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Nanoparticlated Drug Delivery System for Vitreous Humor

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NANOPARTICLATED DRUG DELIVERY SYSTEM FOR VITREOUS HUMOR

By

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[redacted]

MASTER'S PROJECT

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ABSTRACT

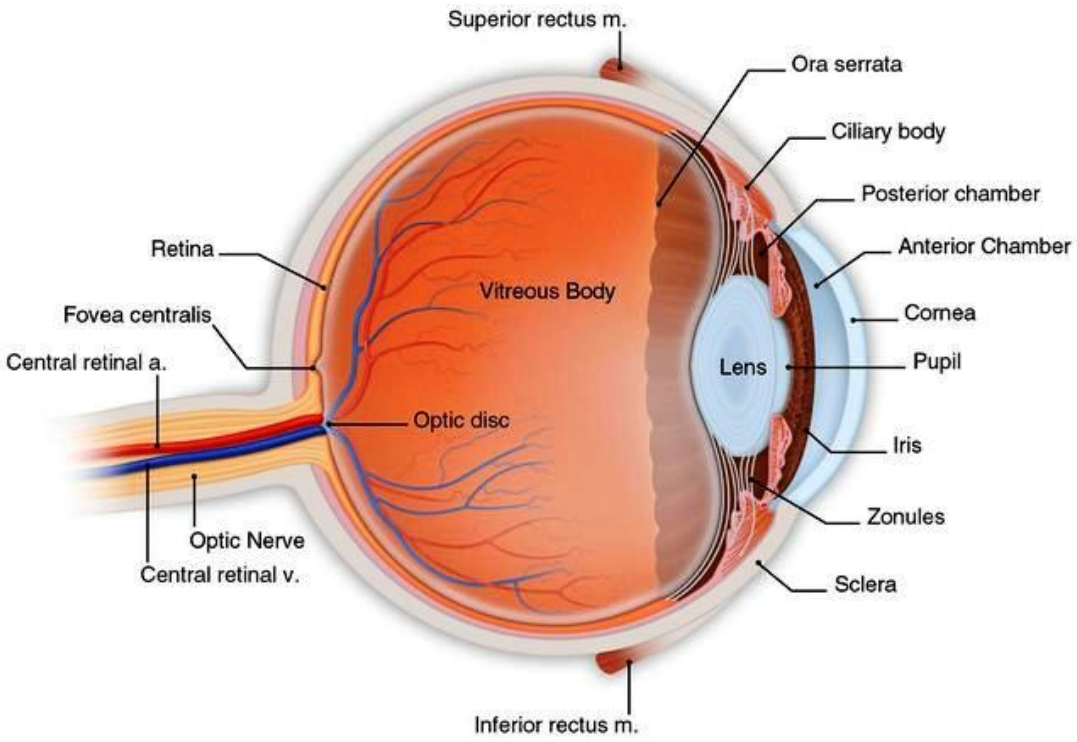
The purpose of this study is to develop a unique nanoparticulated system that has the capability of providing sustained drug delivery into the eyes. In ophthalmic preparations, poor ocular drug delivery of ocular dosage form is due to the production of tears and impermeability through corneal epithelium. The usage of liposomes in ophthalmic disorders shows promising results in ocular drug delivery. Liposomes are bilayered, microscopic vesicles surrounded by the aqueous compartments. Liposomes have the ability to encapsulate both hydrophilic and hydrophobic drugs. This unique property of liposomes helps in delivering the drug at specific site. This invention involves three major components: first, encapsulating of both hydrophilic and hydrophobic drugs into the liposomes. Second, incorporate liposomes into polymeric coating material with a volatile carrier solvent. Finally, apply liposome incorporated coating composite into intraocular lenses. When volatile solvent evaporates, the coated polymer with liposomes will form a thin film upon the intraocular lens. Local application of encapsulated coated intraocular lenses helps in the successful controlled, sustained time release of the drug in to the target site helps in prevention and treatment of ophthalmic diseases.

INTRODUCTION

The eye is a unique and precious organ in human body, characterized by its complex structure and impermeable to foreign substances including drugs. The drug delivery to the specified targeted region of eye is difficult because of the specialized anatomical and physiological nature of the eye. The physiological and anatomical barriers allow only a small fraction of the drug, usually 1-5% of the instilled dose to be effectively absorbed. The two major constraints that result in relatively poor ocular bioavailability are the pre corneal loss factions and the relative impermeability of the corneal epithelium membrane. [1]

The structure of eye can be explained under two segments i.e. anterior segment and posterior segment. Anterior segment consists of front part of the eye which includes pupil, cornea, iris, ciliary body, aqueous humor and lens where as posterior segment includes two third of eye, includes vitreous humor, retina, choroid, macula and optic nerves. [18]

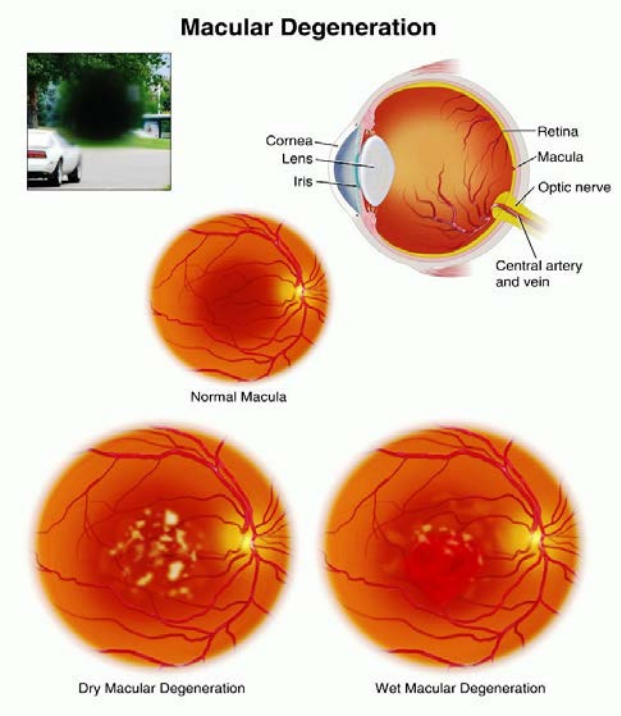
Fig1: The Anatomy of the Eye [15]



Millions of people suffer from a wide variety of ocular diseases, many of which lead to visual impairment and ocular blindness. Certain ocular diseases like Cataracts, Age related Macula and Glaucoma are very common in aging people.

Macular degeneration is the leading cause of severe vision loss in many people over age 60. As the disease develops with person ages, it is often referred to as age-related macular degeneration (AMD). Age-related Macular Degeneration is a chronic, degenerative disease of the macula, the central portion of the retina that results primarily in central vision loss whereas the peripheral vision remains unaffected. It occurs when the small central portion of the retina, known as the macula, deteriorates. It is the third major cause of adult blindness worldwide, and the first in industrialized countries [2].

Fig2: Macular Degeneration [15]



There are two main types of age-related macular degeneration:

Dry AMD:

The "dry" form of macular degeneration is characterized by the presence of yellow deposits, called “drusen” in the macula. As the drusen grow in size and increase in number, they may lead to a dimming of vision. In more advanced stages of dry macular degeneration, there is

also a thinning of the light-sensitive layer of cells in the macula leading to atrophy, or tissue death. In the atrophic form of dry macular degeneration, patients may have blind spots in the center of their vision. [8]

Wet AMD:

The "wet" form of macular degeneration is characterized by the growth of abnormal blood vessels from the choroid underneath the macula. It is called choroidal neovascularization. These blood vessels leak blood and fluid into the retina, causing distortion of vision that makes straight lines look wavy, as well as blind spots and loss of central vision. These abnormal blood vessels eventually scar, leading to permanent loss of central vision. [8]

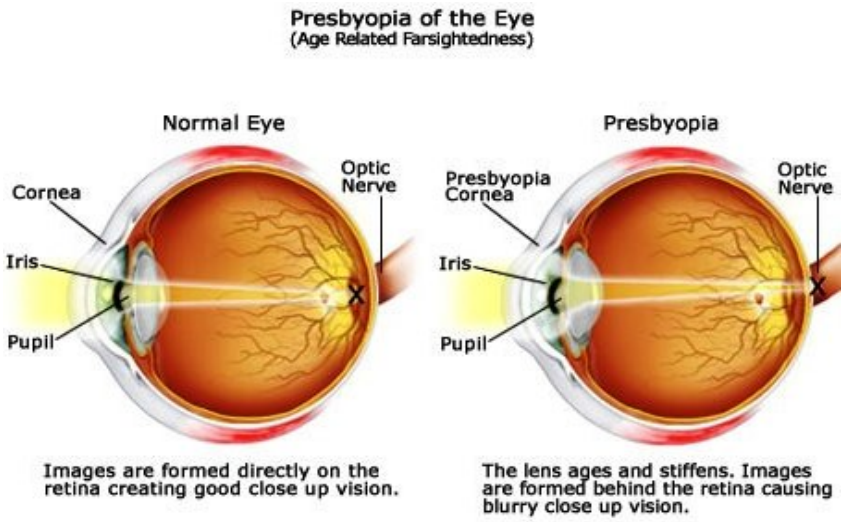
Wet macular degeneration further falls into two categories:

- **Occult**: Vision loss is not as severe as compared to the classic form because of comparatively less new blood vessel growth beneath the retina and the consequent leakage.
- **Classic**: Usually produces more severe vision loss where new blood vessel growth and scarring beneath the retina is more severe. [2]

Other major causes of blindness in older individuals include: Presbyopia, Cataract, Glaucoma and Diabetic Retinopathy.

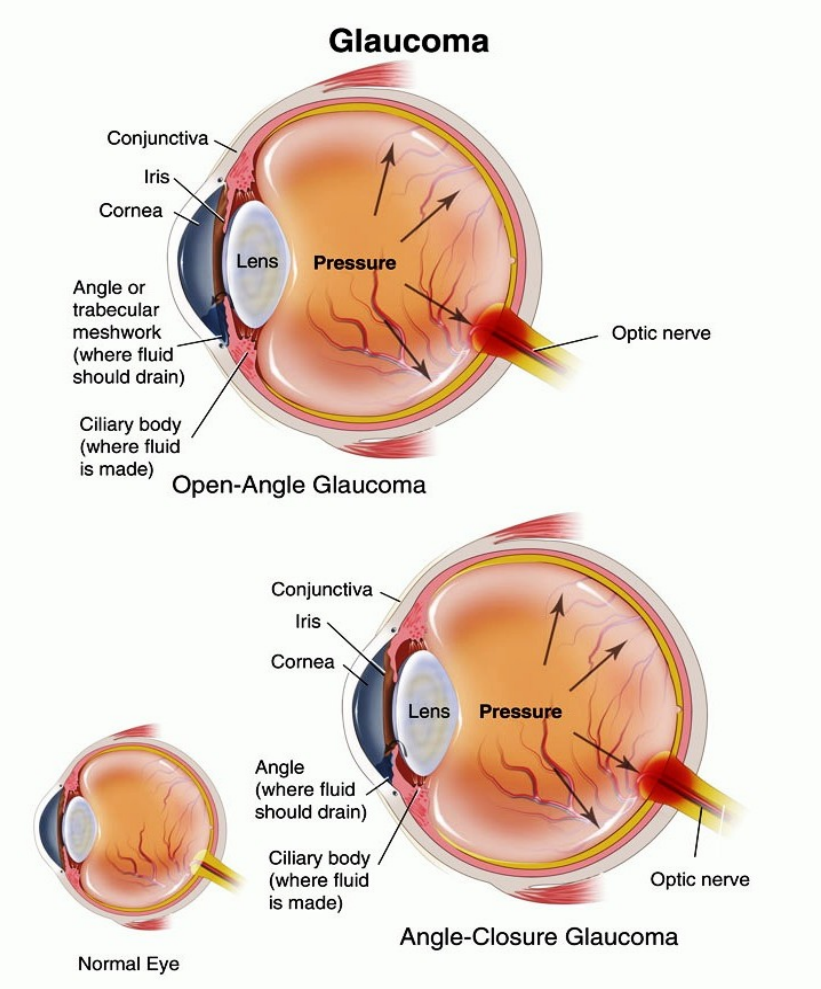
Presbyopia is a condition where there is a difficulty to see the objects close by, because the eye lens loses its ability to focus. These age-related changes occur within the proteins in the lens, making the lens harder and less elastic over time.

Fig3: Presbyopia of the Eye [15]



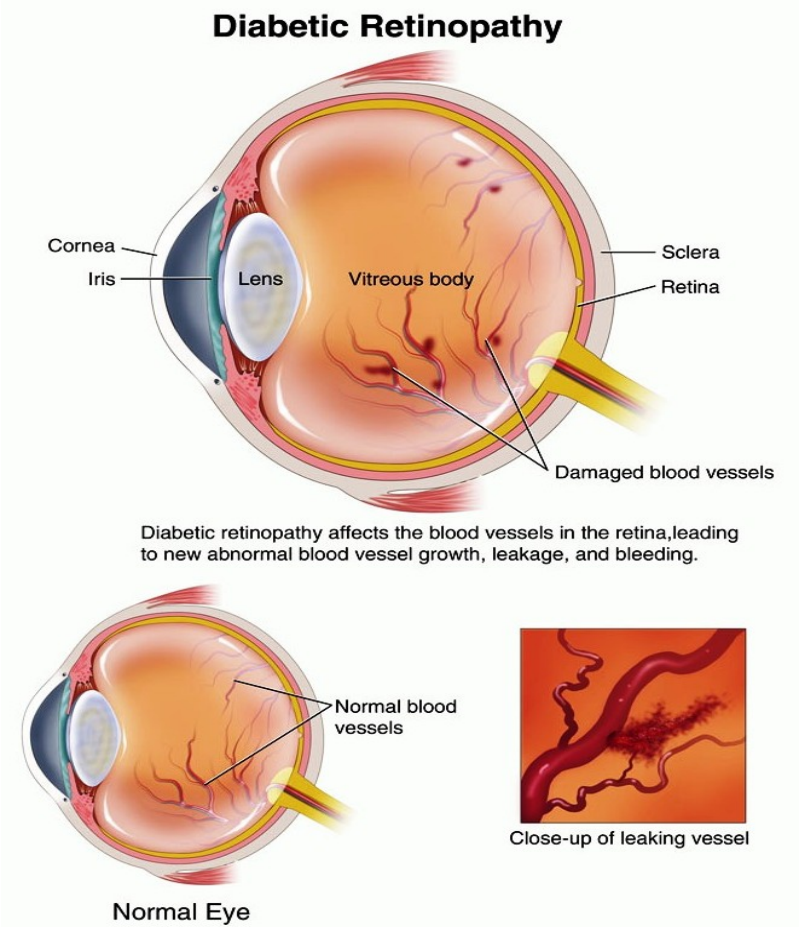
Glaucoma is an eye condition that develops when too much fluid pressure builds up inside the eye. The increased pressure, called intraocular pressure, can damage the optic nerve, which transmits packets of images to the brain. If damage to the optic nerve from high eye pressure continues, glaucoma will cause loss of vision. Without treatment, glaucoma can cause total permanent blindness within a few years.

Fig4: Glaucoma [15]



Diabetic Retinopathy is caused by damage to the fragile blood vessels of the retina. In Diabetic Retinopathy blood vessels may swell and leaks fluids. It could further develop cataracts and glaucoma. It results in changes in veins, arteries and capillaries in the body.

Fig5: Diabetic Retinopathy [15]



Cataracts are changes in clarity of the natural lens inside the eye by the accumulation of turbulent fluid, which gradually degrade visual quality. Cataract surgery is very successful treatment for the restoration of vision. During surgery, the clouded lens i.e. turbulent fluid is removed and replaced it with a clear, intra ocular lens. In Cataract Surgery, by using an operating microscope, a small incision, usually around 3 millimeters long, is made in the eye. Precise surgical instruments are used to break apart and remove the cloudy lens from the eye. In most cases, the natural lens is then replaced with a permanent intraocular lens (IOL) implant. This IOL is clear and is intended to stay in the eye forever. The incision typically does not require stitches. This small incision, no-stitch technique, has drastically reduced the recovery time for modern cataract surgery. [6], [7]

Fig6: Cataracts [15]

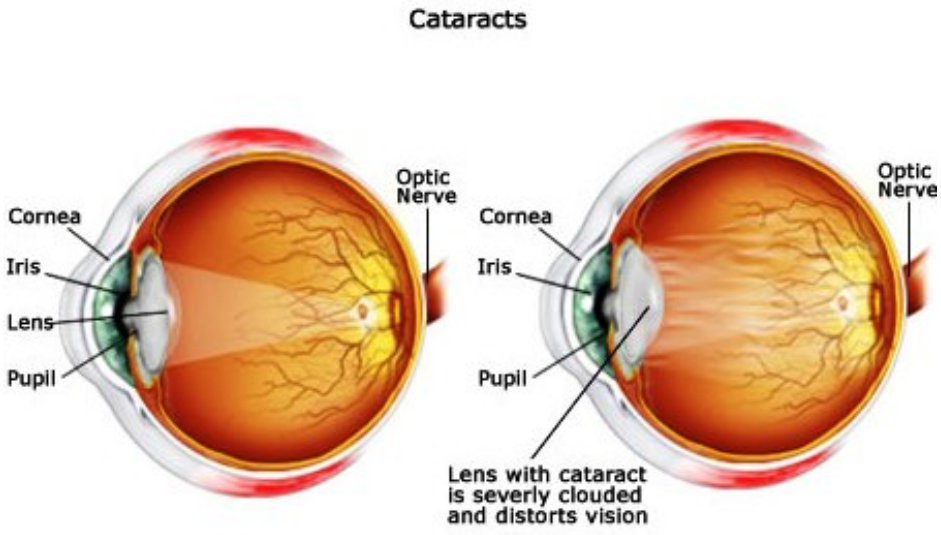
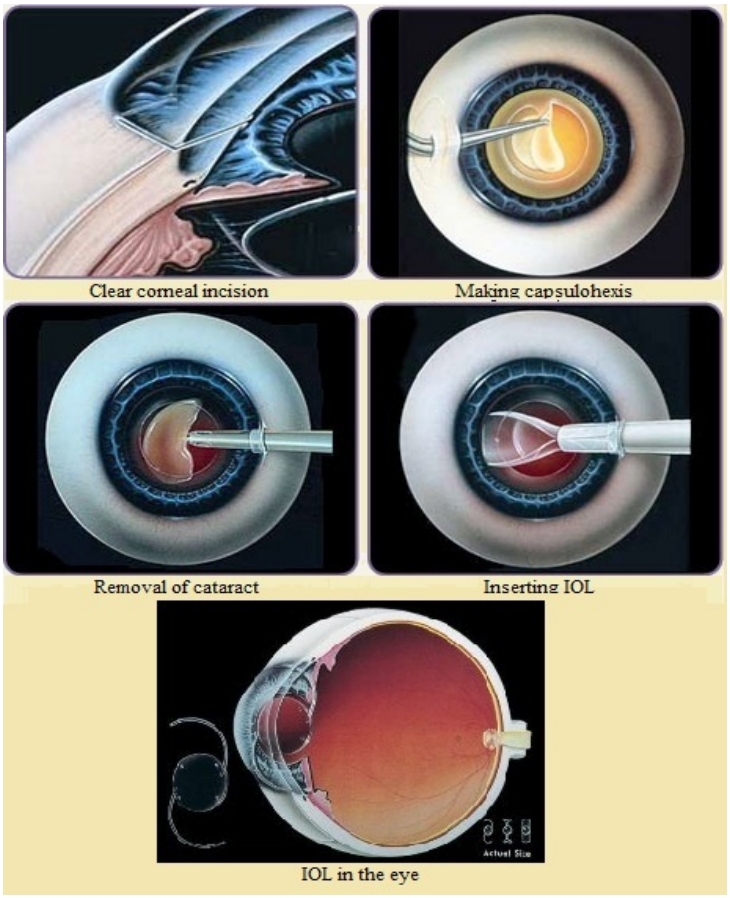
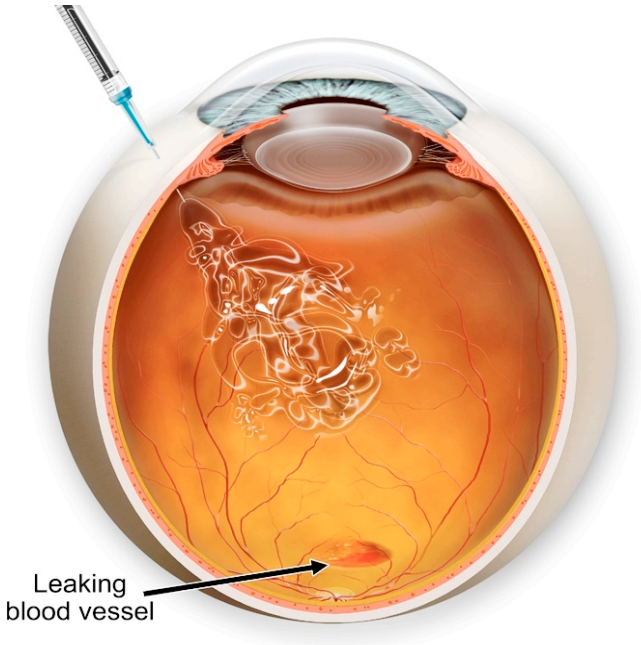


Fig 7: Cataract Surgery [15]



Cataract surgery can increase the risk of retinal detachment slightly. Other complications after the surgery include infection, bleeding, inflammation, loss of vision, double vision, and high or low eye pressure. Intraocular drug delivery is the best mode to achieve highest intraocular bioavailability in the posterior segment tissues such as the macula or fovea. This is possible because it is the only drug delivery which directly circumvents the Blood Retinal Barrier (BRB) and thus highest peak Intravitreal or intraretinal drug concentrations are attained. So far, the main treatment strategy for the diseases of the posterior segment mainly, the Age Related Macular Degeneration is the intravitreal injection, which involves direct injection of the drugs into the vitreous humor. The advantages of this treatment option are that it directly broaches BRB and high drug levels in the retina are achieved. But the main problem is that good patient compliance isn't achieved as this is a very painful technique. Besides, there are several other complications such as increased intraocular pressure, floaters, vitreous hemorrhage, transient blurry vision, retinal tears, retinal detachment, development of endophthalmitis, glaucoma and cataract. Endophthalmitis is the inflammatory condition of the intraocular cavities i.e. the aqueous and vitreous humor usually caused by infection. [1]

Fig 8: Intravitreal Injection [15]



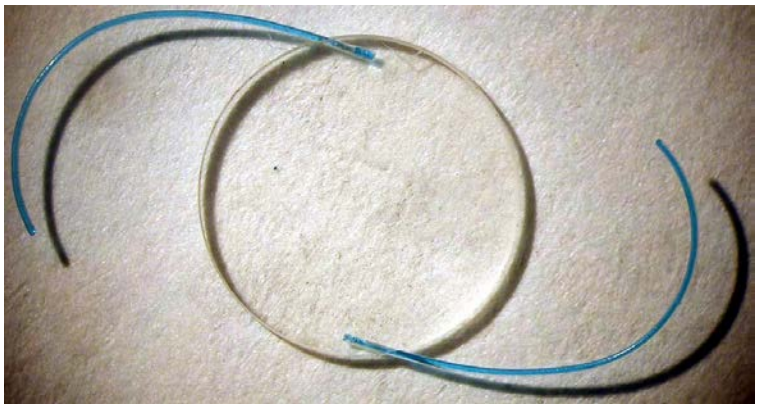
Thus there is a need to develop a simple, non-invasive technique which besides improving the ocular bioavailability and therapeutic efficacy achieves better patient compliance and overcomes the problems encountered with the previous techniques.

Intra Ocular Lens (IOL):

In the past, people had to use very thick eye glasses or special contact lenses to be able to see after cataract surgery. Now, several types of IOL implant are available to help people to improve their vision. IOL is the artificial lens implanted during the Cataract surgery, to replace the natural lens of eye, as it becomes cloudy. Basically natural lens of eye is made up of proteins, the linkage formed between the proteins leads to the formation of the cloudy, turbulent fluid in the eye and this causes the blurred vision of the eye. Intraocular lens works as natural lens of eye. They basically provide a clear, optical path and also restorative refractive capability to the eye [3].

IOLs are made of either hard plastic or soft, foldable polymers such as PMMA, hydrophilic acrylics, poly (2-hydroxyethyl methacrylate) or silicone. In 1981 for the First time FDA approved IOL. [9] The basic function of IOL is to focus the light onto the retina, where the packets of images are converted into electrical impulses that travel to the brain. The basic construction of IOL is shown at the following figure.

Fig 9: Intraocular Lens [17]



The round, corrective central portion acts as the refractive element with two arms which provide structural support for the lens in the lens capsule of the eye [4]. The center viewing zone is called the optic. This is a clear, round disc measuring 5.5 to 6.5 mm in diameter. On opposite

side of the optic, there are two flexible struts are present, which are called as Haptics. These Haptics acts like tension loaded springs to automatically center the lens within the compartment.

There are different types of IOL's are used in cataract surgery. Mainly three types of IOL's are used in cataract surgery. They are

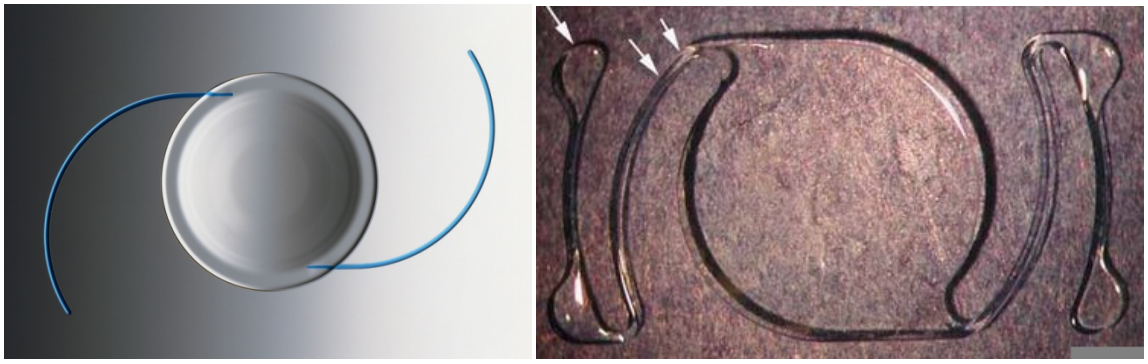
- Monofocal lens
- Multifocal lens
- Toric lens

Monofocal lenses are used for distance vision, by provides a single focal point. Multifocal IOL's are advanced type of IOL's used for multiple focal points and reduces the usage of eyeglasses or contact lenses after the cataract surgery. Toric lenses are the special type of lenses used to reduce the astigmatism of the eye at time of surgery. [9], [10] Examples include: Crystalens, AcrySof IQReSTOR from Alcon's and Technis and ReZoom from Abott Medical Optics.

IOLs are commercially available as:

- Premium IOLs,
- Toric IOLs,
- Blue light-filtering IOLs,
- Aspheric IOLs,
- Piggyback IOLs.

Fig 10: Types of Intraocular lens [17]



The main advantage of using IOLs is that they allow us to study sustained release of therapeutic levels of drug for a desired period of time, thus overcoming Blood Retinal Barrier which is associated with systemic drug delivery. [4], [10]

Nanoparticles:

Nanoparticles are submicron sized colloidal particles with lengths in 2 or 3 dimensions greater than 1nm and smaller than 100nm. This sub-class of ultra-fine particles may or may not exhibit a size-related intensive property. The small size of the nanoparticles allows them to efficiently penetrate across the biological barriers through small capillaries all over the body. This allows nanoparticles, to access into various cells and cellular compartments including the nucleus, thus allowing efficient drug accumulation at the actual targeted site. The unwanted side effects and the toxicity of the drug are thus reduced while enhancing the therapeutic efficiency[5].

There are several other advantages of using nanoparticles as drug delivery systems:

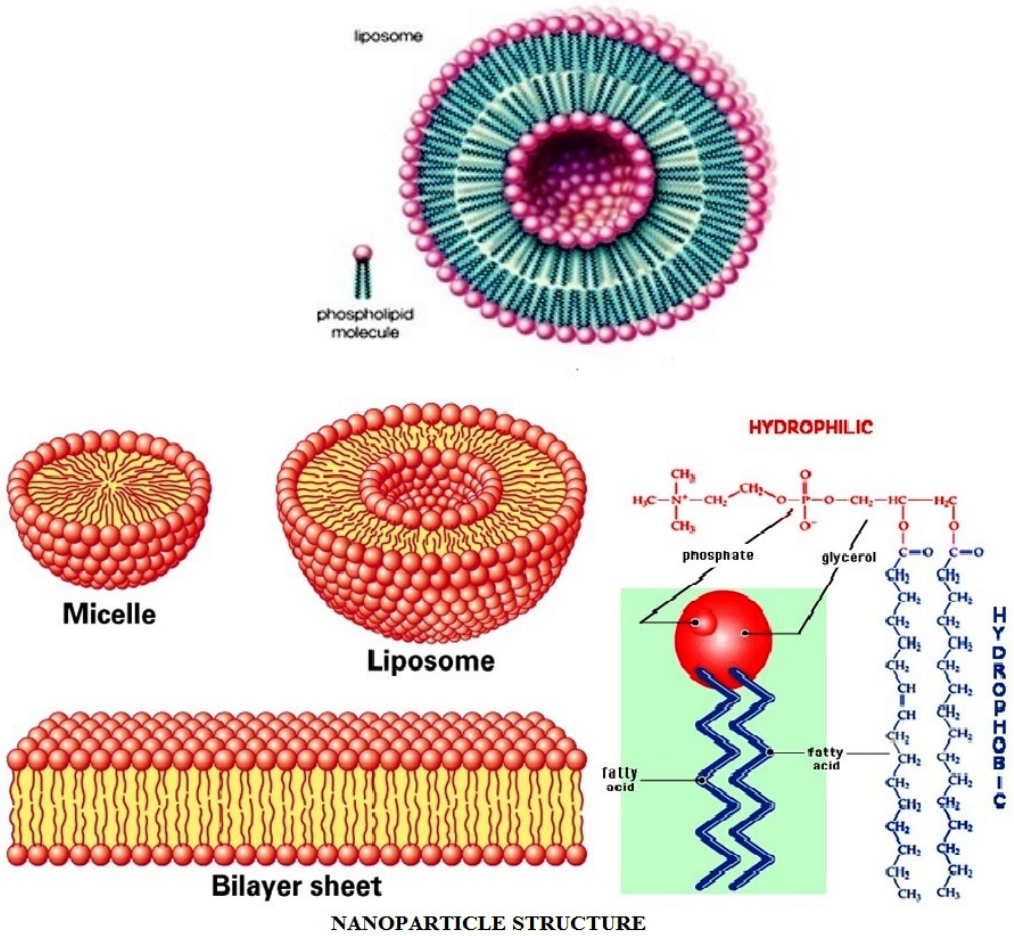
- Solubility of the drug is increased, can be used for all routes of administration.
- Protects the drug from degradation i.e. protect the drug from chemical or enzymatic hydrolysis
- Can be formulated easily and rapidly.
- Sustained drug release and prolonged therapeutic activity
- Novel drug delivery tool for chronic ocular diseases
- Site-specific targeting -surface modification with ligands
- P-glycoprotein circumvention –higher cellular permeability
- Efficient in crossing membrane barriers -blood retinal barrier
- Act as an inert carrier for ophthalmic drugs
- More stable than colloidal system
- Time release studies

In ocular drug delivery, an important consideration would be to obtain therapeutic concentrations within the retina by increasing the relative permeability of the Blood Retinal Barrier(BRB). Use of nanoparticles is an effective strategy to enhance the BRB transport. There are various methods for loading the drugs onto nanoparticles such as encapsulation, surface attachment or entrapment.

In this research, encapsulation is the method followed for loading the drug. As nanoparticles are colloidal systems, the stability of these formulations is very important. This can be improved by the use of polymeric surfactants, or other modifiers. The surfactants are adsorbed or grafted to the particles and form a layer, which produces repulsive force between the nanoparticles and prevent flocculation [5].

It has been shown that the drug delivery using nanoparticles can enhance the bioavailability of drug in target site. In the current research we develop a unique nanoparticle system i.e. liposomes as an ocular drug delivery. Liposomes are microscopic vesicles composed of lipid bilayered membrane surrounded by aqueous compartments. The lipid bilayered membrane mostly composed of phospholipids. These phospholipids are amphiphlic, having hydrophilic head and hydrophobic/lipophilic tail. In the bilayered membrane these nonpolar tails located in interior of the membrane and polar heads are pointing towards the outside of membrane. This property helps in encapsulation of both hydrophilic and lipophilic drugs in liposomes. [11] These liposomes are classified into various types based on their size i.e. unilamellar vesicles (ULV) and multilamellar vesicles (MLV). These unilamellar vesicles again divided into small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). These large size vesicles can be modified into small vesicles by using sonicator. [12]

Fig 11: Nanoparticle Structure [16]



In the current study we used time release technology which is Sustained Release (SR) Technology. The advantages of this time-release technology are uniform release of drug substance over time, reduction in the frequency of drug administration, reduced side effects, and better patient compliance. Sustained release is the slow release of the drug over a time period. In this, the drug concentration will vary with time interval because the initial release of the drug is sufficient to provide a therapeutic dose soon after the administration and then gradual release of the drug takes place over an extended time period.

EXPERIMENTAL METHODS

Fluorescein Sodium Salt from Sigma Chemical Company is used as a biomarker. It is a hydrophilic fluorophore with anhydrous molecular weight of 376.3gm and has an absorption maximum at 494nm and emission maximum at 521nm. It is an orange-red powdered compound that exhibits intense greenish-yellow fluorescence. Major applications of Fluorescein include Nanolithography, nanoparticles, and thin films. It is used in various areas of ophthalmology, especially in retinal vasculature imaging (diagnosis of Diabetic Retinopathy) to reveal corneal lesions and as a systemic biomarker in numerous pharmacokinetic studies. The Flurometer used is Ocean Optics 2000+.from Ocean Optics. The Sonicator used in this study is UP100H from Hielscher ultrasound technology.

Nanoemulsions are dispersions of fine droplets (in nanometer range) in a dispersion medium. So they are thermodynamically unstable. Thus they require considerable mechanical energy during their preparation to enhance the stability. Choosing the oil phase and the surfactants are important consideration in the formulation of nanoemulsions, so as to be able to carry both polar and non-polar drugs and improve control over the release of therapeutic molecules. Most widely used oils are various fixed oils of vegetable origin. Oils used: Castor oil, a hydrophilic non-ionic surfactant is the oil used. Other vegetable oils such as olive oil, canola oil and almond oil are also used.

Surfactants:

Surfynol-465 has a great solubility in water. All the Surfynol 400 Series surfactants have a unique combination of formulating benefits like greater dispersion, emulsification, wetting etc.

Polaxomer 407 is a hydrophilic, non-ionic copolymer, mostly used in cosmetics. This surfactant also used in contact lens cleaning solution. It is non toxic so it is widely used in oral and topical pharmaceutical products.

PEG 3350 is an excipient used as an emulsifier, binder and surfactant. It improves resistance to moisture and oxidation. Polyethylene is a polymerized ethylene resin and glycol is a hydric alcohol. It is highly soluble in water, so it can couple to hydrophobic molecules.

Asolectin is a mixture of phospholipids. It is made from soybean and mainly used as oil in water emulsifier. It is used to reconstitute acetylcholine receptor into liposomes. Asolectin comprises roughly equal proportions of lecithin, cephalin and phosphatidylinositol along with minor amounts of other phospholipids and polar lipids.

Drugs Used:

Two pharmacological classes of drugs have been used:

Antibiotics – Vancomycin

It is a glycopeptides antibiotic. Anti-bacterial mostly used to treat colitis.

Anti-inflammatory – Alclofenac

It is a Non-steroidal Anti-inflammatory drug, analgesic and anti-pyretic.

Coating:

Coating is an important part of the sustained release forms.

Purpose of coating;

- Obtain functional coats.
- Provide chemical stability.
- Enhance patient acceptance

Properties of the coating material for IOL coating:

- Biodegradable,
- Highly flexible polymeric coating with sustained release effect,

- Easy applicability,
- Fast drying or curing,
- Excellent adhesion to IOL,
- Low solvent emission and should be safe in workplace,
- Should leave no residue after removal from the applied surface.

Materials used for the preparation of the coating formulation:

Polycaprolactone is the polymer used. It is a biodegradable, synthetic thermoplastic polymer used in a number of biomedical applications. It is hydrophobic and slow-degrading. It is most commonly used in controlled drug delivery systems and in implants. [14]

Collodion Solution from Fluke Analytical, Contains: Nitrocellulose, Ethyl alcohol and Ether Solution. It is an excellent coating material and when the solvent is evaporated it leaves a tough, clear, transparent film. [14]

Hydroxy Propyl Methyl Cellulose is a biodegradable, base celluloid compound that is highly water soluble. It is also called s hypromellose. Hypromellose 2% solution has been documented to be used during surgery to aid in corneal protection and during orbital surgery. It is a good coating material that forms a clear transparent film on IOL. It is available in different molecular weights. In this experiment we used three different molecular weights i.e. 10000, 90000 and 120000.

Materials for the preparation of PMMA implants:

PMMA:

Poly (methyl methacrylate) is a synthetic, transparent plastic, versatile biocompatible polymeric material for coating used in a number of medical applications. It has a high surface area and low density, which make it an excellent seeding and coating material for antibiotic encapsulated nanoparticles. [14]

MMA:

Methyl methacrylate is a synthetic chemical used in coating formulations. Advantages of biodegradable implants are they don't have to be removed or absorbed or eliminated from the body. [14]

IOL:

Intra ocular lenses supplied by AMO-Inc, Abbott Laboratories.USA

EXPERIMENTAL PROCEDURE

For the preparation of nanoparticles, we need to prepare the stock solution of Fluorescein, and then we need to prepare the micelles followed by the preparation of liposomes.

1M Fluorescein Stock Solution:

37.63 gm of Fluorescein Sodium Salt was taken and dissolved in a small amount of distilled water on a hot plate with continuous stirring. Then make up the volume to 100mL.

Poloxamer 407 Solution:

1gm of Poloxamer 407 + 30ml water under low heat with constant stirring

PEG 3350 Solution:

1gm of PEG 3350 + 30ml water under low heat with constant stirring.

Preparation of PMMA implant:

Formulation 1-A:

18 g of Polymethylmethacrylate (PMMA) + 12 mL methyl methacrylate (MMA)

Formulation 1-B:

18 g of PMMA + 12 mL MMA + 5 mL EMA

Thoroughly mix the above formulation separately in a glass beaker in a chemical hood with constant stirring. The mixture is then cast in a molding plate, made of Al or Sn with the desired thickness. The casting plate is then kept in the oven for over 2 hours at 80^0C in order for the PMMA to cure. [14]

Inverted micelle (W/O Nanoemulsion):

Formulation 2–A:

- 10mL of castor oil is taken and heated gently on a low heat.
- 2mL of Surfynol-465 is added to the above oil and dissolved on a low heat with constant stirring with a magnetic bead, until it forms a homogeneous mixture.

- Aqueous phase i.e. fluorescein is added drop wise to the above mixture, maintaining low heat and continuous stirring until a viscous, homogeneous water-in-oil emulsion is obtained. Approximately 30 drops i.e. 1.5 ml could be incorporated into the mixture.
- The emulsion is then kept for overnight stirring to obtain a clearer, homogeneous emulsion.

Formulation 2-B:

- 10mL of castor oil is taken and heated gently on a low heat.
- 0.1 gm of Asolectin is added to the above oil and dissolved on a low heat with constant stirring until it forms a homogeneous mixture.
- Aqueous phase i.e. fluorescein with 2ml of poloxamer 407 is added drop wise to the above mixture, maintaining low heat and continuous stirring until a viscous, homogeneous emulsion is obtained. Approximately 12 drops added to the mixture.
- The emulsion is then kept for overnight stirring to obtain a clearer, homogeneous emulsion.

Liposomes (O/W/O Nanoemulsions):

Formulation 3-A:

- 10 ml of Poloxamer solution is taken and heated gently under low heat.
- To the above solution inverted micelle i.e. W/O emulsion is added drop wise with continuous stirring under low heat until it forms a homogeneous and clear solution.
- Approximately 18 drops of inverted micelle incorporated in the above solution.

Formulation 3-B:

- 10 ml of PEG 3350 solution is taken and heated gently under low heat.
- To the above solution inverted micelle i.e. W/O emulsion is added drop wise with continuous stirring under low heat until it forms a homogeneous and clear solution.
- Approximately 14 drops of inverted micelle incorporated in the above solution.
- Sonicate for 15 min.

Coating of Nanoemulsions:

- These prepared nanoemulsions are incorporated into a polymeric coating material to study sustained release properties.
- The coating solvent should be non-toxic and it should be easily evaporated once it is coated on to the IOL surface.
- The coating material must be easily dried, so the coating material must prepare just before 10 min, before the coating is done.
- Nano particles were mixed with carrying solvent and a binding agent to prepare slurry which has good binding capacity.

Coating Formulation:

- Dissolve 0.2 g of Polycaprolactone in 2.0 mL of nitrocellulose and 2.0 mL of acetone with constant stirring under very low heat.
- Add acetone drop by drop in the beaker until the polymer is totally dissolved.
- About 1mL of MMA is added to this to improve the adhesive nature of the coating formulation.
- To the above formulation, Liposome (w/o/w Nanoemulsion) is added drop wise with the continuous addition of acetone.
- Approximately 38 drops of liposome i.e. w/o/w emulsion is incorporated in coating formulation.

Coating of PMMA implant:

Approximately 2 mL of the coating formulation is taken and coated on the PMMA implant carefully with a paint brush. The coated PMMA implant is then allowed to dry overnight.

Coating of IOL:

- Intra Ocular Lens is gently held with tweezers by holding one of the Haptics.
- It is then dipped in the coated formulation gently to coat both the sides of the IOL.
- IOL is then allowed to air-dry for 24 hours by covering it with an aluminium foil.

**Throughout the experimental procedure, all the glassware and the test tubes are covered with aluminium foil to prevent any photo activity of Fluorescein.

Testing of IOL for Sustained Release Studies:

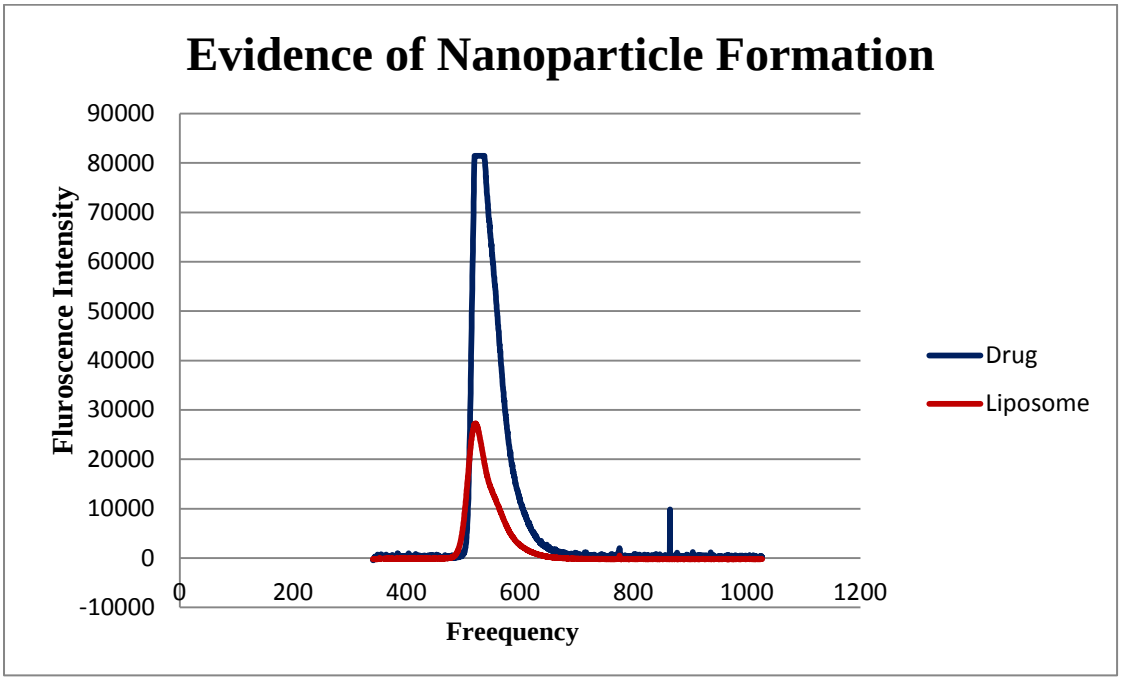
- Simulated vitreous humor and aqueous humor are prepared for testing the sustained release of the drug from the coated IOL.
- The coated IOLs are then placed in them. Samples are collected from the simulated vitreous humor on a daily basis (at 24 hour interval) and hourly basis and the fluorescence intensity is measured.

RESULTS AND DISCUSSIONS

Evidence for Encapsulation of Liposomes:

An evidence for encapsulation of drug in to nanoparticles is provided by the Fluorescence technique by which we can examine the nanoparticles.

Fig 12: Evidence of Nanoparticle Formulation



The graph provides a good evidence for the encapsulation of the drug into the nanoparticles. Fluorescein dye in aqueous solution used as a bio-marker which gives an intensive peak (blue) and some concentration of fluorescein dye solution trapped inside the formulation 3-A and 3-B liposomes (red). Graph shows a decreased intensity of fluorescence of drug in water when compared to that of the drug in the liposomes which indicates that drug is encapsulated in to the liposomes. This is because of the multi lamellar structure of the liposomes. The shift in the graph which is blue shift shows an increase in the polarity of the drug and also represents the drug movement to more hydrophilic environment.

Sustained Release Studies:

Simulated vitreous humor and aqueous humor was prepared to examine the sustained release kinetics. A luminescence bio-marker was encapsulated inside the liposomes instead of the antibiotic drugs in order to monitor the sustained release. We used vancomycin to simulate the hydrophobic drug and fluorescence dye i.e. fluorescein to simulate the hydrophilic drug. The coated Intra ocular lens with encapsulated luminescence bio marker was submerged inside the vitreous humor or aqueous humor and the fluorescence was measured in a time interval of 1 hour for a period of 16hours. The intensity of fluorescence indicates the amount of drug that is released from the coating polymer into the vitreous humor and aqueous humor. Sustained release of fluorescein dye was observed up to 16 days. The liposome thus provides the initial release of drug.

Table 1: Fluorescence intensities taken on an hourly basis

S.No	Time(Min)	Relative Fluorescence Intensity
1	60	748.00
2	120	935.00
3	180	877.00
4	240	954.01
5	300	1031.00
6	360	1040.29
7	420	1123.44
8	480	1245.40
9	540	1293.00
10	600	1388.01
11	660	1483.01
12	720	1620.90
13	780	1823.21
14	840	1848.68
15	900	1869.68
16	960	1989.40

Fig 13: Hourly Drug Release

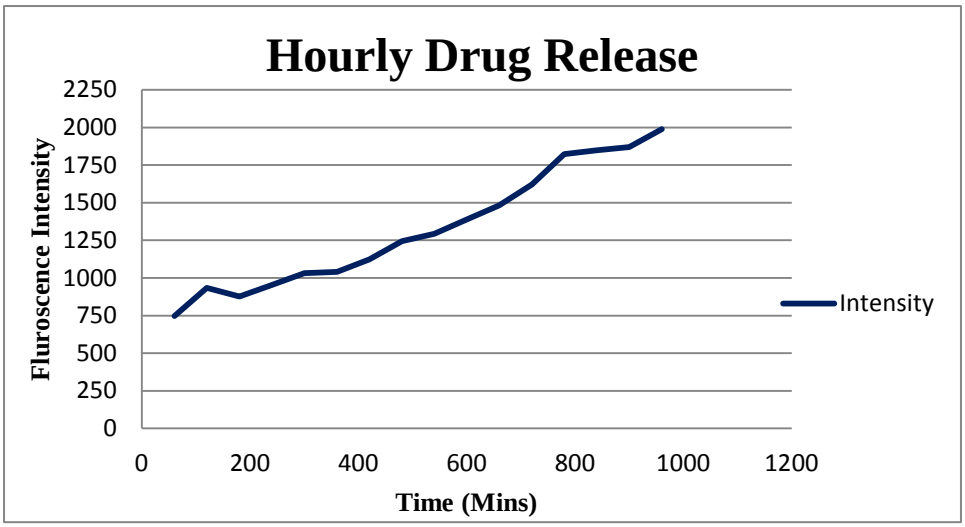


Table 1 shows the Fluorescence intensity studies taken on hourly basis i.e. for every 1hour. We took the fluorescence intensity of the drug measured for a time period of 16 hours. A Graph was plotted on the hourly basis of drug release. This shows the increase in intensity of fluorescence from first hour to sixteenth hour. The Fluorescence intensity increased from **748.00** (1^{st} hour) to **1989.40** (16^{th} hour).

Table 2: Fluorescence intensities taken on daily basis

S.No	Time(Hr)	Relative Fluorescence Intensity
1	24	8107.21
2	48	16550.82
3	72	20032.86
4	96	26157.76
5	120	44001.84
6	144	63652.72
7	168	66919.12
8	192	70573.76
9	216	80996.00
10	240	83583.28
11	264	129129.92
12	288	125849.44
13	312	141469.12
14	336	144108.16
15	360	176436.48
16	384	196057.60

Fig 13: Daily Drug Release

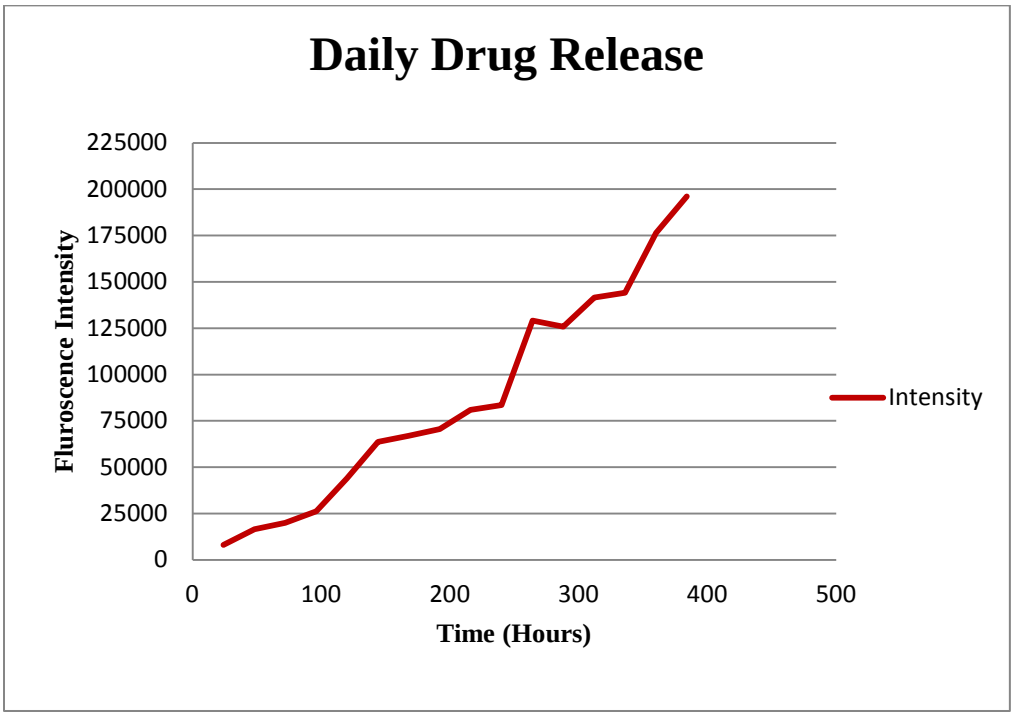


Table 2 shows the Fluorescence intensity studies taken every 24th hour for a period of 16 days. The plotted graph for table 2 shows the drug release profile. The Fluorescence intensity increased from **8107.21** on day 1 to **196057.60** day 16.

Increase in fluorescence intensities supports the prolonged release of the drug at a predetermined rate by maintaining a constant drug level at a specified period of time. The sustained release studies observed up to 16 days which shows more bioavailability of drug for long time. Combination of different liposomes and inverted micelle can give a much prolonged drug release which has to be further investigated.

CONCLUSION

"Nanoparticulate drug delivery system for vitreous humor" has the ability to provide the drug delivery into the eye. Preparation of antibiotics in a nanoparticulate form can be used to prevent infections in treatment of cataract surgery and age related macula. Nanoparticulated drug delivery shows lesser side effects when compared to antibiotics prepared for oral administration and for instillation into the eye. The antibiotic drug prepared in nanoparticulate form can be directly applied to the location of surgery which reduces the first pass metabolism. Intra ocular lens implantation is the main feature of cataract surgery. Fluorescence studies show that the drug is completely encapsulated into the prepared liposome nanoparticles. Sustained release study results indicated that the prepared nanoparticles provided sustained release of the drug for about 16 days. Cytotoxicity test are to be performed in order to test the toxicity of the formulation. Further research should be carried out by preparing different types of formulations of nanoparticles by using different types of drugs.

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