surface of the particles.
- The optimum drug to polymer ratio was found to be in FN-3 depending on the particle size, entrapment efficiency, *and in vitro* release profile. There was no significant increase drug release with increase in drug to polymer ratio.
- Formulation FN-3 showed zero order release and followed Higuchi matrix and showed release through diffusion, it also showed that the diffusion is through Non Fickian mechanism.
- On the basis of drug content, particle size, morphology, *in vitro* release and satisfactory release kinetics, formulation FN-3 was selected as an optimum formulation for *in vivo* and stability studies.
- *In vivo* release profile indicated that polymeric system of Indomethacin has achieved the objectives of increased contact time, prolonged release, and decreased frequency of administration, avoidance of eye-irritation and redness of the rabbit eye.
- The DSC study showed that complete disappearance of the melting endotherm of Indomethacin, which could indicate the complete amorphization of the drug as well as loss of drug crystallinity.
- Zeta potential study proved that the formulation FN-3 have excellent stability. The positive value + 45mv indicate that the Eudragit RS 100 nanosuspension was stabilized by electrostatic repulsion forces.
- By these facts, study can be concluded by saying that nanosuspension prepared from Eudragit RS 100 using different polymer concentration is a promising approach to enhance the bioavailability of Indomethacin

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