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NANOTECHNOLOGY BASED STRATEGIES FOR THE TREATMENT OF ANTERIOR SEGMENT OCULAR DISEASE

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Abstract : The human eye has a unique as well as complex anatomy and physiology due to which there is a great challenge for the researcher as well as for the pharmacologist to provide a controlled drug delivery. Low drug contact time and the poor bioavailability due to the drainage of solution, tear turnover, lacrimation and its dilution are the problems associated with the conventional system. Development of new strategies for delivering of drug to the eye has been continuously done, it has been found that the research advancement in pharmaceutical science have led to the development of new strategies in delivering the drug to anterior segment where as it is difficult for delivering the drug to the posterior segment. Designing of the new delivery system for the anterior segment generates a high drug level, maintains prolonged and effective concentration with minimal or no side effect. Nanotech system are used to deliver the drug to the anterior segment is a new approach. This review provides an overview of anterior segment drug delivery barrier, Nano carriers for anterior segment, disposition of Nano carrier following topical application, Nano-carriers in clinical trials, safety and toxicity as well as the application of the Nano medicines for therapies to the anterior segment diseases.

Keywords: Ocular, Nanotechnology,Nano-carrier,Liposome, solid lipid nanoparticle.

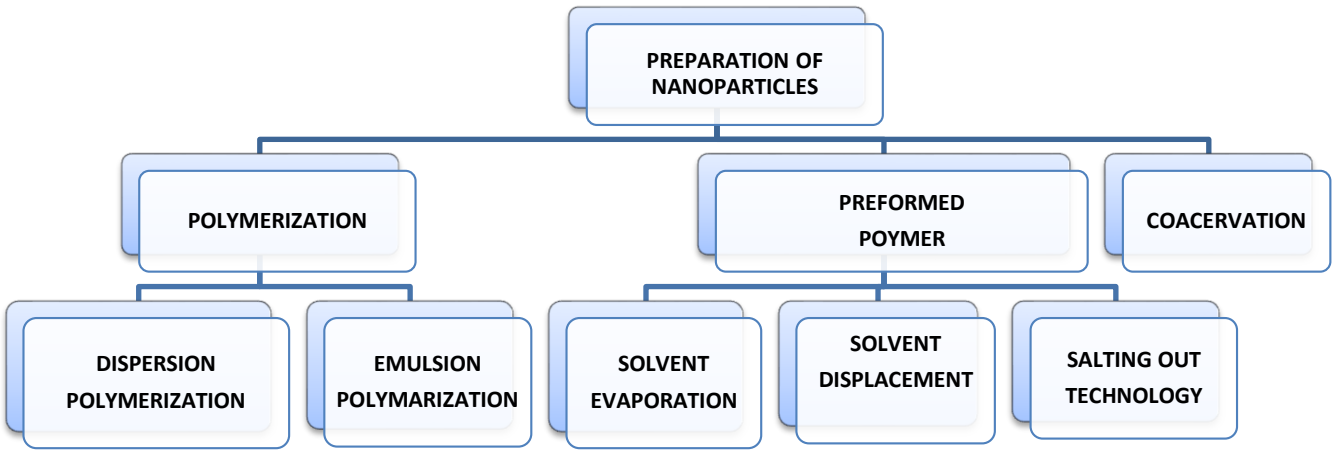
I. INTRODUCTION

The human eye has a complex anatomy and has physiological barriers. The anterior segment of the human eye has cornea, aqueous humor, conjunctivae, ciliary body, iris and lenses whereas the posterior segment consist of choroid, vitreous humor, retina, choroid and optic nerve [1,2].Delivering of drug to the posterior segment is difficult as compared to the anterior segment. The diseases affecting the anterior segment of human eye are allergic conjunctivitis, dry eye syndrome, glaucoma anterior uveitis and cataract [1]. The anterior chamber is 3mm deep and it contains 0.25ml of the aqueous humor [3].

The barriers of the anterior segment drug delivery are dynamic barriers (lymph flow, conjunctival blood flow and tear drainage) and static barriers (corneal epithelium, corneal stroma and blood aqueous barriers). The drug that are delivered tropically involves the conventional dosage form such as ointment (17.4%), suspension (8.7%) and solution (62.4%) [4].

Drugs that are topically administered have ~35-36µL average volume of the formulation and the extra or excess volume drains into the nasolacrimal duct into the systemic circulation. The anterior segment barriers such as the cornea barrier which has tight junction of the epithelium as well as the corneal epithelium has a hydrophobic nature which in order limits the penetration of the hydrophilic drugs, pre corneal barrier which has tear turnover, nasolacrimal drainage and conjunctival barrier where there is drug efflux pump such as P-gp. Nanotech is used in order to deliver the drug to the anterior segment by crossing these barrier as these are sub-Nano sized colloidal structures that are composed of various synthetic and semi-synthetic polymers where the drug is entrapped, encapsulated or attached to a nanoparticle matrix.Drugs delivered by using Nano carriers to the anterior segment of the eye attach to a specific ligand on their surface, improves stability, therapeutic index and reduce toxic effects [5].

The nanoparticles can be prepared by various methods such as: -



PREPARATION TECHNIQUES OF NANOPARTICLES: -

1.

POLYMERIZATION

DISPERSION POLYMERIZATION

Acrylate or methylacrylate monomer is dissolved in aqueous phase

polymerization by γ-radiation or chemical initiation combined with heating to temperature above 65 degree celsius.

The oligomers formed subsequently aggregated and above certain molecular weighed precipitate in the form of nanoparticles.

EMULSION POLYMERIZATION

Monomeris dissolved in aqueous phase which contains as initiator which is a surfactant

Vigrous agitation is done

Emulsion formation takes place

Particle smaller than 10 nm are formed.

2.

COACERVATION

2 different aqueous phases are prepared for polmer:-

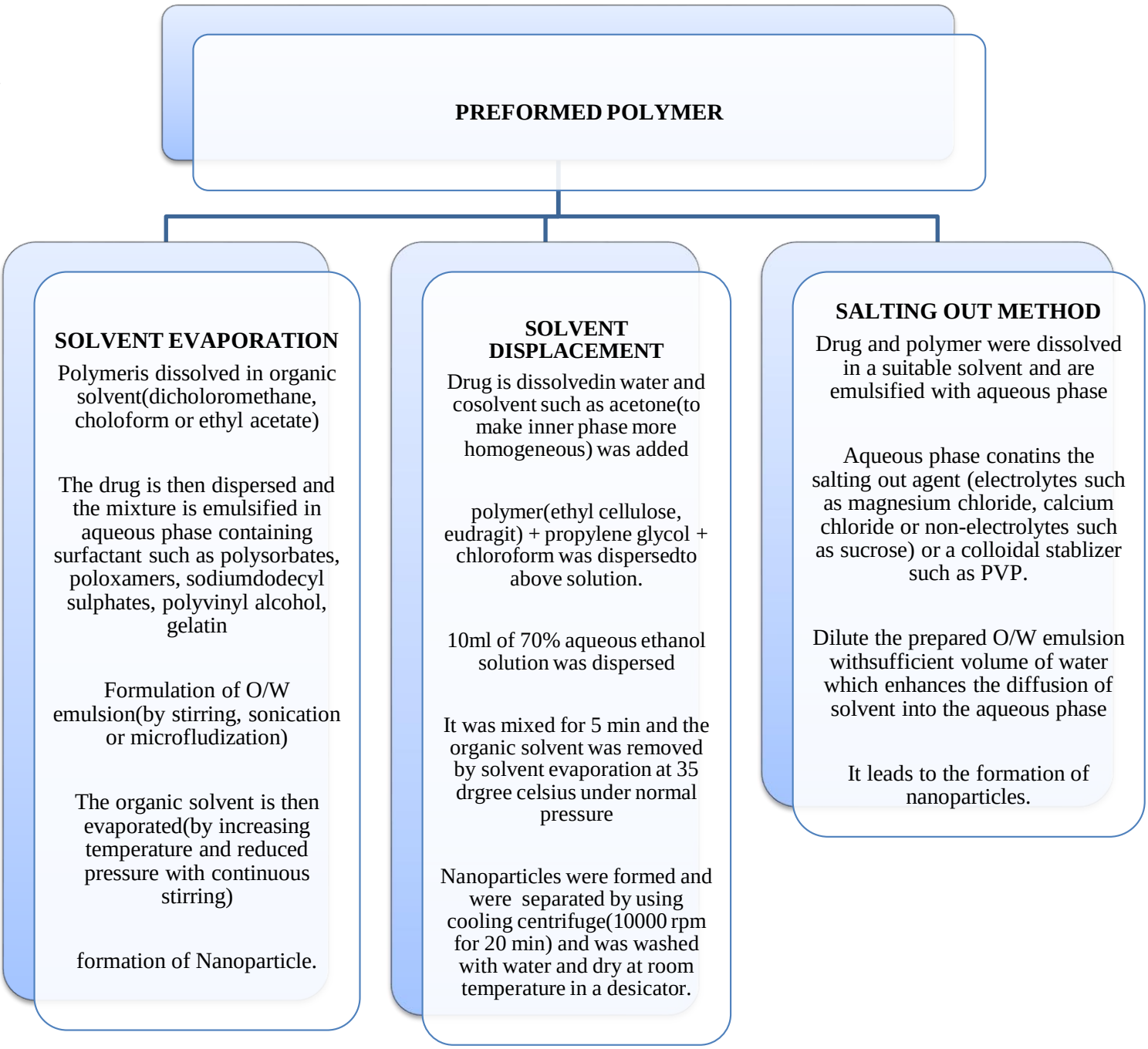
a) chitoson

b) polyanion sodium tripolyphosphate

Strong electrostatic interaction between positively charged amino group of chitoson and negatively charged tripolyphosphate

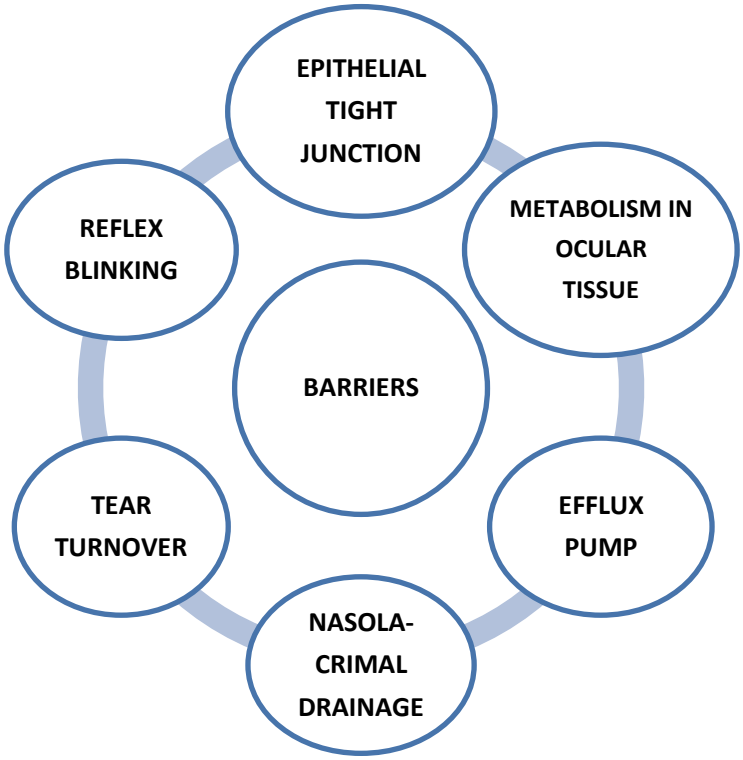
Formation of coacervation with a size in the range of nanometer

3.


PREFORMED POLYMER
SOLVENT EVAPORATION
Polymeris dissolved in organic solvent(dicholoromethane, choloform or ethyl acetate)
The drug is then dispersed and the mixture is emulsified in aqueous phase containing surfactant such as polysorbates, poloxamers, sodiumdodecyl sulphates, polyvinyl alcohol, gelatin
Formulation of O/W emulsion(by stirring, sonication or microfludization)
The organic solvent is then evaporated(by increasing temperature and reduced pressure with continuous stirring)
formation of Nanoparticle.
SOLVENT DISPLACEMENT
Drug is dissolvedin water and cosolvent such as acetone(to make inner phase more homogeneous) was added
polymer(ethyl cellulose, eudragit) + propylene glycol + chloroform was dispersedto above solution.
10ml of 70% aqueous ethanol solution was dispersed
It was mixed for 5 min and the organic solvent was removed by solvent evaporation at 35 drgree celsius under normal pressure
Nanoparticles were formed and were separated by using cooling centrifuge(10000 rpm for 20 min) and was washed with water and dry at room temperature in a desicator.
SALTING OUT METHOD
Drug and polymer were dissolved in a suitable solvent and are emulsified with aqueous phase
Aqueous phase conatins the salting out agent (electrolytes such as magnesium chloride, calcium chloride or non-electrolytes such as sucrose) or a colloidal stablizer such as PVP.
Dilute the prepared O/W emulsion withsufficient volume of water which enhances the diffusion of solvent into the aqueous phase
It leads to the formation of nanoparticles.

ANTERIOR SEGMENT DRUG DELIVERY BARRIERS

The barriers of anterior segment drug delivery are: -



1. EPITHELIAL TIGHT JUNCTION: -

The epithelial tight junction is the primary barrier for the drug to be absorbed through the topical administration. It consists of basal layer of columnar cell, two to three-layer pf wing cells and one or two outer layer of squamous cells [5]. The intracellular tight junction (zonula occludens) are surrounded by the superficial cells. The permeation of the drugs is though the Para cellular routes. These tight junctions are composed of anastomotic strands that provide the resistance to the para cellular drug absorption [6]. The epithelial tight junctions have four proteins (ZO1, CINGULIN, ZO2 [7] and OCCLUDEN [7]), occluden is the most important. Intracellular and extracellular calcium level are present in the tight junctions influence the permeability [8] and if there is any change in the integrity or extracellular calcium ions removed by the EDTA, then the drug permeability is increased throughout the junctions [10,11]. The positive charged molecules permeate faster as compared to negatively charged molecules [12] since the pores present on the corneal epithelial are negatively charged at physiological pH·

2. REFLEX BLINKING: -

Reflex blinking is another barrier of anterior segment of the eye where the drug is lost either by nasolacrimal drainage or reflex blinking that is 5-7 blinks/min which decreases the overall drug for the therapeutic action. The topical formulation such as eyedropper delivers 25-56µl with the average volume of 39µl [13].

3. METABOLISM IN OCULAR TISSUE: -

The metabolism in ocular tissue is yet another barrier where the clearance is through the aqueous humor turnover is low which indicate the majority of the drug delivered is eliminated through the metabolic pathway [14]. Drugs that contains aromatic hydrocarbons are metabolized either through the enzymes that are present in the enzymes that are present in the eye or the pigmented epithelium and ciliary body to their epoxide and phenols [16].

4. TEAR VOLUME: -

A significant hindrance to topical ocular delivery is tear turnover administration of the drug leads to the increase in volume of cul-de-sac that further leads to reflex blinking and increase tear secretion which result in rapid drug loss. The loss is due to tear turnover and nasolacrimal drainage until the tear volume returns to normal range that is 7-9µl [17].

5. NASOLACRIMAL DRAINAGE: -

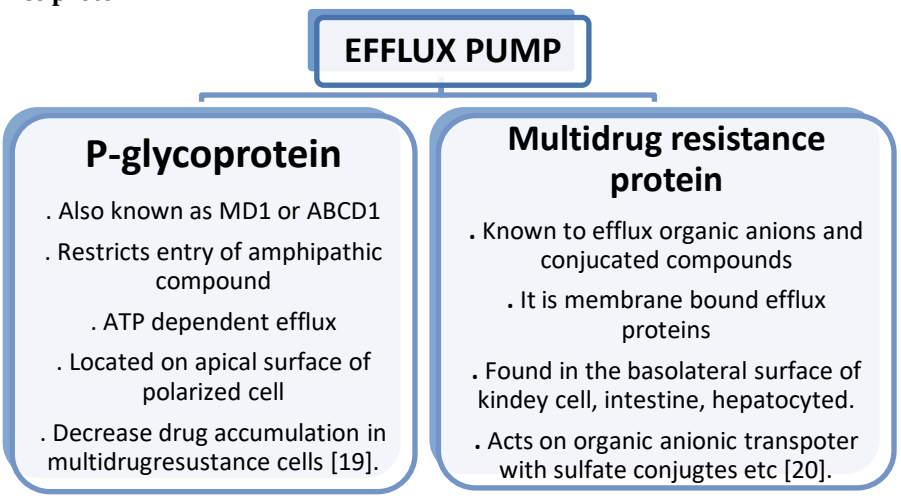
The instilled drug is lost due to nasolacrimal drainage or the tear turnover due to increase volume of the drug in cul-de-sac. 95% of the instilled drug is eliminated systemically or through the nasolacrimal dust [18]. The increase volume of the drug flows into the upper and lower canaliculus that further opens into the nasolacrimal duct further drains into nose. Drugs easily pass through the nasolacrimal sac when their volume is more than the normal range.

6. EFFLUX PUMPS: -

The efflux proteins are the proteins that are located on the basolateral or apical cell membrane and are responsible for enhancing the drug absorption or restricting the drug absorption, depending on their cellular localization [19]. There are two types of efflux pumps that are: -

a) P-glycoprotein

b) Multidrug resistance protein



NANOCARRIERS

FOR ANTERIOR SEGMENT DRUG DELIVERY

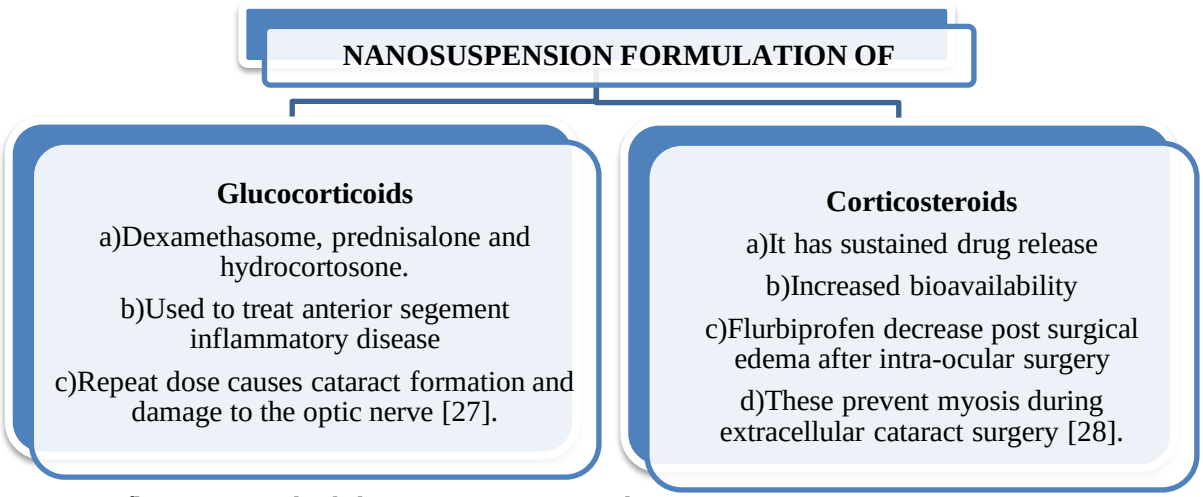
Drug delivering to the eye is a challenge due to its anatomy as well as the barriers because of which there is limited bioavailability, pre-corneal clearance and less duration of action, thus require frequent administration to the patients. The anterior segment drug delivery comprises and novel dosage forms that are liposomes, nanoparticles and implants etc. [21]

>90% of the marketed formulations are the conventional dosage which has less duration of action and limited bioavailability. In order to deliver the drug that can increase the duration of action, increase bioavailability and sustained drug release the drugs [22]. Nano carriers are used to deliver drug to the site of action. The drugs are either encapsulated, entrapped or attached to a nanoparticles matrix. Nano carriers such as liposomes, micro-emulsions, Nano micelles, dendrimers etc. are used as carrier for the drugs.

Pre corneal retention is improved when the drugs are coated with mucoadhesive polymers such as polyethylene glycol, chitosan etc. Recent research is focused on enhancing drug permeability across the cornea through entrapping, encapsulating or attaching the nanoparticle to matrix. The Nano formulations that are used most widely for treating anterior segment diseases are: -

a) MICROEMULSION: - Micro emulsions are a dispersion of water and oil that are clear in appearance and are stabilized by the help of surfactant and co-surfactants. These when added reduce the interfacial tension and are thermodynamically stable with ˜100nm small droplets size [23]. The micro emulsion formulation used to deliver the drug to the anterior segment of eye increases the solubility of the drug. These micro emulsions increase the solubility of the poorly soluble drugs (indomethacin and chloramphenicol). In the presence of co-surfactants and surfactants the oil in water type of micro emulsions have shown to increase the corneal membrane permeability. The micro emulsions have high spreading coefficient and low surface tension due to which the spreading time as well as the mixing of the drug is well with the pre corneal fluid which in order leads to improve in the corneal contact time of drug [24]. Many study done on micro emulsion have shown that there is occurrence of electrostatic attraction between the emulsified cation and the anions. These micro emulsions are stable due to which they have improved solubility as well as improved bioavailability due to which the dosing frequency is reduced as the retention time of the drug in the anterior segment is increased [25].

b) NANOSUSPENSION: - These are sub-micron colloidal dispersion that are poorly water soluble drugs that are stabilized by the use of surfactants or polymers. Nano suspension consist of colloidal carriers such as the polymeric resins that are inert in nature that enhances the drug solubility as well as the bioavailability [26]. These are non-irritant unlike the Nano suspension ad are preferable for ocular drug delivery vehicle. The colloidal carrier used in these Nano suspension are non-irritating to cornea, iris as well as the conjunctival and these also increase the pre corneal residence time, solubility and bioavailability of the drugs.



Study using Lomefloxacin HCL-loaded Nano suspension and

moxifloxacin loaded suspension have shown three folds' increase in the drug permeation across corneal when compared with parent drug solution and also have shown sustain drug release were as an optimized Nano suspension have shown higher antibacterial activity in comparison to conventional eye drops [29].

c) LIPOSOMES: - These are lipid vesicles that are composed of central aqueous compartment having a diameter of 0.025-10µm surrounded by one or more phospholipid bilayer because of the presence of central compartment these liposomes are able to incorporate hydrophilic as well as hydrophobic drugs and show a high degree of biocompatibility than a polymer based system [30]. These liposomes adhere to the cornea and are favorable for the drugs that have high molecular weight, poor absorption, low solubility and partition coefficient. Liposomes having positive charge have tendency to bind with negatively charged mucin coat on the corneal epithelium, these positive charge liposomes show enhanced trans corneal flux and have a controlled drug release, improve bio availability as they are able to adhere on the surface of the cornea as well as have reduced dosing frequency. Depending on size liposomes are known as [31]-

- **Small unilamellar vesicles(SUV)-** Consist of single lipid layer and size range from 25 to 100nm.
- **Large unilamellar vesicles(LUV)-** Consist of single lipid layer and size range from 100-400nm.
- **Multilamellar vesicles(MUV)-** Consist of two or more concentric bilayer and size range from 200nm to several microns.
- **V**esicles above 1µm are known as **giant vesicles.**

These liposomes are considered as a potential functional ocular drug delivery system due to diverse structure and unique physical versatility as well as provide localized drug action in order to maintain the drug activity at the site of action for longer period of time and this minimizes the frequent administration of the drug [32].

d) DENDRIMERS: - These are Nano construct highly branched shaped star shaped structures composed of polymeric macro molecules that are having unique physical & chemical properties such as monodispersity, high-water solubility, encapsulation ability and surface functionalized group were these functional surface group make these dendrimers suitable for delivery of both hydrophilic and lipophilic drugs [33,34]. Dendrimers consisting polyamidoamine (PAMAM) with hydroxyl as well as carboxylic surface group were used to overcome the limitation of the bio adhesive polymers [35] such as poly (acrylic acid) which were used to improve ocular drug delivery by prolonging contact time for good absorption but these polymers when used caused blurring of vision, vision loss due to which PAMAM are used as these polymers not only increase the number of branches in the dendrimers but also improve drug solubility and allows for surface conjugation of the drugs. These dendrimers are suitable for ophthalmic drug delivery as they can solubilize hydrophilic and lipophilic drug in the core and provide sustain drug release [36]. Delivering of drug using dendrimers as a carrier have further improved by PEGylation and can also be altered when an appropriate surface group such as carboxylic, hydroxyl amine or the size or molecular weight of the dendrimers. These show prolonged residence time and improved bioavailability due to which the dosing frequency is decreased.

e) NIOSOMES: - These are Nano sized, bilayer vesicles composed of amphiphilic Nano ionic surfactants that are non-immunogenic, biodegradable & biocompatible. These are chemically stable with 10 to 1000nm in size and can incorporate both lipophilic and hydrophilic drugs [37]. These are preferred carrier system for ocular drug delivery because of their low toxicity that is associated with the Nano ionic surfactant. By definition Niosome can be defined as aqueous core enclosed in a bilayer consisting of cholesterol and Nano ionic surfactants by using Niosome as carrier targeted drug delivery are achieved and the drug is delivered directly to the anterior segment of the eye where it shows the required therapeutic effect. Reduced dose is required to achieve the effect and subsequently improve bioavailability [38] of poorly soluble drugs and has controlled drug release. The Niosome do not require any kind of special handling during their preparation and provide targeted sustained drug release as well as enhanced bioavailability therefore these are considered safe option for sustained trans corneal drug delivery. **For example: -** Nisomal formulation of pH and improved bioavailability.

f) CUBOSOMES: - These are liquid crystalline Nano structured particles with a unique properties of the bulk cubic phase whereas their dispersions have much lower viscosity [39]. Certain properties of the cubic phases that are formed by certain classes of amphiphilis have ability to disperse into particles. These are those Nanoparticles that are self-assembled liquid crystalline particles of some surfactant with proper ratio of water with microstructure and have solid like rheology which provides unique properties of practical interest. Cubosomes have high drug payload due to high internal surface area and cubic crystalline structure and have a very simple method of preparations. These are stable in any dilution level because of they have relative insolubility of cubic phase forming lipid in water. Cubic phase of Cubosomes are colloidally or thermodynamically stable for longer time and provide therapeutic effect and increased bioavailability [40].

g) NANOMICELLES: - These are another type of nanocarriers that are colloidal structured ranging from 5 to 200nm in size and composed of amphiphilic surfactants molecules which can be anionic, cationic or zwitter ionic in nature [41]. These micelles are found in various shapes such as spherical, start-shaped or cylindrical depending on the corona forming blocks and the molecular weight of the core. Nano micelles orient themselves to form reverse micelles or normal micelles. The normal micelles are those in which the hydrophobic portion forms a cluster within the core were as the hydrophilic part align on the outer side that is mainly responsible for increasing the contact with water. In the same way reverse micelles are formed but they have opposite alignment occurs [42]. The normal micelles encapsulated, solubilize and deliver hydrophobic drugs whereas the reverse Nanomicelles are utilized for encapsulating and for delivering of hydrophilic drug. These Nanomicelles have the ability to solubilize less water- soluble drugs in the hydrophobic core in order to form a clear aqueous formulation and because this, they are regarded as a safe alternative for ocular drug delivery. High water solubility, clear aqueous solution, Nano dispersity, ability to form Nano size constructs, ability to minimize drug degradation, reduced toxicity, enhanced permeation through tissue and higher bioavailability are the advantages possessed by Nanomicelles. Mitra et.al described the types of micelles, there method of preparation and the studies that are involving micelles for the anterior segment of the eye. These nanomicelles are known to have following: -

- **Surfactant Nanomicelles: -** Amphiphilic molecules possess hydrophilic head as well as hydrophobic tail.

Carriers either anionic (sodium dodecyl sulfate), cationic charge (ionic surfactant) (dodecyltrimethyl ammonium bromide) or zwitterion surfactant (dioctanoyl phosphatatidyl chloride).

Surfactant used at lower concentration absorb at surface or the interface and lower the surface free energy [43].

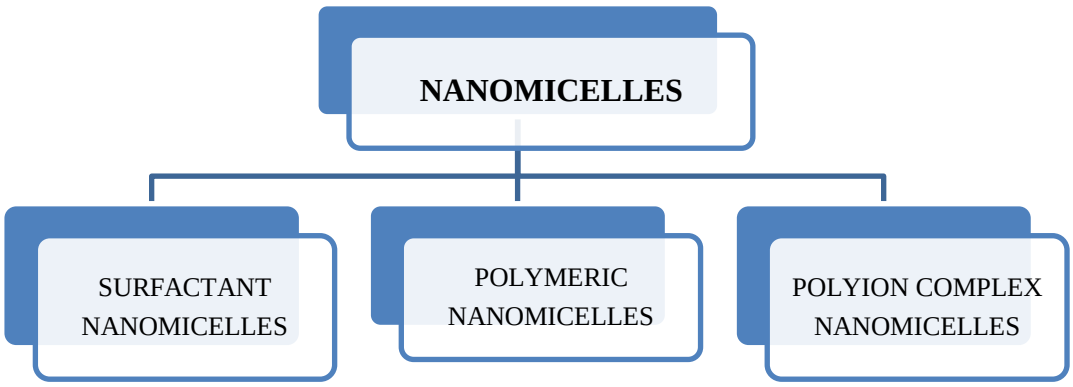
- **Polymeric Nanomicelles:** - Synthetized from block copolymer.

From amphiphilic monomeric unit having hydrophobic core surrounded by hydrophilic shell. Contain polymer chain that are self-assembled [44].

Polymer block are arranged as diblock, triblock.

- **Polyion Complex Nanomicelles:** - Formed by electrostatic interaction between Polyion copolymer and oppositely charged ionic drugs [45].

Employed for gene and antisense oligonucleotide delivery. These are formed when PEG stabilizes the hydrophobic Polyion drug complex. It reduces side effects as they target specific. Successfully tested for gene delivery to the anterior segment ocular tissue.



h) NANOPARTICLES: - These are colloidal carrier that delivers the drug to the anterior segment of the eye and range from 10 to 1000nm [46] whereas 50 to 400nm is the size range of drug loaded nanoparticles and are considered versatile for ocular drug delivery as these can cross the physiological barriers and direct the drug either by ligand or passive mediated mechanism. These nanoparticles are made up of lipid, protein, synthetic or natural polymers, (sodium alginate, albumin, chitosan, polylactic acid and polycaprolactone). These nanocarriers are considered best for delivering the drug to the anterior segment of eye. These carriers provide several advantages such as: -

- Less irritant due to smaller particle size.
- Sustain drug release because of which frequent administration is avoided
- Prevent non-specific uptake
- Provide better absorption
- Improve intracellular penetration

Basically these drug targeting is employed to treat ocular disease such as angiogenesis, choroidal neovascularization and diabetic retinopathy etc. These nanoparticles can incorporate both hydrophobic and hydrophilic, were the hydrophobic drug are entrapped in PLGA nanoparticles that uses an oil-in-water emulsion technique [47] and hydrophilic drugs are entrapped in PLGA nanoparticle which uses water in oil in water for the treatment of anterior segment inflammation sustain drug delivery of polymeric nanoparticles are employed which can be either biodegradable or non-biodegradable and to avoid precorneal elimination. The nanoparticles coated with polyethylene glycol, chitosan-hyaluronic acid. The nanoparticles for anterior segment delivery was published by Dileep.et.al [48].

i) SOLID LIPID NANOPARTICLES: - These solid lipid nanoparticles are solid lipid matrix that are nanometer in size range. The drug is incorporated in the matrix and the drug are stabilized by one or more surfactants. the solid lipid nanoparticles have controlled drug release, long term stability, drug targeting and biocompatibility and limited drug loading capacity. In these nanoparticles the release of the hydrophilic during initial periods is through bursting. SLNs prevent as well as reduce the degradation of the lipophilic drugs. Impediments associated with SLN lead to the modification of nanostructured lipid carrier and these carriers are capable of incorporating large quality of drugs which improves drug- release profile. The incorporation of large quantity of the drug is due to space between the fatty acid chains of glycerides. These NLS consists of 30% of liquid lipids. The SLNs have a tendency to disperse in aqueous media and are formulated into eye drops. The drugs loaded in the SLNs cross the corneal epithelium and anionic nature enables absorption of cationic SLNs.

j) NANOPARTICLES LADEN DEVICES: - The nanoparticles laden devices are the ones where nanoparticles are embedded into a matrix (hydrogel). The devices improve the therapeutics duration and bioavailability. In these devices the drug is incorporated and further these drugs are diffused from the nanoparticles in order to reach to the hydrogel/contact lens matrix to the site of action and because of this the drug release durations of the combined system are longer than the released from nanoparticles. Various nanoparticles such as liposomes, micelles, lipid based nanoparticles polymeric etc. are used for the investigation for extended release in composite system.

TABLE 2: - Current and future drugs for anterior segment of the eye.

Active ingredient	Brand name	Dosage form	Target site
Azithromycin	AzaSite®	Eye-drops	Bacterial conjunctivitis
Azithromycin/Dexamethasone (ISV-502)	AzaSite Plus™	Eye-drops	Blepharoconjunctivitis
Bromfenac (ISV-303)	-	Eye-drops	Post cataract surgery
Timolol maleate	Rysmon® TG	Eye-drops	Glaucoma

Betaxolol	Betoptic S®	Eye-drops	Glaucoma
Tobramycin/Dexamethasone	TobraDex® ST	Eye-drops	Blepharitis
Timolol maleate	Timoptic-XE®	Eye-drops	Glaucoma

TABLE: -3 Summary of some patented nanoformulation[50]

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PATENT	YEAR	NANOFORMULATION TYPE	PATENT NANOFORMULATIONS	CLAIMED DRUG	THERAPEUTIC USE
US7732404	2010	Nanoemulsion	Pro-nanodispersion formulation prepared using solid fat; tricaprin or ethyl stearate, ethyl lactate, macrogolglycerol hydroxystearate, lecithin at room temperature.	Cyclosporine	Dry eye treatment
WO2010144194	2010	Nanomicelles	Mixed nanomicellar formulations (vitamin E TPGS, octoxynol-40) of waterinsoluble drugs.	Prednisolone, methylprednisolone, prednisone, triamcinolone, betamethasone, budesonide, and dexamethasone	Posterior ocular segments disease
US20110008421	2011	Liposome	liposome to target posterior segment of the eye and prepared by phospholipid, a charged substance and a membranereinforcing substance	6-cumarin	Posterior segment US8298568
US8298568	2012	Nanoemulsion	Oil-in-water type emulsion (cetalkonium chloride, tyloxapol and poloxamer) with average particle size of about 300 nm and positive zeta potential.	Sirolimus	Uveitis
US8273366	2012	Nanoemulsion via contact lens	Drug encapsulated polymeric nanoparticles dispersed in contact lens (poly 2hydroxyethylmethacrylate).	Prednisilone acetate, gentamycin, cephalosporin, lidocaine, timolol, ciprofloxacin, cyclosporin A, or pilocarpine	Anti-inflammatory, Antiinfective, Glaucoma
US8153156 B2	2012	Nanoparticles	Nanocomposite by reversible hydrogel embedded nanoparticles	NA*	Substitute of vitreous humor
US8414904 B2	2013	Nanoemulsion	Oil-in-water emulsion with neutral zeta potential	Prostaglandin	Prostaglandin

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