Development of Dexamethasone sodium phosphate nanoparticles for the post cataract treatment

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1.1 NOVEL DRUG DELIVERY SYSTEMS (NDDS) [1]

The basic goal of novel drug delivery system is to achieve a steady state blood or tissue level that is therapeutically effective and non toxic for an extended period of time.

Conventional drug delivery involves the formulation of the drug into a suitable form, such as compressed tablet for oral administration or a solution for IV administration. These dosage forms have been found to have serious limitations in terms of higher doses required lower effectiveness, toxicity & adverse effects.

NDDS are being developed rapidly, so as to overcome the limitations of conventional drug delivery.

Therapeutic Benefits of Novel drug delivery systems over conventional dosage forms

- Increased efficacy of the drug.
- Site specific delivery.
- Decreased toxicity / side effects.
- Increased convenience.
- Shorter hospitalization.
- Viable treatments for previously incurable diseases.
- Potential for prophylactic application.
- Lower health care costs, both short & long term.
- Better patient compliance.

The reasons for intense interest in NDDS is due to

- The recognition of the possibility of repeating successful drugs by applying concepts and techniques of controlled release drug delivery systems, coupled with the increasing expense in bringing new drug entities to market has encouraged the development of NDDS.
- NDDS are needed to deliver the novel, genetically engineered pharmaceuticals, i.e. Peptides and proteins to their sites of action without incurring significant immunogenic or biological inactivation.
- Treating enzyme deficient diseases & cancer therapies can be improved by better drug targeting.
- Therapeutics & safety drug administered by conventional route can be improved by more precise spatial and temporal placement within the body,

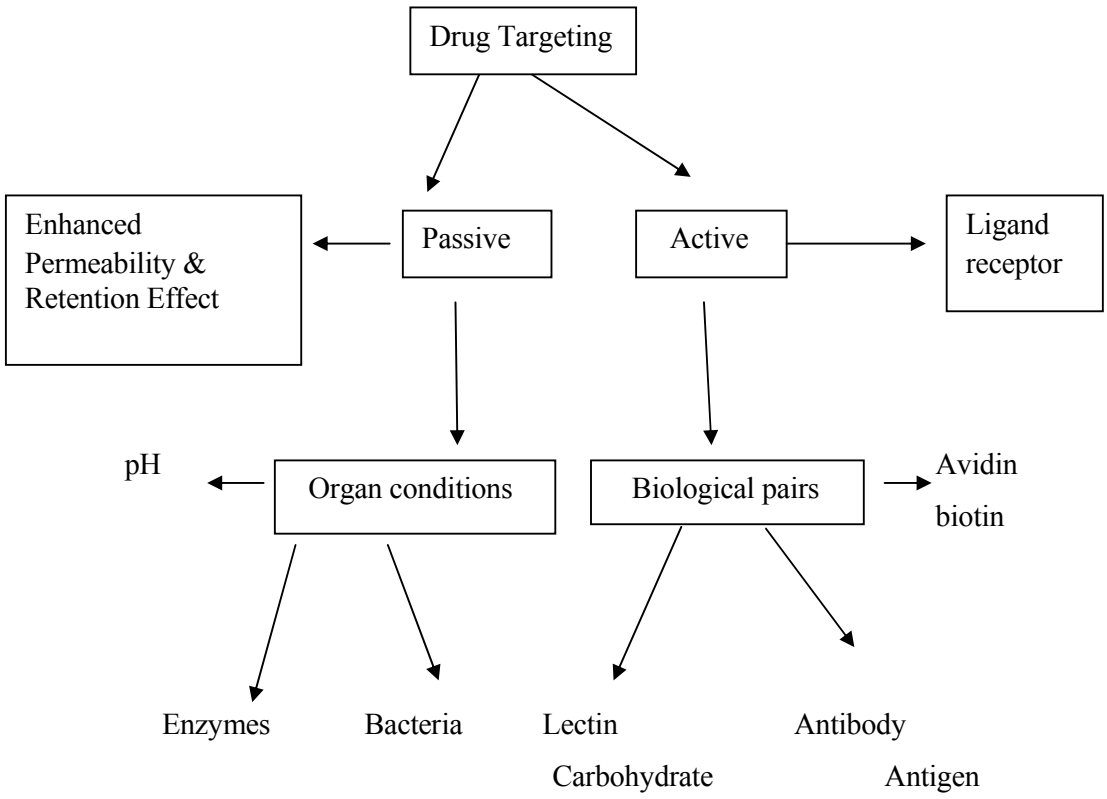
1.2 TARGETING DRUG DELIVERY

Targeted drug delivery may be achieved by using carrier systems, where reliance is placed on exploiting both, intrinsic pathway(s) that these carriers follow, and the bioprotection that they can offer to drugs during transit through the body. The various approaches of vectoring the drug to the target site can be broadly classified as

- Passive targeting
- Inverse targeting
- Active targeting (Ligand mediated targeting and Physical targeting)
- Dual targeting
- Double targeting
- Combination targeting

1.2.1. Passive Targeting

It is a sort of passive process that utilizes the natural course of (attributed to inherent characteristics) biodistribution of the carrier system, through which, it eventually accumulate in the organ compartment(s) of body. The ability of some colloids to be taken up by the RES especially in liver and spleen has made them as ideal vectors for passive hepatic targeting of drugs to these compartments. Passive capture of colloidal carriers by macrophages offer therapeutic opportunities for the delivery of anti-infectives for disease conditions that involve macrophage cells of the reticuloendothelial system (RES) e.g., leishmaniasis, brucellosis and candidiasis. Delivery into lysosomal compartment can also be affected for the treatment of certain lysosomal storage diseases, macrophage neoplasms and macrophage activation.



Schematic representation of the drug targeting

1.2.2. Inverse Targeting

It is essentially based on successful attempts to circumvent and avoid passive uptake of colloidal carriers by reticuloendothelial system (RES). This effectively leads to the reversion of biodistribution trend of the carrier and hence the process is referred to as inverse targeting. One strategy applied to achieve inverse targeting is to suppress the function of RES by a pre-injection of a large amount of blank colloidal carriers or macromolecules like dextran sulphate. This approach leads to RES blockade and as a consequence impairment of host defense system. Alternative strategies include modification of the size, surface charge, composition, surface rigidity and hydrophilicity of carriers for desirable biofate. Recently available literature suggests modification of the surface by imparting distinctive hydrophilicity to the carrier

particles, as an effective mode of targeting of drug(s) to non-RES organs. Phospholipid Microspheres emulsified with Poloxamer 338 showed the' lowest RES uptake in mouse peritoneal macrophages in vitro. Poloxamine 908 is another hydrophilic nonionic surfactant, which diverts normal RES uptake of coated emulsion and coated nanoparticles (polystyrene micro sphere to inflammatory sites in rabbits.

Inverse targeting of drugs to the other than RES rich organs by coating the lipid Micro emulsion (LM) with Poloxamer 308. It has been suggested that surface hydrophilicity may reduce or even eliminate the adhesion of opsonin materials/HDL on to the surface of LM, Which is believed to be an essential step in the process of phagocytosis responsible for ultimate uptake of LM by RES system.

1.2.3. Active Targeting

Active targeting exploits modification or manipulation of drug carriers to redefine its biofate. The natural distribution partum of the drug carrier composites is enhanced using chemical, biological and physical means, so that it approaches and identified by particular biosites. The facilitation of the binding of the drug-carrier to target cells through the use of ligands or engineered homing devices to increase receptor mediated (or in some cases receptor independent but epitope based) localization of the drug and target specific delivery of drug(s) is referred to as active targeting.

The targeting approach can further be classified it into three different levels of targeting, first order targeting (organ compartmentalization), second order targeting (cellular targeting) and third order targeting (intracellular targeting).

♦ First order targeting:

It refers to restricted distribution of the drug-carrier system to the capillary bed of a predetermined target site, organ or tissue. Compartmental targeting in lymphatic, peritoneal cavity, plural cavity, cerebral ventricles, lungs, joints, eyes, etc., represents first order targeting.

♦ Second order targeting:

The selective delivery of drugs to a specific cell type such as tumor cells and not to the normal cells is referred as second order drug targeting. The selective drug delivery to the Kupffer cells in the liver exemplifies this approach.

♦ Third order targeting:

The third order targeting is defined as drug delivery specifically to the intracellular site of target cells. An example of third order targeting is the receptor based ligand-mediated entry of a drug complex into a cell by endocytosis, lysosomal degradation of earner followed by release of drug intracellularly.

1.2.4. Ligand Mediated Targeting

Targeting components, which have been studied and exploited are pilot molecules themselves (bioconjugates) or anchored as ligands on some delivery vehicle (drug-carrier system). All the carrier systems, explored so far, in general, are colloidal in nature. They can be specifically functionalized using various biologically relevant molecular ligands including antibodies, polypeptides, and oligosaccharides carbohydrates), viral proteins and fusogenic residues. The ligands afford specific avidity to drug carrier. The engineered carrier constructs selectively deliver the drugs to the cell or group of cells generally referred to as target. The

cascade of events involved in ligand negotiated specific drug delivery is termed as ligand driven receptor mediated targeting.

1.2.5. Combination Targeting

These targeting systems are equipped with carriers, polymers and homing devices of molecular specificity that could provide a direct approach to target site. Modification of proteins and peptides with the natural polymers, such as polysaccharides containing natural polymers, such as polysaccharides, or their tissues within the vasculature.

1.2.6. MERITS OF TARGETED DRUG DELIVERY SYSTEM (TDDS) [2]

♦ Targeted drug delivery system provides a reduction in drug blood level fluctuation. It provides a control in the rate of drug release in case of controlled drug delivery system i.e. the "Peaks and valleys" of drug in blood or serum levels are eliminated.

♦ There is a reduction in dosing frequency i.e. the rate-controlled products deliver more than a single dose of medication and then are taken less often than conventional forms.

♦ Enhanced patient convenience and compliance with less frequency of dose administration, the patient is less apt to neglect take mg, a dose. There is a\so greater patient convenience with day time and night time medication, and acute or chronic illness.

♦ Reduction is adverse side effects. Because there are seldom drug blood level peaks above the drugs therapeutics range, and into the toxic range, adverse side effects are less frequently encountered.

♦ Reduction in health care costs (i.e.) economy. Although the initial cost of rate-controlled drug delivery form, the average cost of treatment over extended time period may be less with less frequency of dosing enhanced therapeutic benefit and reduced side effects, the time required for health care personal to dispense, administer and monitor patient is reduced.

♦ The major merit of CDDS is that the duration of action can be extended for days or months sometimes up to one year.

1.2.7. LIMITS OF TARGETED DRUG DELIVERY SYSTEM (TDDS)

♦ Certain other TDDS shows poor site specificity e.g., Microsphere.

♦ Targeted drugs delivery system as liposomes, resealed erythrocytes and platelets suffer serious stability problem.

♦ If the particle size of TDDS is high, they may be rapidly cleared by reticuloendothelial system (RES).

1.3. NANOPARTICLES [2, 3]

Nanoparticles are sub-Nano sized colloidal structures composed of synthetic or semi synthetic polymers. Nanoparticles are solid colloidal particles ranging in size 10 nm to 1000 nm (1μm). They are classified into two groups:

- Nanosphere defined as solid core spherical particulates, which are nano metric in size. They contain drug embedded within or adsorbed on to surface.
- Nanocapsules are vesicular system in which drug is essentially encapsulated within the central volume surrounded by an embryonic continuous polymeric sheath.

Nanoprticles (NPs) based drug delivery system has considerable potential for the treatment of Cancer. The importance technological advantages of NPs used has drug carrier are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances and feasibility of variable routes of administration including oral application, parenteral administration and inhalation.

There has been considerable interest in developing biodegradable nanospheres as effective drug delivery system. The controlled release of pharmacologically active agents to the specific site of action at the therapeutically optimal rate and dose regimen has been a major goal in designing such devices. Various polymers have been explored as sustained release and protective carrier of drugs to a target site and thus increase the therapeutic benefit, while minimizing side effects. Among these polymers, Eudragit have tremendous interest due to their excellent biocompatibility and biodegradability several of formulations using these polymers have received world wide marketing approval. [4]

Synthetic biodegradable polymers have been increasingly used to deliver drugs, since they are free from most of the problems associated with the natural polymers. The natural polymers are limited due to their higher cost and questionable purity. Biodegradable polymers from natural or synthetic origin are degraded either enzymatically or non- enzymatically or both produce biocompatible, toxicologically safe by-products which are further eliminated by the normal metabolic pathways.

1.3.1 Advantage of nanoparticles [2]

- Enhancement of therapeutic effectiveness of the drug i.e. the over all pharmacological response per unit dose in increased.
- Toxicity and adverse drug reactions are reduced to a possible extent e.g. polymethacrylic nanoparticles for targeting anticancer drug Doxorubicin to reduce liver toxicity.
- Nanoparticles posses' better stability as compared to liposomes which makes it more important for many modes of targeting.
- Nanoparticles formulated as amorphous spheres offer solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence the bioavailability.
- The methods of preparation are simple, easier and reproducible.
- A high degree of patient compliance can be achieved.
- Wide range of polymer can be used depending on the nature, of the drug and usage i.e. biodegradable polymer for shorter periods, and non- biodegradable for longer periods.
- It can easily pass through syringe needle and exhibit good rheological properties.

1.3.2. Preparation techniques of nanoparticles [5, 6]

Polymer precipitation methods.

- Emulsification – solvent evaporation
- Solvent displacement (Nanoprecipitation)
- Salting out

1.3.2.1. Emulsification – solvent evaporation method

The method has two alternatives depending on the nature of the drug to be entrapped within the nanospheres: the simple emulsion (w/o) and the double emulsion (w/o/w) techniques. In the first, this is used for the encapsulation of hydrophobic drugs. The polymer and the drug are dissolved in the volatile organic solvent immiscible with water, such as dichloromethane or emulsified under intense shear using homogenization, microfluidisaiton or probe sonication into an aqueous phase contains appropriate amounts of a surfactant 9e.g. sodium cholate or PVA). The organic solvent is allowed to nanoparticles formed are usually collected by centrifugation redispersed in distilled water and freeze dried in the second method, which used for the encapsulation of hydrophilic drug and proteins, the drug is dissolved in a small volume of an aqueous phase and this is emulsified in an organic phase containing the polymer. The w/o emulsion formed is then dispersed in a larger volume of an aqueous phase to form the double w/o/w/ emulsion. The remaining steps of the preparation are the same as in the simple emulsion method.

Advantages:

- The use of non- highly –toxic solvents (i.e. ethyl acetate).
- Additives can be used for nanoparticle size reduction.

- Suitable for hydrophilic (double emulsions) and hydrophobic active components.
- The solvent is removed by evaporation (energy consumption), but the process time for solvent removal is reduced (special with fast evaporation with vacuum).

Disadvantages:

- The addition of active component affects the final size of nanoparticles.
- High consumption of energy by the necessity of high shear stress (i.e. sonication).
- The major drawbacks of this method are the use of relatively toxic organic solvents dichloromethane and chloroform belong to class 2 in the ICH guidelines for residual solvents and the application of intense shear. Both the presence of the organic solvent and the application of intense shear forces may cause degradation of labile drugs, such as proteins.

1.3.2.2. Solvent displacement method [7]

The polymer and the drug are dissolved in the organic solvent miscible with water (e.g. acetone) the organic phase is slowly transferred to an aqueous phase containing a surfactant (e.g. PVA or poloxamer 188) under mild stirring. A submicron o/w emulsion is spontaneously formed drug to immediate reduction of the interfacial tension with rapid diffusion of acetone into the aqueous phase (the Marangoni effect). Following acetone diffusion, the nanospheres are formed by aggregation of polymer and drug. The organic solvent is removed by evaporation and the nanosphere suspension is concentrated under reduced pressure, purified by centrifugation or dialysis (removal of non- entrapped drug and excess surfactant) and freeze dried the

presence of surfactant) facilitate their sedimentation. The term nano precipitation and solvent diffusion have also been used to describe solvent displacement techniques.

To improve the encapsulation of water-soluble drugs changed the dispersing medium from an aqueous solution to a medium chain triglyceride (span 80) in the polymer phase. Another drawback of this method is that many drugs are not well soluble in the semi polar solvents, such as acetone or alcohol. To improve the solubility and entrapment of certain drugs a mixture of acetone with dichloromethane was used instead of acetone alone.

Advantage:

- The use of non – highly –toxic solvents (acetone).
- Reduced energy consumption because it only required regular stirring. The process does not require high stress shear. (I.e. sonication or microfluization).
- Additives can be used for nanoparticle size reduction.

Disadvantages:

- The solvent is removed by evaporation (time consuming).
- The main draw back is the requirement for drugs to be highly soluble in polar solvents (acetone, ethyl acetate) and slightly soluble in water to minimize losses during solvent diffusion.
- Nanoparticle size is very much affected by the polymer concentration, higher nanoparticle sizes are obtained at higher polymer concentrations.

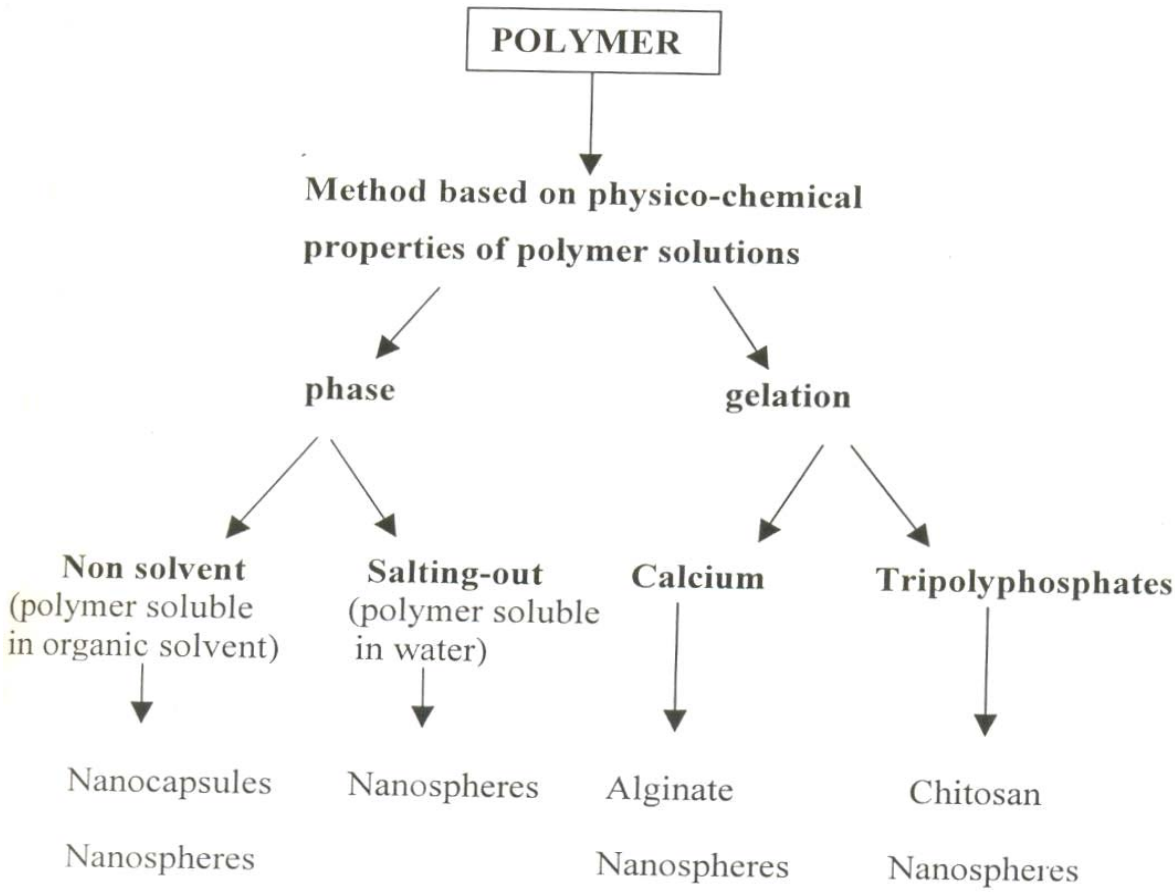
1.3.2.3. Salting out method

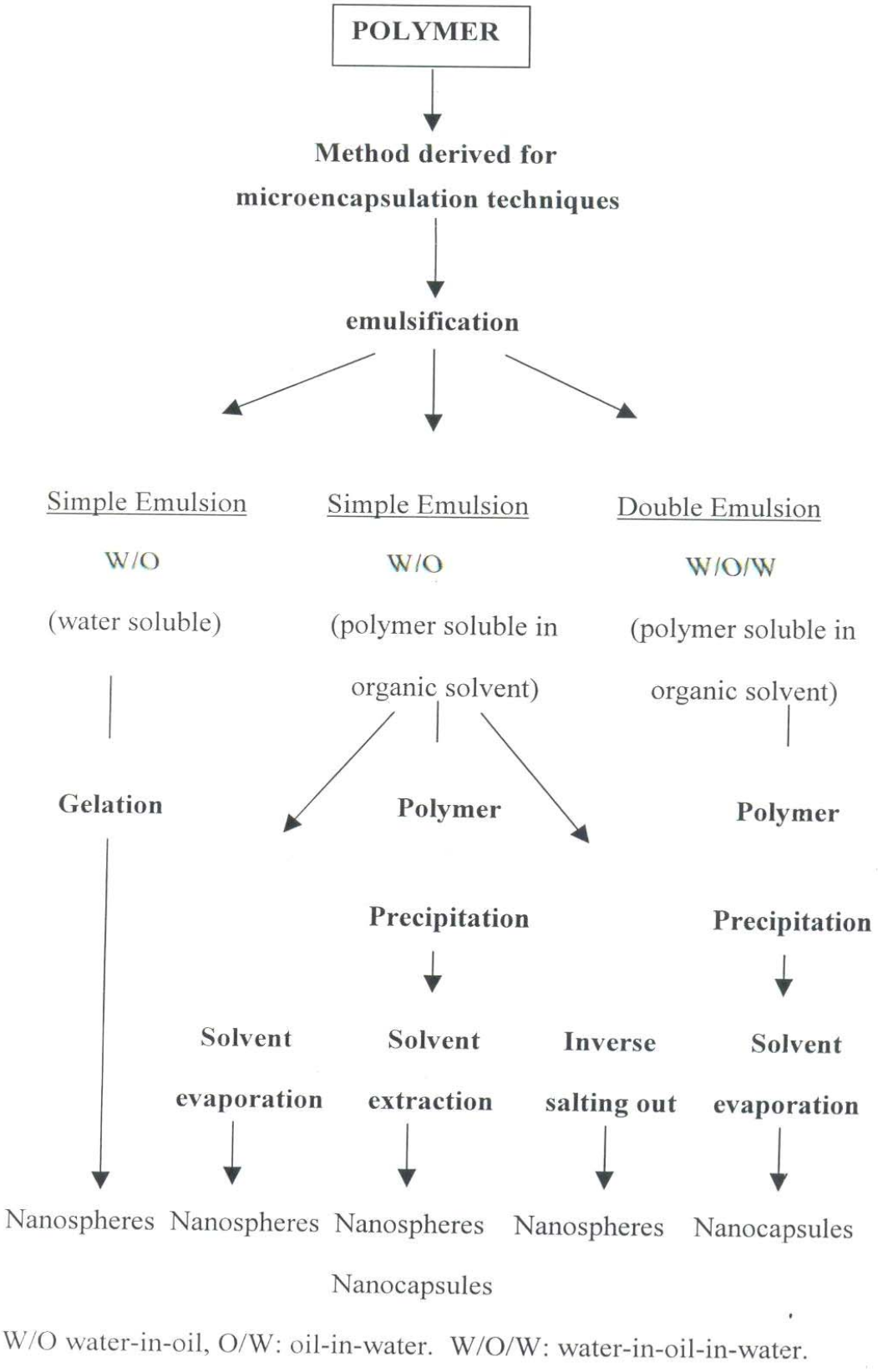
In the salting out method, the polymer and the drug are dissolved in a water-miscible solvent (e.g. acetone or tetrahydrofuran) and the solution is emulsified under

vigorous mechanical stirring in an aqueous gel containing the salting out agent and a colloidal stabilizer (e.g. to enhance the with a sufficient volume of water to enhance the diffusion of acetone in to aqueous phase, thus inducing the formation of nanospheres salting as salting electrolytes, such a sucrose.

The method is available for the preparation of polymeric nanosphere and nanocapsules degradation and drug dissolution or leakage, which are very likely to occur if the nanospheres are stored as aqueous dispersions.

To prevent nanosphere aggregation and retain nanosphere redispersibility after freeze-drying, a suitable cryoprotectant such as trehalose or sucrose has to be added in the nanosphere dispersion lyophilization.





W/O water-in-oil, O/W: oil-in-water. W/O/W: water-in-oil-in-water.

Summary of the different methods to prepare nanospheres and the nanocapsules from polymer. [8]

By increasing the concentration of the nanopraticles suspension at a very low temperature improved the lyoprotective effect of trehalose. They also reported that most of the lyoprotected nanosphere remained stable for at least 12 weeks when stored at $+4^0$C and -25^0C under dry argon.

Advantages:

- Reduced emerge consumption because it only requires normal stirring. The process does not require high stress shear (i.e. sonication or microfluidization).
- Low time- consuming process.
- Suitable for hydrophobic components because the salting out agent is water soluble.

Disadvantages:

- The use of not highly toxic, but explosive solvents (i.e. acetone).
- The main drawback is the requirement of a purification step for salting out agent elimination, which is the in higher amount (at least three times more amount of salting out than polymer).

1.4. NANOPARTICLES IN OCULAR DRUG DELIVERY [9]

1.4.1. Conventional Dosage Forms and Restrictions

The main objective in ocular therapeutics is to provide and maintain adequate concentration of the drug at the site of action. Most ocular diseases are treated with topical applications of solutions administered as eye drops. The major deficiencies of this conventional dosage form include poor ocular drug bioavailability, pulse drug entry, systemic exposure due to the nasolacrimal duct drainage, and poor entrance to the posterior segments of the eye due to the lens diaphragm.

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constrains, which protect the eye and maintain visual functions. After instillation of an ophthalmic drug, most of it is rapidly eliminated from the pre corneal area due to drainage by the nasolacrimal duct, blinking and dilution by the tear turnover (approximately 1µl/min). it has been determined that as much as 90% of the 50ml dose administered as eye drops is cleared within 2 minutes and only 1-5% of the administered dose permeates to the eye. Furthermore, the cornea is a highly selective barrier with five different layers that exclude compounds from the eye. The main corneal barriers are the (i) lipophilic epithelium layers (50µm) with its tight junctions and high turnover of one cell layer per day and (ii) the hydrophilic stroma (450 µm) which represents a rate limiting barrier for absorption of lipophillic drugs. The conjuctival uptake of topically applied drugs is typically in an order of magnitude greater than the corneal uptake due to the relative leakiness of the membrane, the rich blood flow, and the large surface area. Most drugs however are rapidly removed by systemic uptake through the vessels embedded in that tissue before diffusion to the intraocular tissues. Enzymatic metabolism may also account for further loss which can occur in the precorneal space or in the cornea. Therefore, high administration

frequency and large doses of drug are required, resulting with fluctuations in ocular drug concentrations and local / systemic side effects. These systemic side effects after topical administration, mainly due to the nasolacrimal drainage, can cause severe systemic toxicity when using potent drugs such as timolol.

Systemically administered drugs have poor access to the eye because of the blood-ocular barrier, which physiologically separates the eye from the rest of the body by epithelial and endothelial components whose tight junctions limit transport from blood vessels to the eye. The barrier is comprised of two systems; (i) the blood-aqueous barrier, which prevents drugs from entering the aqueous humor, and (ii) the blood-retinal barrier, which prevents drugs from entering into the extra vascular space o retina and into the vitreous body. Subconjuctival and intravitreal injections of drugs are also applied clinically, generating elevated intraocular concentrations with minimal systemic effects. These methods, however, are painful, inconvenient, and associated with severe complications such as perforation of the globe and scarring of the conjunctiva.

1.4.2. Ocular Pharmacokinetics: [10]

The main routes of drug administration and elimination from the eye have been shown schematically in Fig. 1.

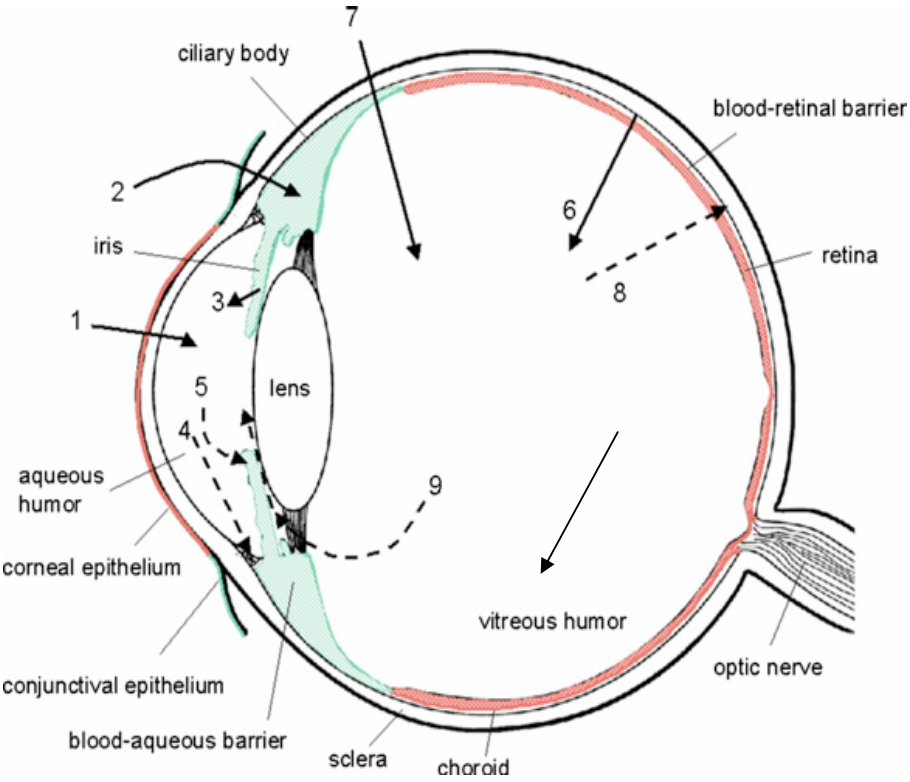


Fig1. Schematic presentation of the ocular structure with the routes of drug kinetics illustrated. The numbers refer to following processes: 1) transcorneal permeation from the lacrimal fluid into the anterior chamber, 2) non-corneal drug permeation across the conjunctiva and sclera into the anterior uvea, 3) drug distribution from the blood stream via blood-aqueous barrier into the anterior chamber, 4) elimination of drug from the anterior chamber by the aqueous humor turnover to the trabecular meshwork and Sclemm's canal, 5) drug elimination from the aqueous humor into the systemic circulation across the blood-aqueous barrier, 6) drug distribution from the blood into the posterior eye across the blood-retina barrier, 7)

intravitreal drug administration, 8) drug elimination from the vitreous via posterior route across the blood-retina barrier, and 9) drug elimination from the vitreous via anterior route to the posterior chamber.

1.4.3. The Barriers

1.4.3.1. DRUG LOSS FROM THE OCULAR SURFACE

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity. Anyway, most of small molecular weight drug dose is absorbed into systemic circulation rapidly in few minutes. This contrasts the low ocular bioavailability of less than 5%. Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid extensively. Therefore, constant drug release from solid delivery system to the tear fluid may lead only to ocular bioavailability of about 10%, since most of the drug is cleared by the local systemic absorption anyway.

1.4.3.2. LACRIMAL FLUID-EYE BARRIERS

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal barrier is formed upon maturation of the epithelial cells. They migrate from the limbal region towards the center of the cornea and to the apical surface. The most apical corneal epithelial cells form tight junctions that limit the Para cellular drug permeation. Therefore, lipophilic drugs have typically at least an order of

magnitude higher permeability in the cornea than the hydrophilic drugs. Despite the tightness of the corneal epithelial layer, transcorneal permeation is the main route of drug entrance from the lacrimal fluid to the aqueous humor (Fig. 1). In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea. Drug absorption across the bulbar conjunctiva has gained increasing attention recently, since conjunctiva is also fairly permeable to the hydrophilic and large molecules. Therefore, it may serve as a route of absorption for larger bio-organic compounds such as proteins and peptides. Clinically used drugs are generally small and fairly lipophilic. Thus, the corneal route is currently dominating. In both membranes, cornea and conjunctiva, principles of passive diffusion have been extensively investigated, but the role of active transporters is only sparsely studied.

1.4.3.3. BLOOD-OCULAR BARRIERS

The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier (Fig. 1). The anterior blood-eye barrier is composed of the endothelial cells in the uvea. This barrier prevents the access of plasma albumin into the aqueous humor, and limits also the access of hydrophilic drugs from plasma into the aqueous humor. Inflammation may disrupt the integrity of this barrier causing the unlimited drug distribution to the anterior chamber. In fact, the permeability of this barrier is poorly characterized. The posterior barrier between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extra vascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia. Despite its

high blood flow the choroidal blood flow constitutes only a minor fraction of the entire blood flow in the body. Therefore, without specific targeting systems only a minute fraction of the intravenous or oral drug dose gains access to the retina and choroid. Unlike blood brain barrier, the blood-eye barriers have not been characterized in terms of drug transporter and metabolic enzyme expression. From the pharmacokinetic perspective plenty of basic research is needed before the nature of blood-eye barriers is understood.

1.4.4. Early Attempts to Enhance Ocular Bioavailability: [11]

Initial attempts to overcome the poor bioavailability of topically instilled drugs typically involved the use of ointments based on mixtures of white petrolatum and mineral oils and suspensions. Ointments ensure superior drug bioavailability by increasing the contact time with the eye, minimizing the dilution by tears, and resisting nasolacrymal drainage. Because these vehicles have the major disadvantage of providing blurred vision, they are nowadays mainly used for either nighttime administration or for treatment on the outside and edges of the eyelids. Use of suspensions as ophthalmic delivery systems relies on the assumption that particles may persist in the conjunctival sac. The efficiency of suspensions has shown high variability, which occurred as a result of inadequate dosing, probably mainly due to the lack of patients compliance in adequately shaking the suspension before administration. These disadvantages have led to other approaches being investigated. One of the common methods to optimize prolonged precorneal residence time is to use hydro gels, liposomes, and micro- and nanocarrier systems. In comparison with traditional formulations, these systems have the advantages of increased contact time, prolonged drug release, reduction of systemic side effects, reduction of the number of applications, and better patient's compliance.

1.4.5. New Ocular Drug Delivery Systems: [9]

Various attempts have been made to improve drug bioavailability by increasing both the retention of the drug in the pre-corneal area and the penetration of the drug through the cornea. In addition, patient compliance and comfort considerations in drug administration are important factors that may impact the drugs therapeutic efficacy.

These attempts can be divided into two main categories: bioavailability improvement and controlled- release drug delivery. The first category includes gels, viscosity enhancers such as Carboxy Methyl Cellulose, Polyvinyl alcohol and Carbomers, penetration enhancers, pro-drugs and liposomes. The second category includes various types of inserts and nanoparticles.

The effect of gels and viscosity enhancers on drug bioavailability was found to be a minimal, and vision blurring limits their chances of becoming popular dosage forms, although marketed products are available. Penetration enhancers caused ocular irritation and toxicity, thus no commercial product was developed, and pro-drugs are difficult to design and develop as evidenced by the fact that probably only one ophthalmic pro-drug has been commercialized. Also, inserts are not very popular as drug delivery systems due to patient discomfort and difficulty in administering and maintaining, especially by elderly patients. Liposomes have been extensively investigated as ocular drug delivery vehicles for over a decade because they offer potential benefits of controlled release and protection from metabolic process. However, many problems are associated with liposomes administration to the eye such as; toxicity, irritability, low drug loading, manufacturing difficulties for sterile preparation and inadequate aqueous stability.

To address the above stated problems, micro and nanotechnology involving drug-loaded polymer particles have been proposed as ophthalmic drug delivery techniques providing sustained release of drug targeted to the ocular tissues.

1.5. CATARACT [12]

Any opacity in the lens either developmental or acquired either in its capsule or substance of lens is called cataract.

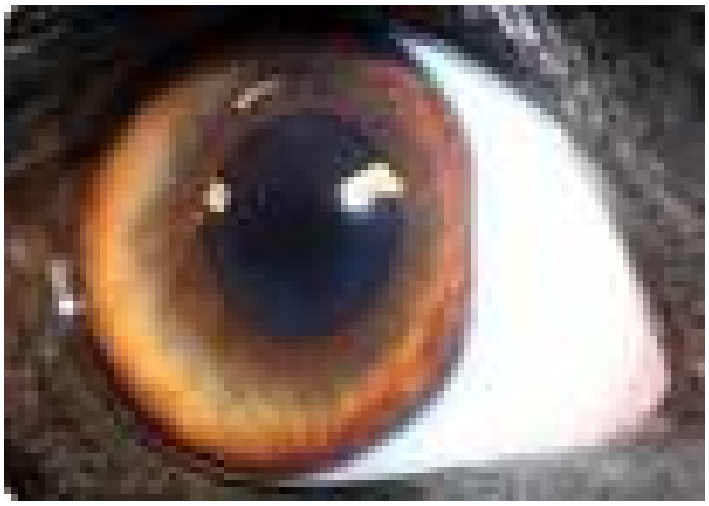
Forty year and above; sexes are equally affected common causes of blindness in the population; 80% of blindness is caused by cataract.

1.5.1. Aetiopathogenesis of Cataract

Exact cause of cataract is unknown. There is a derangement of metabolism of lens, like interference in nutrition as occurs in local eye disease, penetrating trauma which might injure the lens and cause water accumulation leading to cataract. But the common cataract is senile cortical cataract, which occur commonly after 50-60 years of ago the cause, of which is not known.

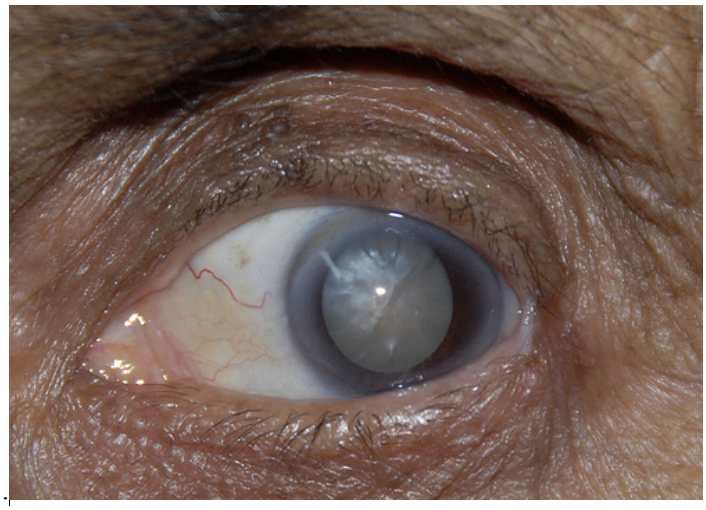
1.5.2. Senile Cataract

Lens fibres are constantly formed from anterior lens capsule and the fibres are arranged regularly in the lens. The older fibres are pushed to centre and condensed to form nucleus. The peripheral or cortical fibres undergo lamellar separation in which fluid droplets appear in between lens fibres. This leads to disturbance in lens metabolism and coagulation of protein of lens fibres. This leads to wedge shaped grey areas as opacity in the cortex. This stage is called immature cataract.



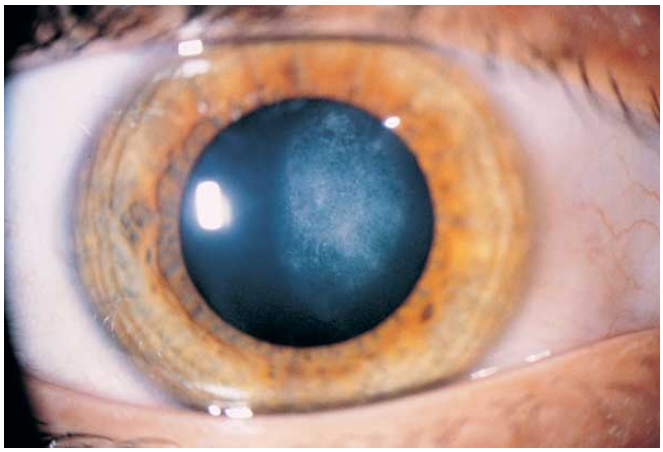
Immature Cataract

Occur initially in the lower and nasal quadrants of lens. Gradually more and more opacity formed between nucleus and cortex and lens appears grey in colour gradually more and more opacity formed and leads to total opacity of lens and colour become grayish-white or purple or pearly-white colour. This stage is called mature cataract.



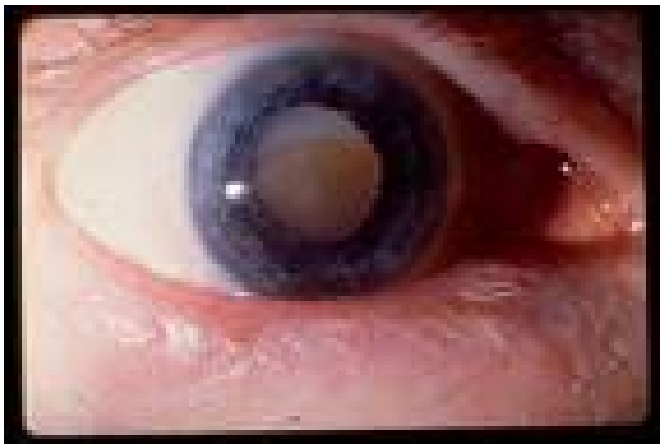
Mature Cataract

During accumulation of water droplets, sudden accumulation of more water gets clogged and whole lens becomes intumescent; swollen and pushes the iris diaphragm forward and cause secondary glaucoma in few cases rarely. In senile cataract some times the opacity strats in the central portion of immediately beneath the posterior capsule and later involve the entire cortex and called posterior sub scapular cataract.



Posterior sub scapular Cataract

When the cortical mature cataract becomes hyper mature if not treated. The cortical lens matter is liquefied and the nucleus sinks down into capsule and this stage is called Morgagnian cataract.



Morgagnian Cataract

1.5.3. Senile Nuclear Cataract

The central nucleus is sclerosed and cortical fibres remain transparent. Then nucleus becomes cloudy and cloudiness spreads to cortex also. There is formation of brown pigment and deposition yellow-pigmented lipoprotein.

1.5.4. Senile Cortical Hypermature Cataract or Shrunken Cataract

In this variety cortical fibres become sclerosed, hardened, dry and flattened leading to the tremulousness of lens, and deep chamber.

1.5.5. Symptoms of Cataract

- Black spots in the field of vision
- Dashing of light while driving in night
- Polyopia
- Haloes around the light
- Detective vision
- Improvement in near vision (due to lenticular myopia)
- Repeated changing of glasses
- Loss of vision (gradual onset)

1.5.6. Signs of Cataract

1. 50 years above common, 40 to 50 years relatively uncommon
2. Both sexes are equally affected
3. Grayish opacity in the papillary area.
4. Iris shadow – when light is thrown at the side of eye iris casts a shadow over pupil in the area between lens opacity and clear lens-in immature cataract.
5. White or grayish-white opacity in papillary area (leucocorea) in mature

1.6. POLYMERS FOR DRUG DELIVERY

The choice of appropriate polymer, particle size, and manufacturing process will primarily depend on the bioacceptabiliy of the polymer,

1.6.1. Characteristics of Ideal polymer system:

An ideal polymer system should possess the following characteristics:

1. It should be inert and compatible with the environment.
2. It should be non-toxic.
3. It should be easily administered.
4. It should be easy and inexpensive to fabricate.
5. It should have good mechanical strength.

Polymers have been classified broadly as,

- **Natural polymers** – Albumin, Starch, Dextran, Gelatin, Fibrinogen, Chitosan.
- **Synthetic polymers** – Polymethyl methacrylate,

Polymethyl methacrylate copolymers:

- Polymethyl cyanoacrylate.
- Polyacrylamide.
- Polyacryl starch.
- Polylactic acid.

Polymers are further classified on the basis of their interaction with water:

- **Non-biodegradable polymers**

  They are inert in the environment of use and eliminated intact from the site of administration and are the limiting factor.

  Ex: Polyethylene vinyl acetate (PVA), Polydimethyl siloxane (PDS)

  Poly urethane (PEU), Ethyl cellulose (EC), Cellulose acetate (CA).

- **Hydro gels**

  These type of polymers swell but do not dissolve when brought in contact with water. They are inert and remover intact from the site of administration.

  Ex: Poly hydroxyl ethyl methacrylate (PHEMA), Cross linked polyvinyl alcohol (PVA)

  Cross linked poly vinyl pyrrolidone (PVP), Polyacrylamide, Dextran.

- **Soluble polymers**

  They are moderate weight uncross-linked polymers that dissolve in water.

  Ex: Poly ethylene glycol (PEG), Hydroxy propyl methyl cellulose (HPMC).

- **Biodegradable polymers**

  These slowly disappear from the site of administration into a chemical reaction like hydrolysis.

  Ex: Polymethyacrylates (EUDRAGIT), Poly lactic acid (PLA).

1.6.2. Drug release mechanisms for polymeric drug delivery devices:

Two broad categories of polymer systems have been studies. The reservoir device involves the encapsulation of a drug within a polymer shell, while the matrix device describes a system in which a drug is physically entrapped within a polymer network the drug will be released over time either by diffusion out the polymer matrix or by erosion (due to degradation) of the polymer or by a combination of two mechanisms.

REVIEW OF LITERATURE

The pre-administration of Dexamethasone (DEX) has previously been shown to enhance the antitumor efficacy of chemotherapeutic agents. The delivery of anti-inflammatory agents specifically to tumors via nanoparticle carriers is expected to promote the effectiveness of chemotherapeutic agents while avoiding systemic toxicities. The process for preparing solid lipid nanoparticles containing anti-inflammatory agents using the nanotemplate engineering method was optimized. Due to the solubilization of DEX in the bulk aqueous phase, its more lipophilic palmitate ester was synthesized and incorporated in nanoparticles that included a pegylating agent, PEG 6000 mono-stearate, as part of the formulation. The stealth properties of these nanoparticles were demonstrated to be enhanced compared to latex particles by measuring the adsorption of radio iodinated IgG (185 μg vs. 6.7 μg IgG/mg NP). In addition, the uptake of 14C-labeled nanoparticles by murine macrophages was shown to decrease from 36.6% to 14.7% of the nanoparticles/mg cell protein as the amount of pegylating agent in the formulation increased from 0 to 4 mg/mL. The high loading values and low burst effect observed for this DEX palmitate-containing nanoparticles in addition to their stealth properties are expected to allow for the delivery of sufficient amounts of DEX to tumors to enhance the uptake of chemotherapeutic agents. [13]

Although eye drops are the most common form of ocular drugs, they have several limitations. Drug absorption into the eye is, in general, less than 5%, addition of preservatives is often necessary, and many drugs cannot be formulated as eye drops. Formulating ocular drugs as powder may solve these problems. The aim of this study was to investigate ocular irritation in rabbits following powder administration.

Timolol maleate (TM) powder was administered to pigmented lop rabbits. Both pure TM powder and freeze-dried with PVP-polymer (2.4% of mass) were tested in 1.0- and 0.1-mg doses. Additionally, 4 rabbits received 0.1 mg of the pure powder 3 times a day for 8 d. Redness of the bulbar conjunctiva and the amount of discharge was rated from photographs (0–3 points, randomized and masked evaluation). The 8-d experiment additionally included examination with a slit lamp and examination of heamatoxylin-eosin stained sections of eyes with light microscopy. No serious or irreversible signs of irritation were noted. Doses of 1.0 mg were more irritating than 0.1-mg doses. There was no detectable difference in irritation between pure or freeze-dried powder. Slit-lamp examination, surface photographs and histology showed a negligible difference between drug and control eyes following the 8-d experiment. The results suggest that 0.1 mg of timolol powder does not irritate the eye and that testing topical timolol powder in humans is feasible. [14]

The present paper concerns both the optimization of Dexamethasone (DXM) entrapment and its release from biodegradable poly (d, l-lactide-coglycolide) (PLGA) nanoparticles prepared by the solvent evaporation process. Since the addition of DXM induced the formation of drug crystals beside the nanoparticle suspension, the influence of several parameters on DXM encapsulation was investigated such as the type of organic solvent and polymer, the DXM initial mass, the evaporation rate of the solvent, the continuous phase saturation and the incorporation of a lipid in the polymer. Nanoparticle size and zeta potential were not modified in the presence of DXM and were respectively around 230 nm and −4mV. The highest drug loading was obtained using 100 mg PLGA 75:25 in a mixture of acetone-dichloromethane 1:1 (v:v) and 10 mg of DXM. The drug was completely released from this optimized formulation after 4 h of incubation at 37 ◦C. Neither the evaporation rate of the

organic solvent, nor the aqueous phase saturation with salt or the incorporation of 1mg 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) within the nanoparticles modified the encapsulation efficiency. Differential scanning calorimetric (DSC) and X-ray diffraction (XRD) demonstrated that the drug was molecularly dispersed within the nanoparticles whereas the non-encapsulated DXM crystallized. These results demonstrate the feasibility of encapsulating Dexamethasone and its subsequent delivery. [15]

The release of the anti-inflammatory agent Dexamethasone (DEX) from nanoparticles of poly (lactic-co-glycolic acid) (PLGA) embedded in alginate hydro gel (HG) matrices was investigated. DEX-loaded PLGA nanoparticles were prepared using a solvent evaporation technique and were characterized for size, drug loading, and in-vitro release. The cross linking density of the HG was studied and correlated with drug release kinetics. The amount of DEX loaded in the nanoparticles was estimated as _13 wt%. The typical particle size ranged from 400 to 600 nm. The in-vitro release of DEX from NPs entrapped in the HG showed that 90% of the drug was released over 2 weeks. [16]

To assess the potential of Chitosan (CS) nanoparticles for ocular drug delivery by investigating their interaction with the ocular mucosa in vivo and also their toxicity in conjunctival cell cultures. Fluorescent (CS-fl) nanoparticles were prepared by ionotropic gelation. The stability of the particles in the presence of lysozyme was investigated by determining the size and their interaction with mucin, by measuring the viscosity of the mucin dispersion. The in vivo interaction of CS-fl nanoparticles with the rabbit cornea and conjunctiva was analyzed by spectrofluorimetry and confocal microscopy. Their potential toxicity was assessed in a human conjunctival

cell line by determining cell survival and viability. CS-fl nanoparticles were stable upon incubation with lysozyme and did not affect the viscosity of a mucin dispersion. In vivo studies showed that the amounts of CS-fl in cornea and conjunctiva were significantly higher for CS-fl nanoparticles than for a control CS-fl solution, these amounts being fairly constant for up to 24 h. Confocal studies suggest that nanoparticles penetrate into the corneal and conjunctival epithelia. Cell survival at 24 h after incubation with CS nanoparticles was high and the viability of the recovered cells was near 100%.CS nanoparticles are promising vehicles for ocular drug Delivery. [17]

The aim of this study was to evaluate solid lipid nanoparticles (SLN) as carriers for topical ocular delivery of tobramycin (TOB). The SLN were in the colloidal size range (average diameter below 100 nm; polydispersity index below 0.2) and contained 2.5% TOB as ion-pair complex with hexadecyl phosphate. The preocular retention of SLN in rabbit eyes was tested using drug-free, fluorescent SLN (F-SLN): these were retained for longer times on the corneal surface and in the conjunctival sac when compared with an aqueous fluorescent solution. A suspension of TOB-loaded SLN (TOB-SLN) containing 0.3% w/v TOB was administered topically to rabbits, and the aqueous humour concentration of TOB were determined up to six hours. When compared with an equal dose of TOB administered by standard commercial eye drops, TOB-SLN produced a significantly higher TOB bioavailability in the aqueous humour. [18]

The objective of present study was to prepare positively charged ciprofloxacin-loaded nanoparticles providing a controlled release formulation. The particles were prepared by water-in-oil-in-water (w/o/w) emulsification and solvent

evaporation, followed by high-pressure homogenization. Two non-biodegradable positively charged polymers, Eudragit® RS100 and RL100, and the biodegradable polymer poly (lactic-co-glycolic acid) or PLGA were used alone or in combination, with varying ratios. The formulations were evaluated in terms of particle size and zeta potential. Differential scanning calorimetric measurements were carried out on the nanoparticles and on the pure polymers Eudragit® and PLGA. Drug loading and release properties of the nanoparticles were examined. The antimicrobial activity against Pseudomonas aeruginosa and Staphylococcus aureus was determined. During solvent evaporation, the size and zeta potential of the nanoparticles did not change significantly. The mean diameter was dependent on the presence of Eudragit® and on the viscosity of the organic phase. The zeta potential of all Eudragit® containing nanoparticles was positive in ultra pure water (around +21/+25 mV). No burst effect but a prolonged drug release was observed from all formulations. The particles' activity against P. aeruginosa and S. aureus was comparable with an equally concentrated ciprofloxacin solution. [19]

Different types of in situ gelling systems of indomethacin, in sodium alginate vehicle, were prepared and evaluated for their pharmaceutical properties including viscosity, sterility and drug content uniformity. The gelling efficacy of the prepared systems was evaluated by using an in house fabricated gelation cell. The in vitro release kinetics of the prepared systems was determined in simulated tear. The gelling time and the nature of the gel formed were dependent on the concentration of sodium alginate present in the systems. The drug release from these systems was extended up to 8 h and predominately followed zero-order kinetics. [20]

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, gatifloxacin, based on the concept of ion-activated systems. Sodium alginate was used as the gelling agent in combination with hydroxy propyl methyl cellulose (Methocel E50LV), which acted as a viscosity enhancing agent. The developed formulations were therapeutically efficacious, stable, and non-irritant and provided sustained release of the drug over an eight hour period. The developed system is thus a viable alternative to conventional eye drops. [21]

Intra ocular implants of sodium alginate alone and in combination with hydroxy propyl methyl cellulose with or without calcium chloride were formulated with indomethacin as a model drug. The drug release from the implants was evaluated using static method, continuous flow through apparatus (developed in house), and USP dissolution and agar diffusion. Except in the static method, indomethacin particle size did not impart any effect on the drug release. In agar diffusion method, an increase in agar concentration from 1 to 2% resulted in a significant decrease ($P<0.005$) in the amount of drug released. Inclusion of hydroxypropylmethylcellulose (33.3, 41.6 and 50% w/w), resulted in decrease of indomethacin release irrespective of the method of dissolution study. The agar diffusion method and the continuous flow through methods seem to simulate to a certain extent the in vivo conditions as far as the placement of the device and the hydrodynamic diffusion layer around the intra ocular implant is concerned. The static method and USP method affected the hydrodynamic diffusion layer either too slowly or too fast. [22]

Hypertonic ophthalmic solutions are used to treat ocular diseases associated with edema. In this study, we developed a chloramphenicol hypertonic ophthalmic solution. These drops were developed based on the co solvency and additional dielectric constant concepts. Two different solvents: PEG 300 and glycerol were used as co solvents. Solubility curves were plotted. Based on the solubility curves, two different solutions were selected. These solutions were evaluated for physical parameters and accelerated stability. The results indicated that chloramphenicol was stable in these formulations. The selected blend of solutions was hypertonic. Thus, the solubility and stability of chloramphenicol was enhanced using a co solvency technique so as to develop a chloramphenicol hypertonic ophthalmic solution. [23]

An ophthalmic gel of Diclofenac sodium, a potent non steroidal anti inflammatory drug was formulated using polymers of hydroxyl propyl methylcellulose. The gels were sterilized and assessed for various parameters like clarity, pH, physical appearance, physical stability, viscosity and uniformity of drug content. Almost 96% of drug was released from the hydroxyl propyl methyl cellulose formulation within a period of 9 h is expressed in the decreasing order as follows hydroxyl propyl methyl cellulose gel > methyl cellulose gel > sodium Carboxy methyl cellulose gel. Ocular irritation on score basis study in rabbits reveals that none of the gel formulation showed any redness, inflammation or increased tear production when compared to placebo. Diclofenac formulation was found to be more stable in hydroxyl propyl methyl cellulose gel compared to methyl cellulose gel & sodium Carboxy methyl cellulose gel at ambient, refrigerator and incubator temperature. The stability of the gel was evidenced by the degradation rate constant. Formulated ophthalmic gel with hydroxyl propyl methyl cellulose proves to be a viable alternative

to conventional eye drops as it offers longer pre corneal residence time and excellent ocular tolerance. [24]

Polymeric micelles made of copolymer of N-isopropylacrylamide (NIPAAM), vinyl pyrrolidone (VP) and acrylic acid (AA) having cross-linkage with N,N%-Methylene bis-acrylamide (MBA) were used as host carrier in which up to 30%w:w ketorolac (free acid) was entrapped to make the formulation. The lyophilized powder was used for physical characterization. The drug entrapment was found to be about 80% and the formulation was stable for 8–10 days at room temperature. The smaller the amount of ketorolac dissolved into the micelles, the longer was the formulation shelf life. The size of the particles as measured by dynamic light scattering was found to be around 35 nm diameter at 25°C. TEM picture showed spherical particles. The structure of the polymer and its morphology were characterized by FTIR, NMR and XRD measurements. IR data indicated weak interaction between polymer and ketorolac in the encapsulated system. NMR spectra indicated rigid polymer backbone with intermittent iso-propyl group in the chain. XRD spectra showed significant loss of crystallinity of the drug while being entrapped in the polymeric micelles. The releases of drug in aqueous buffer (pH 7.2) from the polymeric micelles at 25°C were 20 and 60% after 2 and 8 h respectively and are temperature and pH dependent. In vitro corneal permeation studies through excised rabbit cornea indicated two fold increases in ocular availability with no corneal damage compared to an aqueous suspension containing same amount of drug as in nanoparticles. The formulation showed significant inhibition of lid closure up to 3 h and PMN migration up to 5 h compared to the suspension containing non-entrapped drug, which did not show any significant effect. [25]

Diclofenac sodium ophthalmic inserts were prepared by using methyl cellulose (MC), sodium carboxy methyl cellulose (SCMC) alone and in combination. Weight variation, thickness, drug content, ocular irritation and stability of medicated inserts were evaluated. In vitro study was carried out by using a semi permeable dialysis membrane. According to the results, 97% of drug was released from the formulation containing 4% SCMC and 1% MC in combination over a period of 12 h. release followed zero order kinetics. Medicated inserts were subjected to UV radiation and in vivo drug release studies. No significant change was observed in the drug content and physical features during storage at 30^{o}C and 40^{o}C for 2 months. From this study it was concluded that ophthalmic inserts prepared with 4% SCMC and 1% MC in combination showed sustained release and were found to be stable. [26]

The main objective of this study is to examine the therapeutic activity of hydrophilic glucocorticoid encapsulated in PLGA nanoparticles, which have shown slow release and are targeted to inflamed joints after intravenous administration, in experimental arthritis models. Methods: Betamethasone sodium phosphate (BSP) encapsulated in PLGA nanoparticles with a size of 100–200 nm (PLGA-nanosteroid) was prepared using modified oil in water emulsion solvent diffusion method with Zn ions and coated with lecithin. Rats with adjuvant arthritis (AA rats) and mice with anti-type II collagen antibody induced arthritis (AbIA mice) were treated intravenously with PLGA-nanosteroid after the initial sign of arthritis. Results: In AA rats, a 30% decrease in paw inflammation was obtained in 1 day and maintained for 1 week with a single injection of 100 mg of PLGA-nanosteroid. Soft X ray examination 7 days after this treatment showed decreased soft tissue swelling. Moreover, the PLGA-nanosteroid was also highly effective in AbIA mice. A single injection of 30 mg of the PLGA-nanosteroid resulted in almost complete remission of the

inflammatory response after 1 week. In contrast, the same dose of free BSP after three administrations only moderately reduced the severity of inflammation. In addition, a histological examination 7 days after the treatment showed a significant decrease of the inflammatory cells in the joints. Conclusion: The observed strong therapeutic benefit obtained with PLGA-nanosteroid may be due to the targeting of the inflamed joint and its prolonged release in situ. Targeted drug delivery using a sustained release PLGA-nanosteroid is a successful intervention in experimental arthritis. [27]

Nanoparticles have found wide spread application in varied fields of engineering. Recently, core/shell nanostructures have been found to have improved properties when compared to their other alternatives are patented. These core/shell structures also interest researchers in the field of biomedical engineering and some potential applications have been identified. The classification of core/shell nanoparticles, the synthesis of these structures and their applications in the field of biomedical engineering are discussed in this article. The future work points at the possibilities of improvement and the material that might be preferred for specific applications. [28]

The purpose of this study is to test an aqueous eye drop solution containing a high concentration of Dexamethasone in a cyclodextrin-based drug delivery system. This system increases both drug solubility in aqueous eye drops and drug permeability into the eye, through drug-cyclodextrin- polymer co-complexes. 2-hydroxypropyl-/?-cyclodextrin is a water-soluble oligosaccharide that can be used to dissolve lipophilic drugs, such as Dexamethasone, in aqueous solutions. Co-complexation with a polymer further increases the solubility and increases drug permeability through biologic membranes. Eye drops containing Dexamethasone (0.32% and 0.67%), 2-

hydroxypropyl- /3-cyclodextrin, and polymer were given to patients before cataract surgery, and the resultant Dexamethasone concentration was measured from aqueous humor samples. The Dexamethasone -cyclodextrin drops give a significantly higher concentration of Dexamethasone in aqueous humor than Dexamethasone alcohol 0.1% (Maxidex). Heating of the Dexamethasone-cyclodextrin- polymer co-complexes appears to enhance the permeability of the drug into the eye. The cyclodextrin-based drug delivery system enhances both the solubility of Dexamethasone in aqueous eye drops and the permeability of the drug into the human eye. Dexamethasone concentration levels in the human aqueous humor exceed those reported with currently available steroid eye drops. [29]

Patients with glaucoma and/or dry eye disease often require frequent dosing of topical medications to control their ocular disease. Detergents (such as Benzalkonium chloride) and oxidants (such as stabilized oxychloro complex) are commonly used as preservatives in multidose ophthalmic medications. A literature review was performed to investigate the clinical implications of preservatives in ophthalmic medications that are used for chronic diseases such as glaucoma and/or dry eye disease. Animal histopathology studies and a recent patient study found that ophthalmic medications with Benzalkonium chloride, unlike medications without this agent, were associated with lymphocyte infiltration of the conjunctiva. Ocular damage seemed to be correlated with the level of preservative. A recent rabbit study comparing Benzalkonium chloride preserved medication with stabilized oxychloro preserved medication, found significantly less corneal damage in the group treated with the latter agent. The type and amount of ophthalmic preservative that patients' eyes are exposed to should be taken into account when treating patients with chronic ocular conditions such as glaucoma and/or dry eye disease. [30]

The purpose of the study is to assess the potential of Chitosan (CS) nanoparticles for ocular drug delivery by investigating their interaction with the ocular mucosa in vivo and also their toxicity in conjunctival cell cultures. Fluorescent (CS-fl) nanoparticles were prepared by ionotropic gelation. The stability of the particles in the presence of lysozyme was investigated by determining the size and their interaction with mucin, by measuring the viscosity of the mucin dispersion. The in vivo interaction of CS-fl nanoparticles with the rabbit cornea and conjunctiva was analyzed by spectrofluorimetry and confocal microscopy. Their potential toxicity was assessed in a human conjunctival cell line by determining cell survival and viability. CS-fl nanoparticles were stable upon incubation with lysozyme and did not affect the viscosity of mucin dispersion. In vivo studies showed that the amounts of CS-fl in cornea and conjunctiva were significantly higher for CS-fl nanoparticles than for a control CS-fl solution, these amounts being fairly constant for up to 24 h. confocal studies suggest that nanoparticles penetrate into the corneal and conjunctival epithelia. Cell survival at 24 h after incubation with CS nanoparticles was high and the viability of the recovered cells was near 100%. [31]

Dexamethasone sodium phosphate (DSP) is the most common corticosteroid used in the treatment of edema paired with brain tumors (1, 2). As with other corticosteroids, DSP has some adverse effects on the cardiovascular, immune and nervous systems. The objective of this study was to minimize the adverse effects of DSP and to extend the release time of the drug from Microspheres by encapsulating with Bovine Serum Albumin (BSA). The Microspheres were prepared by emulsion polymerization. An aqueous solution of glutaraldehyde (25% w/v) was used as the cross linking agent in two different amounts. The release time DSP was found to be extended in the series containing 15% DSP with the increase in the amount of

glutaraldehyde used. Also it was observed that the release time is extended in series prepared using 0.5 mL glutaraldehyde with the amount of DSP. [32]

The development of zero-order release systems capable of delivering drug(s) over extended periods of time is deemed necessary for a variety of biomedical applications. We hereby describe a simple, yet versatile, delivery platform based on physically cross-linked poly (vinyl alcohol) (PVA) micro gels (cross-linked via repetitive freeze/thaw cycling) containing entrapped Dexamethasone-loaded poly (lactic co-Glycolic acid) (PLGA) Microspheres for controlled delivery over a 1-month period. The incorporation of polyacids, such as humic acids, Nafion, and poly (acrylic acid), Was found to be crucial for attaining approximately zero order release kinetics, releasing 60% to 75% of Dexamethasone within 1 month. Microspheres alone entrapped in the PVA hydro gel resulted in negligible drug release during the 1-month period of investigation. On the basis of a comprehensive evaluation of the structure-property relationships of these hydrogel/microsphere composites, in conjunction with their in vitro release performance, it was concluded that these polyacids segregate on the PLGA microsphere surfaces and thereby result in localized acidity. These surface-associated polyacids appear to cause acid-assisted hydrolysis to occur from the surface inwards. Such systems show potential for a variety of localized controlled drug delivery applications such as coatings for implantable devices. [33]

The pre-administration of Dexamethasone (DEX) has previously been shown to enhance the antitumor efficacy of chemotherapeutic agents. The delivery of anti-inflammatory agents specifically to tumors via nanoparticle carriers is expected to promote the effectiveness of chemotherapeutic agents while avoiding systemic toxicities. The process for preparing solid lipid nanoparticles containing anti-

inflammatory agents using the nanotemplate engineering method was optimized. Due to the solubilization of DEX in the bulk aqueous phase, its more lipophilic palmitate ester was synthesized and incorporated in nanoparticles that included a pegylating agent, PEG6000 mono-stearate, as part of the formulation. The stealth properties of these nanoparticles were demonstrated to be enhanced compared to latex particles by measuring the adsorption of radio iodinated IgG (185 μg vs. 6.7 μg IgG/mg NP). In addition, the uptake of 14C-labeled nanoparticles by murine macrophages was shown to decrease from 36.6% to 14.7% of the nanoparticles/mg cell protein as the amount of pegylating agent in the formulation increased from 0 to 4 mg/mL. The high loading values and low burst effect observed for this DEX palmitate-containing nanoparticles in addition to their stealth properties are expected to allow for the delivery of sufficient amounts of DEX to tumors to enhance the uptake of chemotherapeutic agents. [34]

The objective of the present study was to develop a novel solid lipid nanoparticle (SLN) for the lung-targeting delivery of Dexamethasone acetate (DXM) by intravenous administration. DXM loaded SLN colloidal suspensions were prepared by the high pressure homogenization method. The mean particle size, drug loading capacity and drug entrapment efficiency (EE %) of SLNs were investigated. In vitro drug release was also determined. The biodistribution and lung-targeting efficiency of DXM-SLNs and DXM-solutions (DXM-sol) in mice after intravenous administration were studied using reversed-phase high-performance liquid chromatography (HPLC). The results (expressed as mean ± SD) showed that the DXM-SLNs had an average diameter of 552 ± 6.5 nm with a drug loading capacity of 8.79 ± 0.04% and an entrapment efficiency of 92.1 ± 0.41%. The in vitro drug release profile showed that the initial burst release of DXM from DXM-SLNs was about 68% during the first 2 h,

and then the remaining drug was released gradually over the following 48 hours. The biodistribution of DXM-SLNs in mice was significantly different from that of DXM-sol. The concentration of DXM in the lung reached a maximum level at 0.5 h post DXM-SLNs injection. A 17.8-fold larger area under the curve of DXM-SLNs was achieved compared to that of DXM-sol. These results indicate that SLN may be promising lung-targeting drug carrier for lipophilic drugs such as DXM. [35]

Vascular endothelial growth factor (VEGF) and Dexamethasone (DX) release from hydrogel coatings were examined as a means to modify tissue inflammation and induce angiogenesis. Antibiofouling hydrogels for implantable glucose sensor coatings were prepared from 2-hydroxyethyl methacrylate, N-vinyl pyrrolidinone, and polyethylene glycol. Micro dialysis sampling was used to test the effect of the hydrogel coating on glucose recovery. VEGFreleasing hydrogel-coated fibers increased vascularity and inflammation in the surrounding tissue after 2 weeks of implantation compared to hydrogel-coated fibers. DXreleasing hydrogel-coated fibers reduced inflammation compared to hydrogel-coated fibers and had reduced capsule vascularity compared to VEGF-releasing hydrogelcoated fibers. Hydrogels that released both VEGF and DX simultaneously also showed reduced inflammation at 2 weeks implantation; however, no enhanced vessel formation was observed indicating that the DX diminished the VEGF effect. At 6 weeks, there were no detectable differences between drug-releasing hydrogel-coated fibers and control fibers. From this study, hydrogel drug release affected initial events of the foreign body response with DX inhibiting VEGF, but once the drug depot was exhausted these effects disappeared. [36]

The ocular bioavailability of timolol increased in sorbic acid solution due to ion pair formation. Its octanol/water partition coefficient also increased, suggesting the formation of a more lipophilic complex. The concentration of timolol in rabbit aqueous humor was determined after instillation of timolol ophthalmic solution containing sorbic acid. When the molar ratio of sorbic acid to timolol was two or higher, the concentration of timolol in the aqueous humor was higher than with timolol alone. In the presence of sorbic acid the maximal aqueous humor concentration and the area under the curve were more than two-fold higher than those of Timoptol®, a timolol maleate ophthalmic solution, and similar in value to TIMOPTIC-XE®, a gel-forming ophthalmic solution. To investigate the transcorneal absorption mechanism, in vitro permeation profiles across the intact and de-epithelialyzed cornea were analyzed on the basis of the bilayer diffusion model. The partition coefficient in the epithelium was about twice as high in the presence of sorbic acid than with timolol alone, although the diffusion coefficient in the epithelium did not change. We conclude that the improved ocular bioavailability in the presence of sorbic acid is due to increased partitioning of timolol in the corneal epithelium. [37]

Topical drug delivery systems of Fluconazole, antifungal drug in the form of gels were formulated using polymers like HPMC, Carbopol 934, Methylcellulose and Sodium alginate. The gels were evaluated for various physicochemical parameters like pH, viscosity, rheology, drug content, spreadibility and skin irritation test. In addition, in vitro drug release by diffusion using cellophane membrane and permeation through hairless rat skin using modified Kiesery Chien Diffusion cell was performed. The rheological behavior and apparent viscosity values for different gel bases were measured before and after storage under freezing condition at 2-8 0C and

were taken as measure for stability of gel network structure. Also accelerated stability testing at 45±2 0C and 75%±5% R.H for 3 months were performed. Among the four formulations, gel prepared using HPMC shows desired properties and exhibit better release pattern when compared with other formulations prepared with Carbopol, Sodium alginate and Methylcellulose. [38]

The poor bioavailability and therapeutic response exhibited by the conventional ophthalmic solutions due to pre-corneal elimination of the drug may be overcome by the use of *in situ* gel forming systems, which upon instillation as drops into the eye undergo a sol-gel transition in the cul-de-sac. This may result in better ocular availability of the drug. The purpose of this work was to develop an ophthalmic delivery system of the NSAID indomethacin, based on the concept of ion activated *in situ* gelation. Gelrite_ gellan gum, a novel ophthalmic vehicle, which gels in the presence of mono or divalent cations present in the lacrimal fluid, was used as the gelling agent. The developed formulations were therapeutically efficacious (in a uveitis induced rabbit eye model) and provided sustained release of the drug over an 8-hour period *in vitro*. [39]

Present limitations in the management of extra ocular diseases include the inability to provide long-term extra ocular drug delivery without compromising intraocular structures and/or systemic drug exposure. In the present study, the potential of Chitosan (CS) nanoparticles as a new vehicle for the improvement of the delivery of drugs to the ocular mucosa was investigated. Cyclosporine a (CyA) was chosen as a model compound because of its potential usefulness for the treatment of these local diseases. An ionic gelation technique was conveniently modified in order to produce CyA-loaded CS nanoparticles. These nanoparticles had a mean size of 293

nm, a zeta potential of +37 mV and high CyA association efficiency and loading (73 and 9%, respectively). In vitro release studies, performed under sink conditions, revealed a fast release during the first hour followed by a more gradual drug release during a 24-h period. In vivo experiments showed that, following topical instillation of CyA-loaded CS nanoparticles to rabbits, it was possible to achieve therapeutic concentrations in external ocular tissues (i.e., cornea and conjunctiva) during at least 48 h while maintaining negligible or undetectable CyA levels in inner ocular structures (i.e., iris/ciliary body and aqueous humour), blood and plasma. These levels were significantly higher than those obtained following instillation of a CS solution containing CyA and an aqueous CyA suspension. From these results, we can conclude that CS nanoparticles may represent an interesting vehicle in order to enhance the therapeutic index of clinically challenging drugs with potential application at extra ocular level. [40]

Although eye drops are the most common form of ocular drugs, they have several limitations. Drug absorption into the eye is, in general, less than 5%, addition of preservatives is often necessary, and many drugs cannot be formulated as eye drops. Formulating ocular drugs as powder may solve these problems. The aim of this study was to investigate ocular irritation in rabbits following powder administration. Timolol maleate (TM) powder was administered to pigmented lop rabbits. Both pure TM powder and freeze-dried with PVP-polymer (2.4% of mass) were tested in 1.0- and 0.1-mg doses. Additionally, 4 rabbits received 0.1 mg of the pure powder 3 times a day for 8 d. Redness of the bulbar conjunctiva and the amount of discharge was rated from photographs (0–3 points, randomized and masked evaluation). The 8-d experiment additionally included examination with a slit lamp and examination of heamatoxylin-eosin stained sections of eyes with light microscopy. No serious or

irreversible signs of irritation were noted. Doses of 1.0 mg were more irritating than 0.1-mg doses. There was no detectable difference in irritation between pure or freeze-dried powder. Slit-lamp examination, surface photographs and histology showed a negligible difference between drug and control eyes following the 8-d experiment. The results suggest that 0.1 mg of timolol powder does not irritate the eye and that testing topical timolol powder in humans is feasible. [41]

Eye-drops are the conventional dosage forms that account for 90% of currently accessible ophthalmic formulations. Despite the excellent acceptance by patients, one of the major problems encountered is rapid precorneal drug loss. To improve ocular drug bioavailability, there is a significant effort directed towards new drug delivery systems for ophthalmic administration. This chapter will focus on three representative areas of ophthalmic drug delivery systems: polymeric gels, colloidal systems, and cyclodextrin and collagen shields. Hydrogels generally offer a moderate improvement of ocular drug bioavailability with the disadvantage of blurring of vision. *In situ* activated gel-forming systems are preferred as they can be delivered in drop form with sustained release properties. Colloidal systems including liposomes and nanoparticles have the convenience of a drop, which is able to maintain drug activity at its site of action and is suitable for poorly water-soluble drugs. Among the new therapeutic approaches in ophthalmology, cyclodextrin represent an alternative approach to increase the solubility of the drug in solution and to increase corneal permeability. Finally, collagen shields have been developed as a new continuous-delivery system for drugs that provide high and sustained levels of drugs to the cornea, despite a problem of tolerance. It seems that new tendency of research in ophthalmic drug delivery systems is directed towards a combination of several drug delivery technologies. There is a tendency to develop systems which not only prolong the

contact time of the vehicle at the ocular surface, but which at the same time slow down the elimination of the drug. Combination of drug delivery systems could open a new directive for improving results and the therapeutic response of non-efficacious systems. [42]

In the present update on mucoadhesive ocular dosage forms, the tremendous advances in the biochemistry of mucin, the development of new polymers, the use of drug complexes and other technological advances are discussed. This review focuses on recent literature regarding mucoadhesive liquid (viscous solutions, particulate systems), semi-solid (hydrogel, in situ gelling system) and solid dosage forms, with special attention to in vivo studies. Gel-forming minitablets and inserts made of thiomers show an interesting potential for future applications in the treatment of ocular diseases. [43]

Ocular bioavailability after instillation of carteolol was investigated by ion pair formation, taking into consideration a balance between lipophilicity and water solubility. The octanol/ water partition coefficient (PCO/W) and the aqueous humor concentration in rabbits after instillation of carteolol containing fatty acids having not more than 6 carbons were measured. The longer carbon chain fatty acid showed the higher PCO/W of carteolol. The aqueous humor concentration of carteolol increased with carbon chain length of fatty acid and was clearly correlated with logPCO/W. The increment of counter ion also increased both the log- PCO/W and aqueous humor concentration of carteolol. The findings suggested that the transcorneal absorption of carteolol would be designed by coordinating with quality and quantity of counter ions. The area under concentration (AUC) in aqueous humor applied by ion pair formulation containing 2% carteolol with sorbate was 2.6 times higher than that by

2% carteolol ophthalmic solution (control), whereas the AUC applied by 4% carteolol ophthalmic solution was 1.4 times higher. The plasma level after instillation of ion pair formulation was almost the same as that of 2% ophthalmic solution. The ratio of AUC (aqueous humor/ plasma) of ion pair formulation was markedly higher, as compared with those of 2% and 4% ophthalmic solution. These results showed that the ion pair formation with sorbate improved the ocular bioavailability of carteolol without enhancing systemic absorption. [44]

The aim of this study was to evaluate the effect of BAY 57-1293, a helicase-primase inhibitor, on herpes simplex virus type 1 (HSV-1) reactivation in mice and its efficacy on established disease in rabbits. BALB/c mice latent for McKrae-strain HSV-1 were reactivated via heat stress, treated with BAY 57-1293, and their corneas were swabbed for virus or the trigeminal ganglia (TG) obtained for quantification of viral DNA. New Zealand white rabbits were infected and treated topically or orally in comparison with trifluridine or valacyclovir. Oral BAY 57-1293 suppressed reactivation in HSV-1-infected mice and reduced the viral load in TG up to four orders of magnitude. In the rabbits, the therapeutic efficacies of topical BAY 57-1293 and trifluridine were similar. Once-daily oral BAY 57-1293 was significantly more effective than valacyclovir and as effective as twice a day topical trifluridine. BAY 57-1293 may be more effective than valacyclovir, without the cytotoxicity or potential healing retardation seen with trifluridine. Oral BAY 57-1293 may be a substitute for eye drops as an effective treatment for herpetic keratitis and might be useful in treating stromal keratitis and iritis, as well as preventing recurrences of ocular herpes. [45]

Dexamethasone is a corticosteroid with proven efficacy for treating both anterior- and posterior-segment ocular diseases. Delivery of drugs to the back of the eye has always been a challenge, with Dexamethasone being no exception. There are multiple delivery routes to the retina, with each exhibiting different pharmacokinetics, depending on the drug molecule and specific route of administration. In this study, we used intravenous (IV), subconjunctival (SC), and intravitreal (IVT) injections in rabbits to determine the pharmacokinetics of Dexamethasone phosphate and its metabolic product, Dexamethasone, at low (25 μg/kg) and high (250 μg/kg) doses. Plasma samples were collected from each group of animals at different time points up to 24 h after the injection. Using a liquid chromatographic mass spectrometric method with a limit of detection of 0.5 ng/ml, the plasma concentration for Dexamethasone and its prodrug compound were quantified. IV delivery showed the fastest plasma elimination, followed by SC delivery. IVT delivery exhibited a depot effect, with very low plasma levels throughout the 24-h time course. At 24 h post injection, only the high-dose IVT and low- and high-dose SC Dexamethasone injections were still detectable in the plasma. [46]

1.7.1. Summary

Nanoparticles act as a controlled release formulation, which establishes and sustains the drug release at target site. The size ranges between 1 to 100 nanometers. The rationale behind the development and use of Nanoparticles are,

- Decrease the toxicity
- Controlling the rate and site of drug release
- Provides a more predictable drug delivery system
- It gives greater convenience and better patient compliance
- They are reproducible
- They can be freeze dried so obtained in dry powder form
- Non- toxic and biodegradable

Our aim is to sustain the action of medicament for more hours using nano sized formulation, for the post operative cases of cataract surgery. There by increasing the intact time of drug in eye which in turn helps to heal the wound faster and increases the patient compliance.

DRUG PROFILE

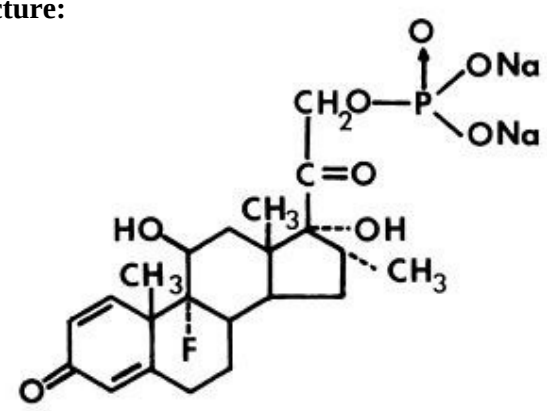
DEXAMETHASONE SODIUM PHOSPHATE

Synonym: Dexamethasone Phosphate Sodium, Dexamethasone 21-(Disodium Phosphate)

Chemical Name: 9-fluoro-11β, 17-dihydroxy-16α-methyl-21-(phosphonooxy) pregna-1, 4-diene-3, 20-dione Disodium salt

Chemical family: Corticosteroid

Structure:



Dexamethasone sodium phosphate

Molecular Formula: $C_{22}H_{28}F\,Na_2O_8P$

Molecular Weight: 516.41

Physical properties: White to almost white powder, very hygroscopic.

Solubility: Freely soluble in water, slightly soluble in alcohol, practically insoluble in ether and Methylene chloride.

Melting Point: 255°C with decomposition

268-271°C

262-264°C

Biological half-life in plasma: 2-5 hours

Use

Systemically and locally for chronic swelling; allergic, hematological, neoplastic, and autoimmune diseases; may be used in management of cerebral edema, septic shock, as a diagnostic agent, antiemetic.

Contraindications

Hypersensitivity to Dexamethasone or any of the component of the formulations are active when untreated infections; ophthalmic use in viral, fungal, or tuberculosis diseases of the eye.

Warnings/Precautions

Use with caution in patients with hypothyroidism, cirrhosis, hypertension, CHF, ulcerative colitis, or thromboembolic disorders. Corticosteroids should be used with caution in patients with diabetes, osteoporosis, peptic ulcer, glaucoma, cataracts, or tuberculosis. Use caution following acute MI (corticosteroids have been associated with myocardial rupture). Use caution in hepatic impairment. Because of the risk of adverse effects, systemic corticosteroids should be used cautiously in the elderly in the smallest possible effective dose for the shortest duration.

Adverse Reactions

Cardiovascular: Edema, hypertension, arrhythmia, cardiomyopathy, myocardial rupture (post-MI), syncope, thromboembolism, thrombophlebitis, vasculitis

Central nervous system: Insomnia, nervousness, vertigo, seizure, psychosis, pseudo tumor cerebra (usually following discontinuation), headache, mood swings, delirium, hallucinations, euphoria

Dermatologic: Hirsutism, acne, skin atrophy, bruising, hyper pigmentation, pruritus (generalized), perianal pruritus (following I.V. injection), urticaria.

Gastrointestinal: Appetite increased, indigestion, peptic ulcer, nausea, vomiting, abdominal distention, ulcerative esophagitis, pancreatitis, intestinal perforation

Genitourinary: Altered (increased or decreased) spermatogenesis *Neuromuscular & skeletal:* Arthralgia, muscle weakness, osteoporosis, fractures, myopathy (particularly in conjunction with neuromuscular disease or neuromuscular blocking agents), tendon rupture, vertebral compression fractures, neuropathy, neuritis, parasthesia.

Ocular: Cataracts, glaucoma, exophthalmos, and intraocular pressure increased.

Miscellaneous: Infections, anaphylactic reaction, anaphylaxis, angioedema, a vascular necrosis, secondary malignancy, Kaposi's sarcoma, intractable hiccups, impaired wound healing, abnormal fat deposition, moon face

Mechanism of Action

Decreases inflammation by suppression of neutrophil migration, decreased production of inflammatory mediators, and reversal of increased capillary permeability; suppresses normal immune response. Dexamethasone mechanism of antiemetic activity is unknown.

Actions

Important to distinguish between physiological effects (replacement therapy) and pharmacological effects (occur at higher doses)

Mineralocorticoid

- Na retention by renal tubule
- increased K excretion in urine

Glucocorticoid

CHO metabolism: increased gluconeogenesis, ± peripheral glucose uptake may be decreased with resultant hyperglycemia ± glycosuria protein metabolism: anabolism is decreased but catabolism continues unabated or is increased resulting in negative N balance and muscle wasting. Osteoporosis occurs, growth slows in children, skin atrophies (together with increased capillary fragility leads to bruising and striae), healing and fibrosis delayed fat deposition: increased on shoulders, face and abdomen inflammatory response depressed allergic response depressed antibody production reduced by large doses lymphoid tissue reduced (including leukemia lymphocytes) decreased eosinophils renal urate excretion increased euphoria or psychotic states may occur. ? due to CNS electrolyte changes anti-vitamin D action reduction of hypercalcaemia (chiefly where this is due to increased absorption from gut: vit D intoxication, sarcoidosis) increased urinary Ca excretion. Renal stones may form growth reduction where new cells are being added (eg in children) but not where they are replacing cells as in adult tissues suppression of HPA axis. NB steroid suppressed adrenal continues to secrete aldosterone

Pharmacodynamics/Kinetics

Onset of action: Prompt

Duration of metabolic effect: 72 hours; acetate is a long-acting repository preparation

Metabolism: Hepatic

Half-life elimination: Normal

Renal function: 1.8-3.5 hours;

Biological half-life: 36-54 hours

Time to peak, serum: Oral: 1-2 hours;

I.M.: ~8 hours

Excretion: Urine and feces

Dosage

Adults:

Anti-inflammatory:

Oral, I.M., I.V. (injections should be given as sodium phosphate): 0.75-9 mg/day in divided doses every 6-12 hours

Intra-articular, intralesional, or soft tissue (as sodium phosphate): 0.4-6 mg/day

Ophthalmic:

Instill 2 drops into conjunctival sac every hour during the day and every other hour during the night; gradually reduce dose to every 3-4 hours, then to 3-4times/day.

Topical: Apply 1-4times/day. Therapy should be discontinued when control is achieved; if no improvement is seen, reassessment of diagnosis may be necessary.

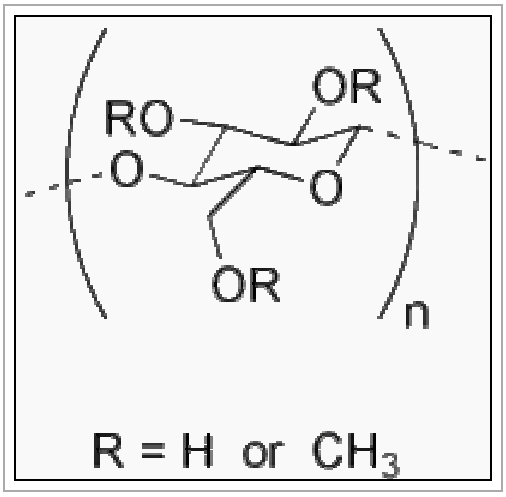
TOXICITY: LD_{50}: Oral (mouse) -1800 mg/kg

Intravenous (mouse)-112mg/kg

POLYMER PROFILE

4.1. METHYL CELLULOSE

Structure:



Other names: Cellulose, Methyl ether; Methylated cellulose; Methylcellulose

Nature: Methyl cellulose (or methylcellulose) is a chemical compound derived from cellulose. Like cellulose, it is not digestible, not toxic, and not allergenic.

Solubility: It is a hydrophilic white powder in pure form and dissolves in cold (but not in hot) water, forming a clear viscous solution or gel.

Chemistry: Chemically, methyl cellulose is a methyl ether of cellulose, arising from substituting the hydrogen atoms of some of cellulose's hydroxyl groups -OH with methyl groups -CH_3, forming -OCH_3 groups.

Different kinds of methyl cellulose can be prepared depending on the number of hydroxyl groups so substituted. Cellulose is a polymer consisting of numerous linked glucose molecules, each of which exposes three hydroxyl groups. The *Degree of Substitution* (DS) of a given form of methyl cellulose is defined as the average

number of substituted hydroxyl groups per glucose. The theoretical maximum is thus a DS of 3.0; however more typical values are 1.3 - 2.6.

Source: Methyl cellulose does not occur naturally and is synthetically produced by heating cellulose with caustic solution (e.g. a solution of sodium hydroxide) and treating it with methyl chloride.

Solubility and temperature:

Methyl cellulose dissolves in cold water. Higher DS-values result in lower solubility, because the polar hydroxyl groups are masked.

The chemical is not soluble in hot water, which has the paradoxical effect that heating a saturated solution of methyl cellulose will turn it solid, because methyl cellulose will precipitate out.

The temperature at which this occurs depends on DS-value, with higher DS-values giving lower precipitation temperatures.

Preparing a solution of methyl cellulose with cold water is difficult however: as the powder comes into contact with water, a gluey layer forms around it, and the inside remains dry. A better way is to first mix the powder with hot water, so that the methyl cellulose particles are well dispersed in the water, and cool down this dispersion while stirring, leading to the dissolution of those particles.

Uses

Methyl cellulose has an extremely wide range of uses, of which several are described below.

1. Scientifically Advanced Cookery

Methyl cellulose, as a gel, has the unique property of setting when hot and melting when cold. This technique is currently being developed at the University of Nottingham.

2. Treatment of constipation

When eaten, methyl cellulose is not absorbed by the intestines but passes through the digestive tract undisturbed. It attracts large amounts of water into the colon, producing a softer and bulkier stool. It is used to treat constipation, diverticulosis, hemorrhoids and irritable bowel syndrome. It should be taken with sufficient amounts of fluid to prevent dehydration.

A well-known trade name of methyl cellulose when used as a drug is Citrucel by GlaxoSmithKline, but generic versions are also widely available.

3. Lubricant

Methyl cellulose is used as a variable viscosity personal lubricant; it is the main ingredient in K-Y Jelly.

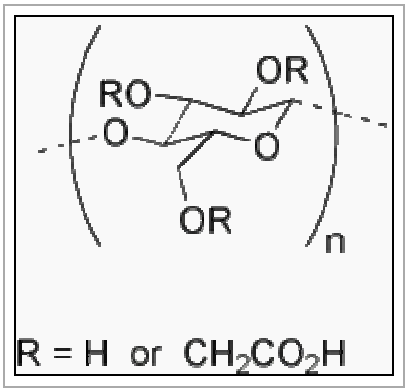
4. Artificial tears and saliva

Solutions containing methyl cellulose or similar cellulose derivatives (see below) are used as substitute for tears or saliva if the natural production of these fluids is disturbed.

4.2. CARBOXY METHYL CELLULOSE

Carboxy methyl cellulose (CMC) is a cellulose derivative with carboxy methyl groups (-CH_2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone. It is often used as its sodium salt, sodium carboxy methyl cellulose.

Structure:



Preparation:

It is synthesized by the alkali-catalyzed reaction of cellulose with chloroacetic acid. The polar (organic acid) carboxyl groups render the cellulose soluble and chemically reactive.

The functional properties of CMC depend on the degree of substitution of the cellulose structure (i.e., how many of the hydroxyl groups have taken part in the substitution reaction), as well as the chain length of the cellulose backbone structure.

Uses

CMC is used in food science as a viscosity modifier or thickener, and to stabilize emulsions in various products including ice cream. As a food additive, it has E number E466.

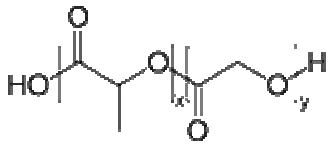
It is also a constituent of many non-food products, such as K-Y Jelly, toothpaste, laxatives, diet pills, water-based paints, detergents, textile sizing and various paper products. It is used primarily because of it has high viscosity, is non-toxic, and is non-allergenic.

CMC is used as a lubricant in non-volatile eye drops (artificial tears). Sometimes it is methyl cellulose (MC) which is used, but its non-polar methyl groups ($-CH_3$) do not add any solubility or chemical reactivity to the base cellulose.

Insoluble micro granular carboxy methyl cellulose is used as a cation-exchange resin in ion-exchange chromatography for purification of proteins.[1] Presumably the level of derivatization is much lower so that the solubility properties of micro granular cellulose are retained while adding sufficient negative charged carboxylate groups to bind positively charged proteins.

4.3. POLY (LACTIC-*CO*-GLYCOLIC ACID) PLGA

Structure:



x= number of units of lactic acid; *y*= number of units of glycolic acid.

PLGA or **poly(lactic-*co*-glycolic acid)** is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility.

Synthesis:

PLGA is synthesized by means of random ring-opening co-polymerization of two different monomers, the cyclic dimers (1, 4-dioxane-2, 5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this polymer include tin (II) 2-ethylhexanoate, tin (II) alkoxides, or aluminum isopropoxide. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, aliphatic polyester as a product.

Depending on the ratio of lactide to glycolide used for the polymerization, different forms of PLGA can be obtained: these are usually identified in regard to the monomers' ratio used (e.g. PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid. All PLGA are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60 °C. Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubility, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.

PLGA degrades by hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production: the higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is the copolymer with 50:50 monomers' ratio which exhibits the faster degradation (about two months). In addition, polymers that are end-capped with esters (as opposed to the free carboxylic acid) demonstrate longer degradation half-lives.

PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is very minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. It, however, can cause trouble for lactose intolerant people. Also, the possibility to tailor the polymer degradation time by altering the ratio of the monomers used during synthesis has made PLGA a common choice in the production of a variety of biomedical devices such as: grafts, sutures, implants, prosthetic devices, micro and nanoparticles. As an example, a commercially available drug delivery device using PLGA is Lupron Depot for the treatment of advanced prostate cancer.

Other biodegradable polymers:

- Polycaprolactone
- Polyglycolide
- Polylactic Acid
- Poly-3-Hydroxybutyrate

4.4. SODIUM ALGINATE

Synonyms: Algin, alginic acid, sodium salt, kelcosol, manugel

Algin is a collective term for the hydrophillic substances isolated from certain brown algae commonly used genera algae are macrocystis pyrifera, laminaria digitate, and ascophyllum.

Empirical Chemical Formula: $NaC_6H_7O_6$.

Description:

A white buff colored, which is odorless. Slowly soluble in water forming a colloidal solution, and practically insoluble in chloroform, alcohol and ether.

Its form as a gum, when extracted from the cell walls of brown algae, is used by the foods industry to increase viscosity and as an emulsifier. It is also used in indigestion tablets and the preparation of dental impressions. Sodium alginate has no discernible flavor.

Another major use of sodium alginate is reactive dye printing, where it is used in the textile industry.

Uses:

A major application for sodium alginate is as thickener for reactive dyestuffs (such as the procion cotton-reactive dyes) in textile screen-printing and carpet jet-printing. Alginates do not react with these dyes and wash out easily, unlike starch-based thickeners.

Sodium alginate is a good chelator for pulling radioactive toxins such as iodine-131 and strontium-90 from the body which have taken the place of their non-radioactive counterparts. It is also used in immobilizing enzymes by inclusion.

Alginates in drug delivery system:

As a matrix material, the function of alginate is to release an active ingredient in a controlled, sustained way. This is accomplished either through erosion of tablets in the ventricle or by dissolution of the alginate in the small intestine.

Cross- linking and gelling properties of alginates:

The cross linking and gelling properties of alginates is a function of both the alginate composition and length of the molecules. The affinity of the cross-linking cations for the alginate is also of great importance in gel making. Calcium is the most widely used cross linking agent, it is believed to interact with five different oxygen atoms of two adjacent guluronate units in intrachem binding. In addition, it makes an egg box model through intrachain binding of ca 2+ or two or more alginate chains in gel.

The chemical reaction between water-soluble sodium alginate and calcium chloride gives a water insoluble calcium alginate. But its ammonium, sodium, magnesium salt as well as propylene glycol esters are readily soluble in cold and hot water to form viscous condition. Viscosities of alginates are stable at pH 4-10.

Safety:

Sodium alginate is widely used in food products and topical or oral pharmaceutical formulations. It is generally regarded as a non toxic and non irritant having satisfactory hemocompatability. Repeated systemic administration of alginate solution did not cause any adverse immunoglobulin G (IgG) and IgM humotal response. Also, alginates were not observed to accumulate in any of the major organs. The WHO has set an estimated acceptable daily intake of alginic acid and alginate salts used as food additives upto 25mg/kg body weight.

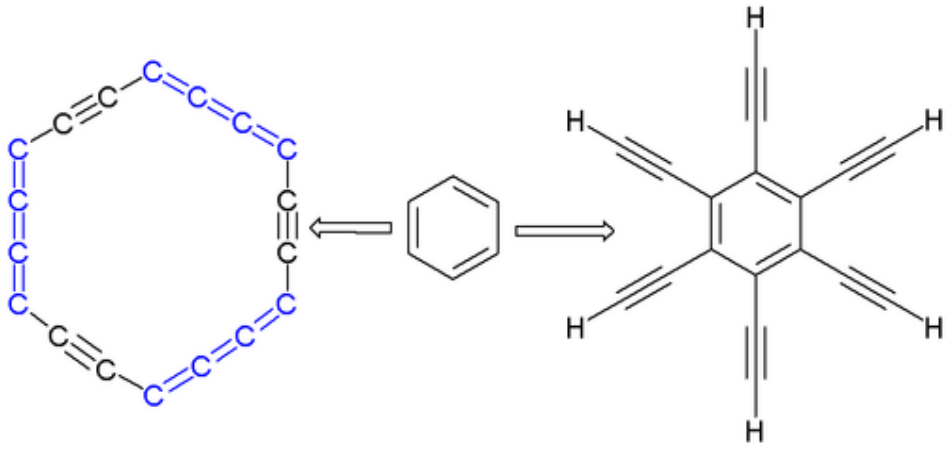
4.5. CARBOPOL

Synonym: Carbomer, Carboxypolymethylene:

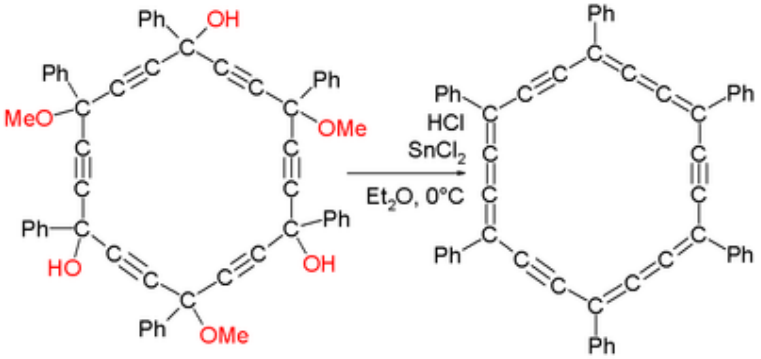
Physical and Chemical Properties

Form:	fluffy powder
Color:	white
Odor:	slightly acidic
Inflammability (solid):	not inflammable
Ignition temperature (dust):	968°F / 520°C
Explosion hazard:	Product is non-ignitable, however development of explosive
Minimum explosive conc.:	0.13 oz/ft3 (130 g/m^3)
Minimum ignition energy:	> 1 Joule at 0.5 kg dust /m^3 air
Vapor density:	Not volatile
Bulk density:	(20°C) 0.19 – 0.24 g/ml
Solubility in water:	dispersible
pH-Value:	2.5 - 3 (10 g/l H20 / 20°C)

In organic chemistry, a **carbomer** is an expanded molecule obtained by insertion of a C_2 unit in a given molecule [1]. Carbomers differ from their templates in size but not in symmetry when each C–C single bond is replaced by at least one alkynes bond, and when a double bond is replaced by an allene bond. The size of the carbomer will continue to increase when more alkyne bonds are introduced and for this reason carbomers are also called **carbo^n-molecules**, where "n" is the number of acetylene or allene groups in an n-expansion unit. This concept, devised by Remi Chauvin in 1995, is aimed at introducing new chemical properties for existing chemical motifs.

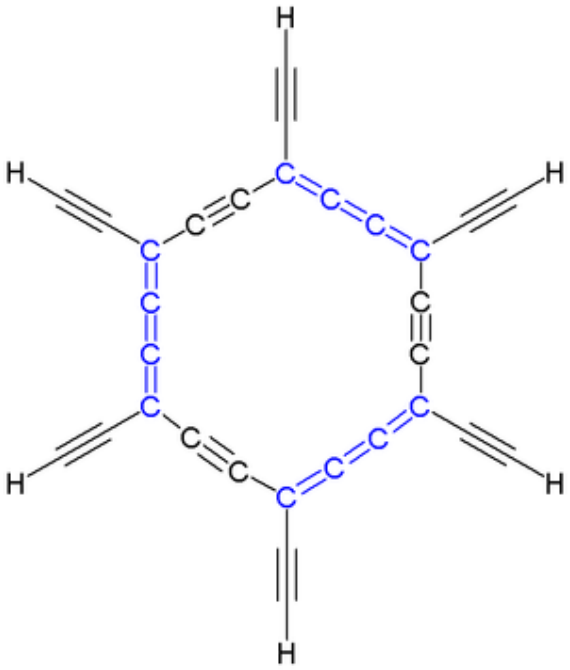


Two representations exist for **carbo-benzene** (written as an [18]annulene derivative: 1,2,4,5,7,8,I0,11,13,14,16,17-dodecadehydro[18]annulene), one has the aromatic core of benzene expanded, and one has the hydrogen substituents expanded. The substituted benzene derivative hexaethynylbenzene is a known compound, [2] and the core-expanded molecule also exists, although with the hydrogen atoms replaced by phenyl groups [3]. The final step in its organic synthesis is organic oxidation of the triol with stannous chloride and hydrochloric acid in diethyl ether.



The proton NMR spectrum for this compound shows that the phenyl protons are shifted downfield compared to a proton position in benzene itself (chemical shift position for the ortho proton is 9.49 ppm), suggesting the presence of a diamagnetic ring current and thus aromaticity.

The total carbomer of benzene with core and periphery expanded ($C_{30}H_6$) only exists in silicon [4].



Calculations predict a planar D_{6h} structure with bond lengths similar to the other two carbobenzenes. Its non-planar isomer is called "hexaethynyl-carbo-[6] trannulene" - a pun on the all-*cis* annulenes - and resembles a cyclohexane ring. This hypothetical molecule is predicted to be more energetic by 65 kcal/mol.

Other carbomer meanings

Carbomer is also a generic name for synthetic polymers of acrylic acid used as emulsion stabilizers or thickening agents in pharmaceuticals and cosmetic products. They may be homopolymers of acrylic acid, cross linked with an allyl ether pentaerythritol, allyl ether of sucrose, or allyl ether of propylene. CarboMer is also a California-based biotechnology firm.

OBJECTIVE OF THE WORK

Cataract is a degradation of the optical quality of the crystalline lens. The development of cataract is therefore a continuum, extending from minimal changes of original transparency in the crystalline lens to the extreme stage of total opacity.

Of the total estimated 45 million blind persons in the World, 7 million are in India. The main objective of National Programme for Control of Blindness is to reduce the prevalence of blindness in the country from 14 per 1000 to 3 per 1000 population. In Tamilnadu, the prevalence of blindness is 4 per 1000 population.

Postoperative regimens of topically applied antibiotics, corticosteroids, and NSAIDs vary among practitioners. Most commonly used Corticosteroids with antibiotics are,

- DEXASONE (Dexamethasone sodium phosphate0.1%, Neomycin Sulphate 0.5%)
- DECADAN (Dexamethasone sodium phosphate0.1%)
- BETNESOL (Betamethasone sodium phosphate0.1%, Neomycin Sulphate 0.5%)
- TOBA-DM (Dexamethasone sodium phosphate0.1%, Tobramycin Sulphate 0.3%)
- CHLOROMET-DM (Dexamethasone sodiumphosphate0.1%, chloramphenicol 0.3%)
- MICROFLOX-DM (Dexamethasone 0.1%, Ciprofloxacin 0.3%)
- OFLACIN-DX (Dexamethasone sodium phosphate0.1%, Ofloxacin 0.3%)
- SCAT-DM (Dexamethasone sodium phosphate0.1%, Sparfloxacin 0.3%)

These are some common conventional dosage forms intended for Post cataract surgery which has to be instilled to eye once an hour till the inflammation suppresses and wound heals. This causes inconvenience to the patients.

There are no controlled investigations that establish optimal regimens for the use of topical agents; therefore, it is the decision of the operating surgeon to use any or all of these products singly or in combination. Complications of postoperative medications include elevated IOP with corticosteroids and allergic reactions to antibiotics, delayed corneal healing due to patient non compliance. In order to reduce the side effects, non compliance, dosing frequency, indirect cost rise in post operative therapy due to the use of higher antibiotics ophthalmic drugs can be incorporated in different carriers such as Microspheres , Nanoparticles , Polymeric micelles and vesicular like Liposomes, Niosomes, Pharmacosomes, Virosomes.

Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment. At present no available drug delivery system behaves ideally achieving lofty goals, but sincere attempts have been made to achieve them through novel approaches in drug delivery systems.

In the treatment of post operative cases of cataract, instillation of eye drops containing corticosteroids with antibiotics (i-e) for every hour installation of medicaments is one of the major draw back for getting compliance from the patient. It needs nursing care.

To overcome the above drawbacks we wish to formulate an ophthalmic preparation containing Dexamethasone sodium phosphate nanoparticles for the Post cataract treatment with the objectives

- To enhance contact time of drug in eye,
- To enhance the bioavailability to corneal epithelium,
- To provide a sustained action,

- To reduce the dosing frequency,
- To improve the patient compliance ,
- To study the effect of bioadhesive property of Dexamethasone sodium phosphate nanoparticle gel in post cataract surgery.

5.1. PLAN OF WORK

1. Construction of Standard Curve.
2. Preparation of Ophthalmic particulate solution by solvent evaporation method.
3. Determination of Particle size by optical Microscopical method.
4. Dispersion of particulate solution in gels.
5. Drug content uniformity.
6. pH of the formulated gels.
7. Determination of Viscosity by Brookfield Viscometer.
8. *In vitro* drug release using dialysis membrane.
9. Sterilization.
10. *In vivo* studies in rabbits.

MATERIALS AND METHODS

6.1. MATERIALS

S.NO	CHEMICALS	SOURCE
1.	Dexamethasone sodium phosphate	Gift sample from Appasamy formulations. Ltd, Puducherry
2.	Sodium alginate	Loba Chemie, Mumbai
3.	Carbopol 934	Loba Chemie, Mumbai
4.	Methyl cellulose (MC)	Loba Chemie, Mumbai
5.	Carboxy Methyl cellulose(CMC)	Loba Chemie, Mumbai
6.	Poly(lactic- Glycolic) acid 85:15 (PLGA)	Sigma-Aldrich, Hyderabad
7.	Phosphotidyl Choline	Sigma-Aldrich, Hyderabad
8.	Poly Vinyl alcohol (PVA)	Loba Chemie, Mumbai
9.	Dichloro methane	Loba Chemie, Mumbai
10.	Dialysis membrane (M.wt.14,000)	Himedia, Mumbai
11.	Polysorbate 80	Loba Chemie, Mumbai
12.	Sodium hydroxide	Himedia, Mumbai
13.	Sodium Edetate	Loba Chemie, Mumbai
14.	Benzalkonium Chloride	Loba Chemie, Mumbai
15.	Potassium dihydrogen phosphate (AR)	Himedia, Mumbai

INSTRUMENTS

S.NO	EQUIPMENTS	MODEL
1.	Magnetic stirrer	2 MLH, Remi instruments
2.	Mechanical Stirrer	RQ- 122, Remi instruments
3.	Eppendroff Centrifuge	5415, Germany
4.	UV spectrophotometer	UV 1650 PC Shimadzu
5.	pH meter	ELCO, LI 120
6.	Optical Microscope	806253, Olympus
7.	Electronic balance	Shimadzu ELB 300
8.	Sonicator	230V AC 50 Hz
9.	Brookfield viscometer	LVDV-E, Brookfield engineering Lab

6.2. EXPERIMENTAL

6.2.1. Construction of standard curve

a. Preparation of Standard Stock Solution

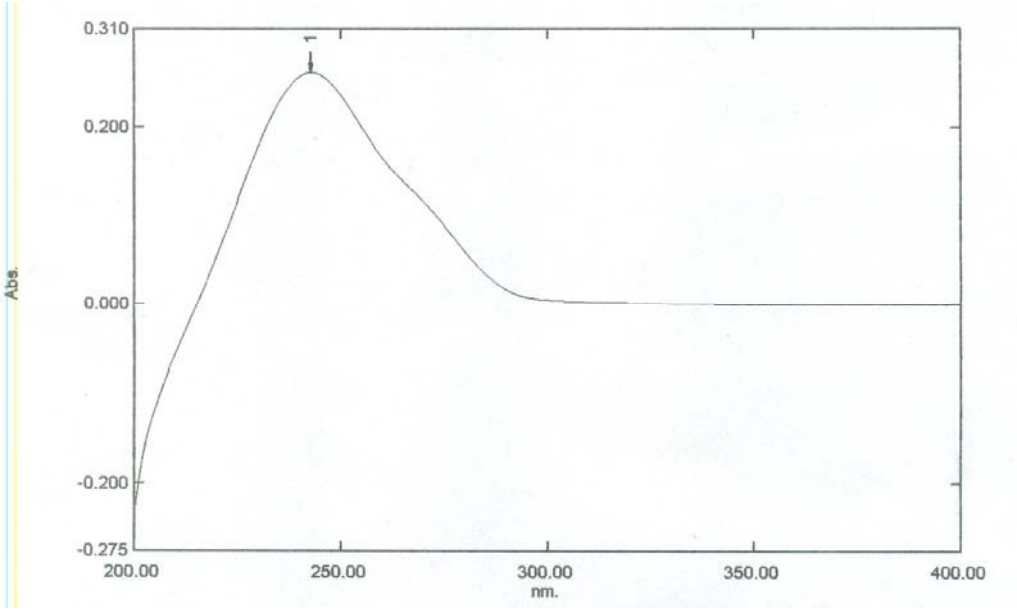
Accurately, about 100 mg of Dexamethasone sodium phosphate was weighed and transferred to a 100 ml volumetric flask. The drug was dissolved in 100 ml of Phosphate buffer pH 7.4 with shaking and then the volume made up to the mark with Phosphate buffer pH 7.4 to obtain a standard stock solution of a drug concentration, 1000 µg/ml.

b. Selection of Analytical Wavelength

With appropriate dilution of the standard stock solution with Phosphate buffer pH 7.4, the solution was scanned using the double beam UV visible spectrophotometer (Model: UV- 1650 PC, SHIMADZU) in the spectrum mode between the wavelength range of 400 nm to 200 nm. The λ_{max} of Dexamethasone sodium

phosphate was found to 242 nm, which was selected as the analytical wavelength for further analysis. (Fig 1)

Fig 1. Peak Pick of Dexamethasone Sodium Phosphate



No.	P/V	Wavelength	Abs.	Description
1		242.80	0.261	
2		328.00	0.002	

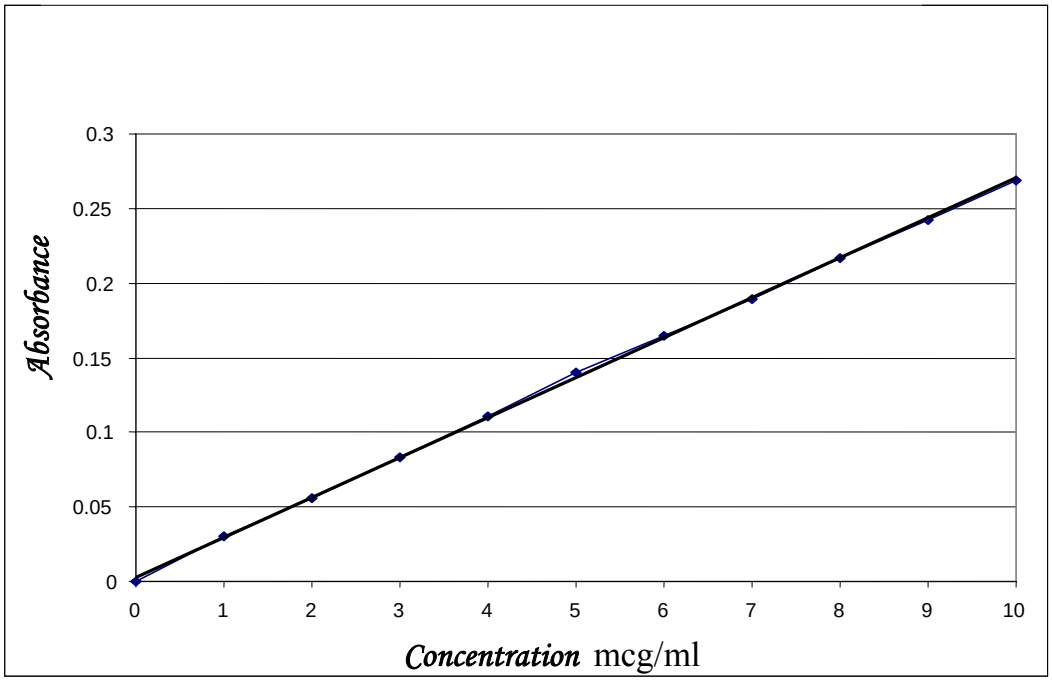
c. Standard Plot

Stock solution was further diluted to different concentrations to determine the linearity range. Linearity was obtained in the concentration between 1-10 µg/ml. The standard samples were analyzed at 242 nm using UV Spectrophotometer. (Table1 & Fig 2)

Table 1: Calibration of standard Curve

S.No	Concentration (µg/ml)	Absorbance at 242 nm
1	1	0.03006
2	2	0.05547
3	3	0.08313
4	4	0.11084
5	5	0.13775
6	6	0.16431
7	7	0.18944
8	8	0.21704
9	9	0.24243
10	10	0.26855

Fig 2: Standard Curve of Dexamethasone sodium phosphate



Y = 0.02602X + 0.00744, Correlation Coefficient r^2 = 0.99780

6.2.2. Formulation of Ophthalmic Particulate Solution by solvent evaporation method. [47, 48]

It was prepared by solvent evaporation method. Dexamethasone sodium phosphate (0.1% by weight) was added to dichloro methane and sonicated for 3 mins. The organic phase was added to corresponding aqueous phase like Methyl cellulose (0.2 or 0.4 %), or Carboxy methyl cellulose (0.2 or 0.4%), or (PLGA (85:15), / 0.25% PVA and 200mg of Phosphotidyl choline solution), then magnetically stirred at 1200 rpm at room temperature to evaporate dichloro methane (about 4 h). The particulate solution was obtained. (Table 2, 3, 4)

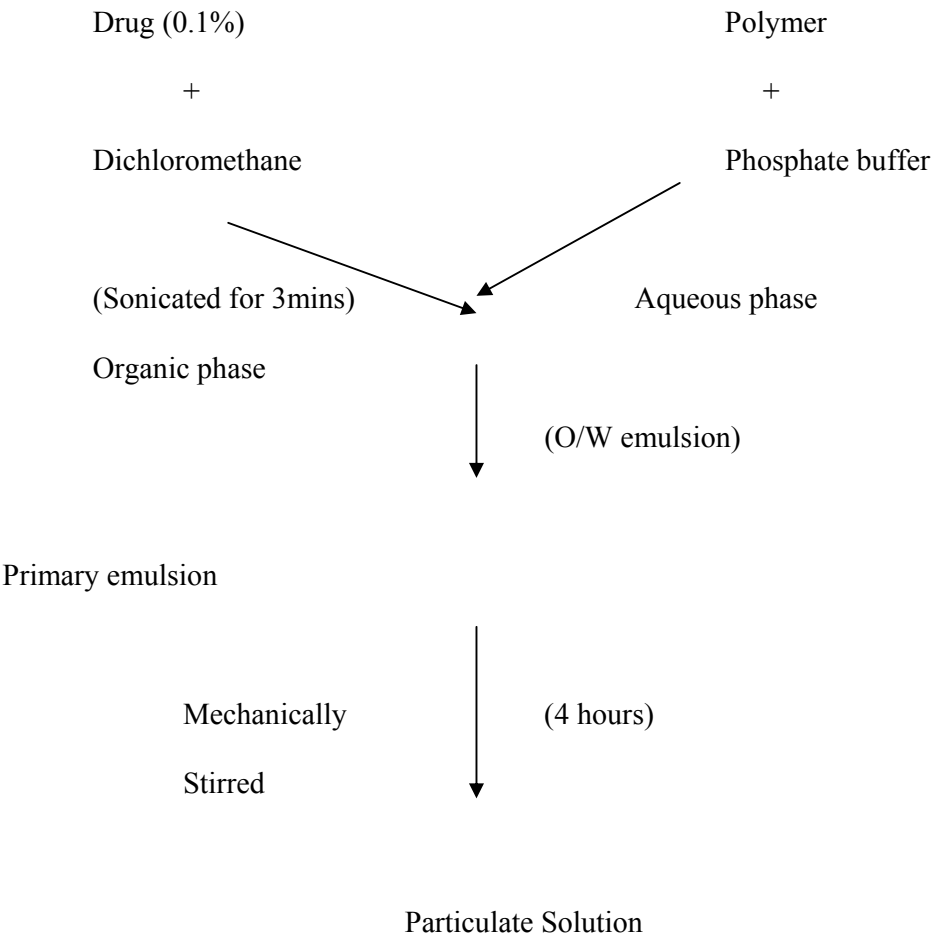


Table 2: Formulation of Particulate Solution with Methyl Cellulose

S.NO	INGREDIENTS	METHYL CELLULOSE			
		0.1%	**0.2%**	**0.3%**	**0.4%**
1.	Dexamethasone Sodium Phosphate	0.01 gm	0.01 gm	0.01 gm	0.01 gm
2.	Sodium Edetate	0.001 gm	0.001 gm	0.001 gm	0.001 gm
3.	Benzalkonium chloride	0.001 gm	0.001 gm	0.001 gm	0.001 gm
4.	Polysorbate 80	0.005 gm	0.005 gm	0.005 gm	0.005 gm
5.	Phosphate buffer (qs)	10 ml	10 ml	10 ml	10 ml

Table 3: Formulation of Particulate Solution with Carboxy methyl cellulose

S.NO	INGREDIENTS	CARBOXY METHYL CELLULOSE			
		0.1%	**0.2%**	**0.3%**	**0.4%**
1.	Dexamethasone Sodium Phosphate	0.01 gm	0.01 gm	0.01 gm	0.01 gm
2.	Sodium Edetate	0.001 gm	0.001 gm	0.001 gm	0.001 gm
3.	Benzalkonium chloride	0.001 gm	0.001 gm	0.001 gm	0.001 gm
4.	Polysorbate 80	0.005 gm	0.005 gm	0.005 gm	0.005 gm
5.	Phosphate buffer (qs)	10 ml	10 ml	10 ml	10 ml

Table 4: Formulation of Particulate Solution with PLGA

S.NO	INGREDIENTS	For 10 ml
1.	Dexamethasone Sodium Phosphate	0.01 gm
2.	PLGA	0.025 gm
3.	Phosphotidyl choline	0.2 gm
4.	Poly vinyl alcohol	0.025 gm
5.	Sodium Edetate	0.001 gm
6.	Benzalkonium chloride	0.001 gm
7.	Polysorbate 80	0.005 gm
8.	Phosphate buffer (qs)	10 ml

6.2.3. Particle Size Determination. [49]

The particulate solution was taken in a glass slide and particle size was determined by optical microscopy using a pre calibrated eye piece. The size of 50 particles was measured randomly using ocular and stage micrometer. Eye piece was calibrated using stage micrometer at 40 X magnification. Size of each division of eye piece micrometer was determined using the formula

$$\text{Size of each division} = \frac{\text{Number of divisions of stage micrometer}}{\text{Number of divisions of eye piece micrometer}} \times 10$$

Each division of eye piece micrometer was found to be 2 μm at 40 X magnification.

Photo Micrographs

A drop of ophthalmic particulate solution was placed on the microscopic glass slide. Photographs of formulations were taken at 40 X magnification using the digital camera (Olympus, 8 mega pixels) attached to the eye piece of the microscope. The particle and its size are showed (Fig 3-10 & Table 5).

Fig .3 0.1% MC

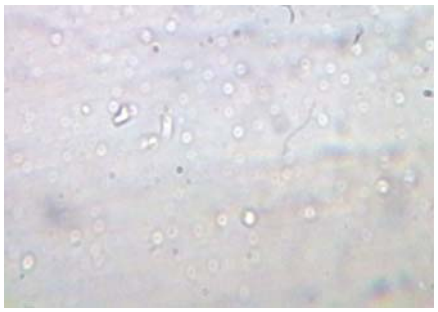


Fig .4 0.2% MC

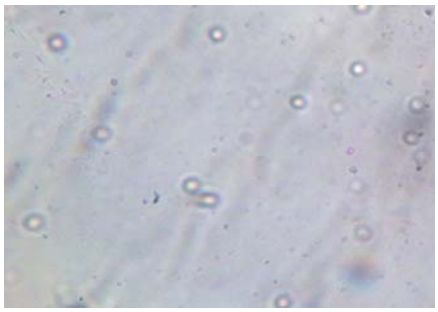


Fig .5 0.3% MC

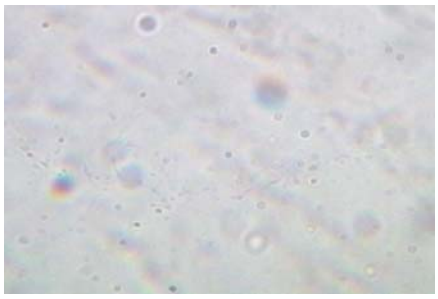


Fig .6 0.4% MC

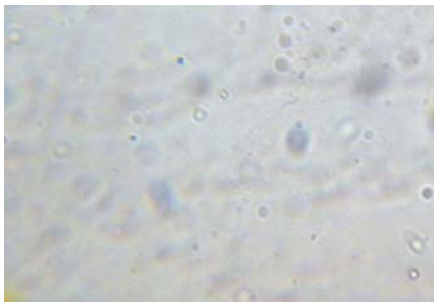


Fig .7 0.2% CMC

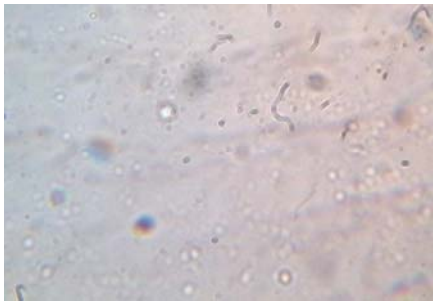


Fig .8 0.3% CMC

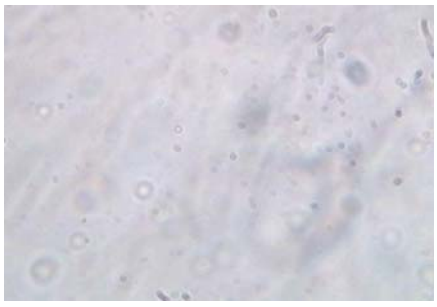


Fig 9. 0.4% CMC

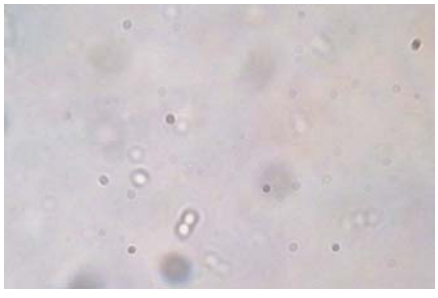


Fig 10. PLGA

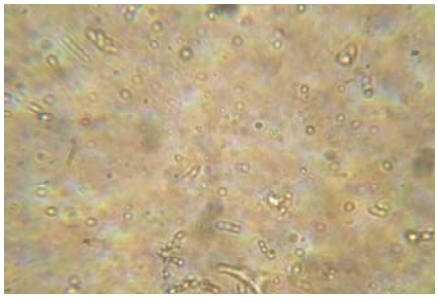


Table 5: AVERAGE PARTICLE SIZE IN FORMULATION

S.NO	POLYMER	PARTICLE SIZE (µm)
1.	0.1% MC	3.88
2.	0.2% MC	2.4
3.	0.3% MC	3.63
4.	0.4% MC	5.26
5.	0.2% CMC	2.28
6.	0.3% CMC	3.56
7.	0.4% CMC	2.34
8.	PLGA	2.22

6.2.4. Dispersion of particulate solution in gels.

The ophthalmic particulate solutions were taken and mixed with the specified quantity of Sodium Alginate (1% or 3%) or Carbopol (0.1%) correspondingly, and trituration was continued for 1 hour till the particulate solution was dispersed and to get a gel consistency.

6.2.5. Drug content uniformity. [20]

The vials containing the preparations were shaken for a few minutes and 100 µl of the preparations were transferred to 25 ml volumetric flasks using a Micro Pipette. Phosphate buffer (pH 7.4) was added in small portions (5 ml), shaken to dissolve the contents, volume was adjusted to 25 ml, and the solutions were assayed for Dexamethasone sodium phosphate content at 242 nm.(Table 13.)

$$\text{Drug Content Uniformity} = \frac{\text{Concentration} \times 1 \times \text{Dilution Factor}}{1000}$$

6.2.6. pH. [38]

2.5 gm of gel was accurately weighed and dispersed in 25 ml of purified water. The pH of dispersions was measured using pH meter. (Table 6)

Table 6: COMPARATIVE PROFILE OF DRUG CONTENT UNIFORMITY AND pH OF FORMULATED GELS

S.NO	CONTENT	% UNIFORMITY	pH
1% SODIUM ALGINATE GEL			
1	0.2% MC	77.36	7.2
2	0.2% CMC	41.8	7.34
3	0.4% CMC	60.56	7.12
4	PLGA	87.57	7.42
3% SODIUM ALGINATE GEL			
5	0.2% MC	79.17	7.18
6	0.2% CMC	64.4	7.32
7	0.4% CMC	14.2	7.16
8	PLGA	75.27	7.38
0.1% CARBOPOL GEL			
9	0.2% MC	18.8	7.22
10	0.2% CMC	13.0	7.3
11	0.4% CMC	14.9	7.1
12	PLGA	24.75	7.4

6.2.7. VISCOSITY AND RHEOLOGICAL STUDIES. [21]

Viscosity of formulated gels was determined using Brookfield Viscometer. Gels were tested for their rheological characteristics at 25^0C using Brookfield Viscometer (LVDV-E Brookfield engineering Lab) spindle no 62. The measurement was made over with the speed 30 rpm. (Table 7)

Table 7: VISCOSITY PROFILE OF OPHTHALMIC GELS

S.NO	CONTENT	VISCOSITY (cps) at 30 rpm
1% SODIUM ALGINATE GEL		
1	0.2% MC	52
2	0.2% CMC	198
3	0.4% CMC	493
3% SODIUM ALGINATE GEL		
5	0.2% MC	189
6	0.2% CMC	588
7	0.4% CMC	994

6.2.8. *IN VITRO* DRUG RELEASE.

In vitro release from formulation was carried out by dialysis membrane diffusion technique (obtained from Hi-Media) having a molecular weight cut off 12000. The membrane used was transparent and regenerated cellulose type, which was permeable to low molecular weight substances. The release study of the gel was performed by 1ml ophthalmic gel in a dialysis bag and then immersed in phosphate buffer (pH 7.4).The receptor compartment was continuously stirred (50 rpm) using a magnetic stirrer. The temperature was maintained between 37° ± 1°C. Samples were withdrawn at predetermined time intervals and the same volume was replaced with

fresh buffer medium to maintain sink condition. The absorbance of the withdrawn sample was measured after suitable dilution. At various time intervals, aliquot samples was withdrawn and assayed for drug content by UV spectrophotometer method. Drug release profiles were plotted by taking time on X axis and percentage release on Y axis. The *in vitro* release data and comparative invitro release profile of corresponding gels are showed in Table 8-15 and Fig 11-14. .

Table 8: *IN VITRO* RELEASE DATA OF 1% SODIUM ALGINATE GEL:

Table 8.1: 0.2% MC

n = 2

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.01501	0.29093	0.000291	0.29093	29.0930±1.49
60	0.0199	0.478862	0.000479	0.478862	47.8862±1.38
90	0.02625	0.722905	0.000723	0.722905	72.2905±1.39
120	0.03088	0.900846	0.000901	0.900846	90.0845±1.52

Table 8.2: 0.2% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.03503	1.060338	0.00106	0.106034	10.60338
60	0.05383	1.782859	0.001783	0.178286	17.82859
90	0.06445	2.191007	0.002191	0.219101	21.91007
120	0.07532	2.608762	0.002609	0.260876	26.08762
150	0.09375	3.317064	0.003317	0.331706	33.17064
180	0.10364	3.697156	0.003697	0.369716	36.97156

Table 8.3: 0.4% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.00781	0.01422	1.42E-05	0.01422	1.421983
60	0.01704	0.368947	0.000369	0.368947	36.8947
90	0.02551	0.694466	0.000694	0.694466	69.44658
120	0.03308	0.985396	0.000985	0.985396	98.53958

Table 8.4: PLGA

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.02148	0.539585	0.00054	0.053958	5.395849
60	0.02649	0.732129	0.000732	0.073213	7.321291
90	0.03491	1.055726	0.001056	0.105573	10.55726
120	0.03528	1.069946	0.00107	0.106995	10.69946
150	0.047	1.520369	0.00152	0.152037	15.20369
180	0.05298	1.750192	0.00175	0.175019	17.50192
210	0.05518	1.834743	0.001835	0.183474	18.34743
240	0.06226	2.106841	0.002107	0.210684	21.06841
270	0.06763	2.313221	0.002313	0.231322	23.13221
300	0.09501	3.365488	0.003365	0.336549	33.65488

Fig 11: COMPARATIVE RELEASE PROFILE OF 1% SODIUM ALGINATE GELS

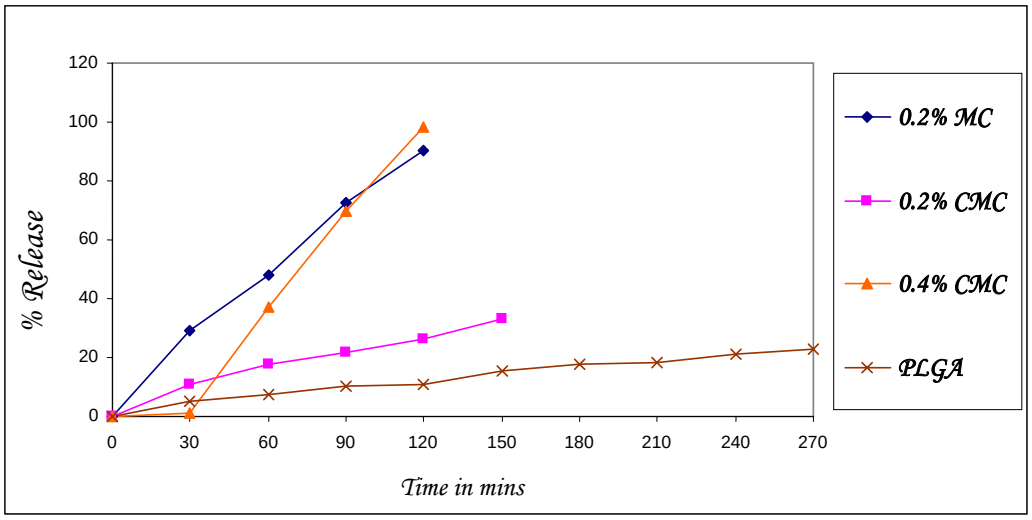


Table 9: COMPARATIVE RELEASE PROFILE OF 1% SODIUM ALGINATE GELS

TIME IN MINS	CUMULATIVE % DRUG RELEASE OF 1% SODIUM ALGINATE GEL			
	0.2% MC	0.2% CMC	0.4% CMC	PLGA
0	0	0	0	0
30	29.0930±1.49	10.60338	1.421983	5.395849
60	47.8862±1.38	17.82859	36.8947	7.321291
90	72.2905±1.39	21.91007	69.44658	10.55726
120	90.0845±1.52	26.08762	98.53958	10.69946
150	-	33.17064	-	15.20369
180	-	-	-	17.50192
210	-	-	-	18.34743
240	-	-	-	21.06841
270	-	-	-	23.13221
300	-	-	-	33.65488

Table 10: *IN VITRO* RELEASE DATA OF 3% SODIUM ALGINATE GEL:

Table 10.1: 0.2% MC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.0415	1.308993	0.001309	0.130899	13.08993
60	0.09338	3.302844	0.003303	0.330284	33.02844
90	0.11194	4.016141	0.004016	0.401614	40.16141
120	0.18835	6.952729	0.006953	0.695273	69.52729

Table 10.2: 0.2% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.00159	-0.22483	-0.00022	-0.22483	0
60	0.00089	-0.25173	-0.00025	-0.25173	0
90	0.01599	0.328593	0.000329	0.328593	32.85934
120	0.02942	0.844735	0.000845	0.844735	84.47348
150	0.03345	0.999616	0.001	0.999616	99.96157

Table 10.3: 0.4% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.02283	0.591468	0.000591	0.591468	59.14681
60	0.02734	0.764796	0.000765	0.764796	76.47963
90	0.00281	0.00281	0.00281	0.00281	0.00281

Table 10.4: PLGA

n = 2

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.01221	0.183321	0.000183	0.183321	18.3320±1.36
60	0.01965	0.469254	0.000469	0.469254	46.9254±1.89
90	0.02734	0.764796	0.000765	0.764796	76.4796±1.9
120	0.2229	8.280553	0.008281	0.828055	82.8055±1.68
150	0.22583	8.393159	0.008393	0.839316	83.9315±1.54
180	0.24976	9.312836	0.009313	0.931284	93.1283±1.06
210	0.26624	9.946195	0.009946	0.99462	99.4619±0.38

Fig 12: COMPARATIVE RELEASE PROFILE OF 3% SODIUM ALGINATE GELS

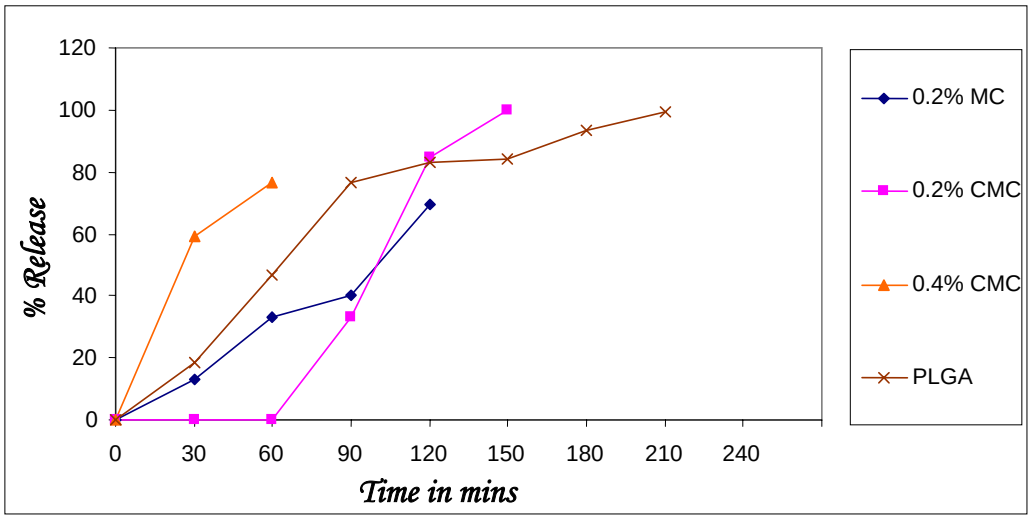


Table 11: COMPARATIVE RELEASE PROFILE OF 3% SODIUM ALGINATE GELS

TIME IN MINS	CUMULATIVE % DRUG RELEASE OF 3% SODIUM ALGINATE GEL			
	0.2% MC	0.2% CMC	0.4% CMC	PLGA
0	0	0	0	0
30	13.08993	0	59.14681	18.3320±1.36
60	33.02844	0	76.47963	46.9254±1.89
90	40.16141	32.85934	-	76.4796±1.9
120	69.52729	84.47348	-	82.8055±1.68
150	-	99.96157	-	83.9315±1.54
180	-	-	-	93.1283±1.06
210	-	-	-	99.4619±0.38
240	-	-	-	-
270	-	-	-	-
300	-	-	-	-

Table 12: *IN VITRO* RELEASE DATA OF 0.1% CARBOPOL GEL:

Table 12.1: 0.2% MC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.0365	1.116833	0.001117	0.111683	11.16833
60	0.07373	2.547656	0.002548	0.254766	25.47656
90	0.09534	3.378171	0.003378	0.337817	33.78171
120	0.10889	3.898924	0.003899	0.389892	38.98924
150	0.12231	4.414681	0.004415	0.441468	44.14681
180	0.13501	4.902767	0.004903	0.490277	49.02767
210	0.15625	5.719062	0.005719	0.571906	57.19062
240	0.17749	6.535357	0.006535	0.653536	65.35357
270	0.21167	7.848962	0.007849	0.784896	78.48962

Table 12.2: 0.2% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.01123	0.145657	0.000146	0.014566	1.456572
60	0.02368	0.624135	0.000624	0.062414	6.241353
90	0.02686	0.746349	0.000746	0.074635	7.46349
120	0.03345	0.999616	0.001	0.099962	9.996157
150	0.03711	1.140277	0.00114	0.114028	11.40277
180	0.08203	2.866641	0.002867	0.286664	28.66641
210	0.08838	3.110684	0.003111	0.311068	31.10684
240	0.09717	3.448501	0.003449	0.34485	34.48501
270	0.11511	4.137971	0.004138	0.413797	41.37971

Table 12.3: 0.4% CMC

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.01184	0.169101	0.000169	0.01691	1.691007
60	0.00708	-0.01384	-1.4E-05	-0.00138	-0.13836
90	0.02173	0.549193	0.000549	0.054919	5.491929
120	0.04639	1.496925	0.001497	0.149693	14.96925
150	0.0614	2.073789	0.002074	0.207379	20.73789
180	0.073	2.5196	0.00252	0.25196	25.196
210	0.08704	3.059185	0.003059	0.305919	30.59185

Table 12.4: PLGA

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
30	0.02698	0.750961	0.000751	0.075096	7.509608
60	0.0448	1.435819	0.001436	0.143582	14.35819
90	0.07373	2.547656	0.002548	0.254766	25.47656
120	0.06897	2.364719	0.002365	0.236472	23.64719
150	0.08228	2.876249	0.002876	0.287625	28.76249
180	0.0968	3.434281	0.003434	0.343428	34.34281
210	0.10693	3.823597	0.003824	0.38236	38.23597
240	0.11182	4.01153	0.004012	0.401153	40.1153

Fig 13: COMPARATIVE RELEASE PROFILE OF 0.1% CARBOPOL GEL

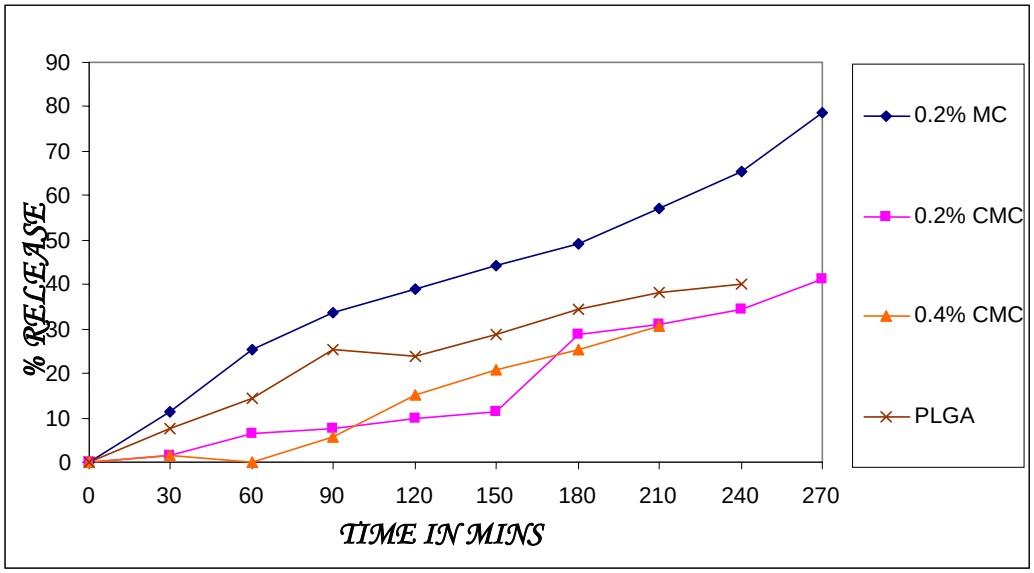


Table 13: COMPARATIVE RELEASE PROFILE OF 0.1% CARBOPOL GEL

TIME IN MINS	CUMULATIVE % DRUG RELEASE OF 0.1% CARBOPOL GEL			
	0.2% MC	0.2% CMC	0.4% CMC	PLGA
0	0	0	0	0
30	11.16833	1.456572	1.691007	7.509608
60	25.47656	6.241353	-0.13836	14.35819
90	33.78171	7.46349	5.491929	25.47656
120	38.98924	9.996157	14.96925	23.64719
150	44.14681	11.40277	20.73789	28.76249
180	49.02767	28.66641	25.196	34.34281
210	57.19062	31.10684	30.59185	38.23597
240	65.35357	34.48501	-	40.1153
270	78.48962	41.37971	-	-

Table 14: *IN VITRO* RELEASE DATA OF DRUG IN SOLUTION:

n = 2

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
15	0.0681	2.331284	0.002331	0.233128	23.3128 ± 0.93
30	0.1244	4.495004	0.004495	0.4495	44.9500 ± 1.34
45	0.1996	7.385088	0.007385	0.738509	73.8508 ± 0.52

Fig 14: RELEASE PROFILE OF DRUG IN SOLUTION

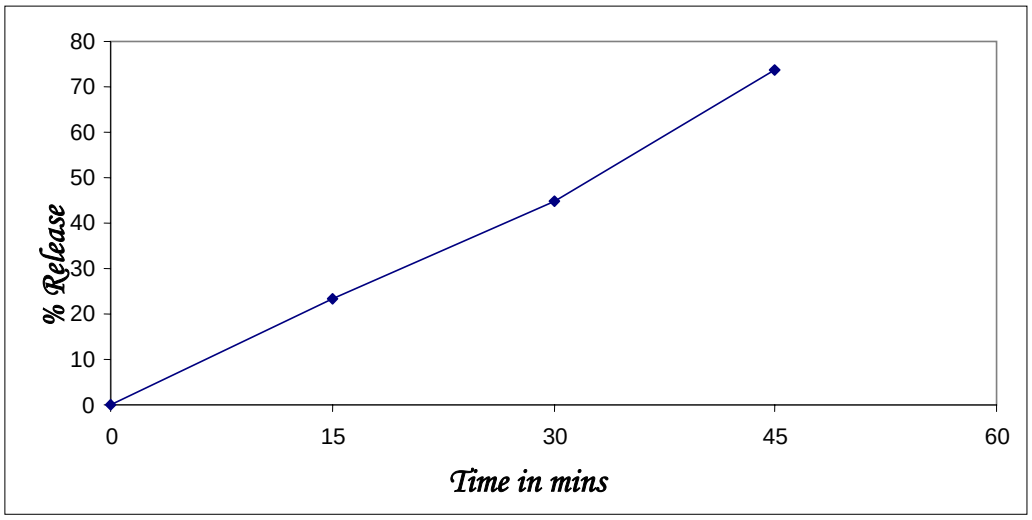


Table 15: RELEASE PROFILE OF DRUG IN SOLUTION

TIME IN MINS	CUMULATIVE % RELEASE
0	0
15	23.3128 ± 0.93
30	44.9500 ± 1.34
45	73.8508 ± 0.52

DRUG RELEASE KINETICS

6.2.8.1. 0.2% MC IN 1% SODIUM ALGINATE GEL.

Fig 15: ZERO ORDER RELEASE of 0.2% MC

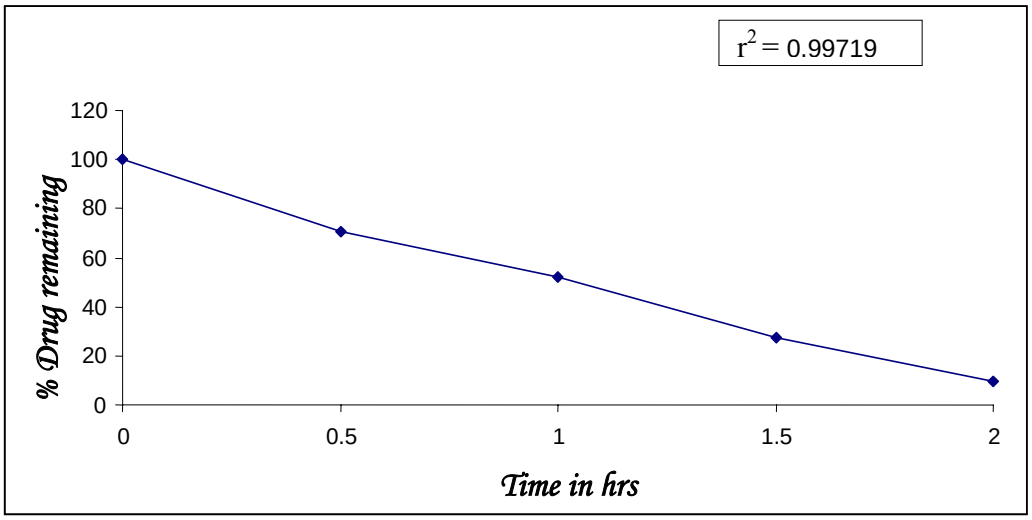


Fig 16: FIRST ORDER RELEASE of 0.2% MC

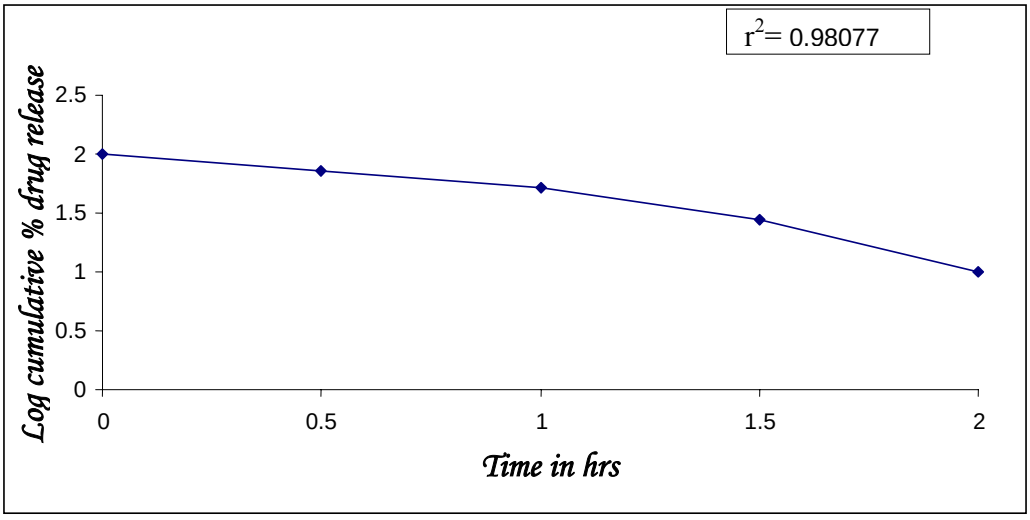


Fig 17: HIGUCHI PLOT of 0.2% MC

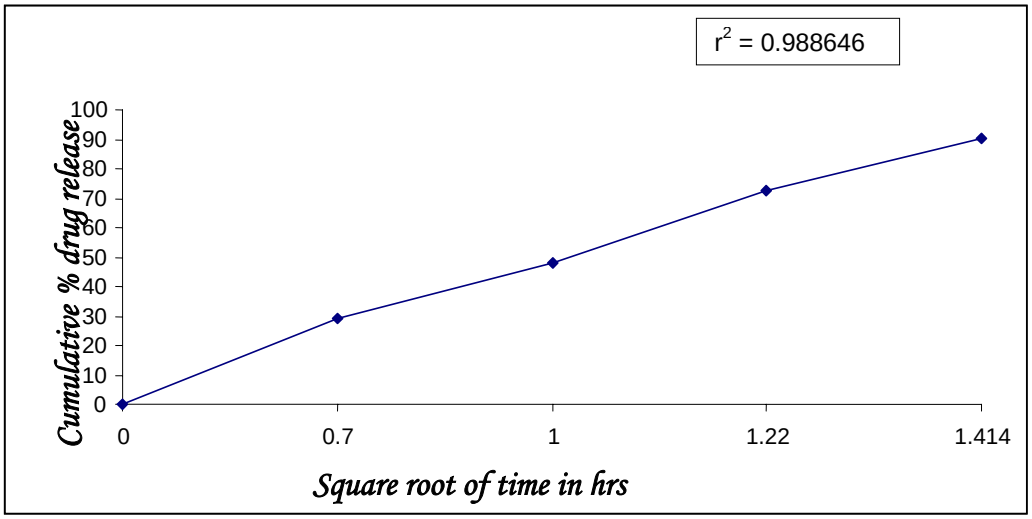
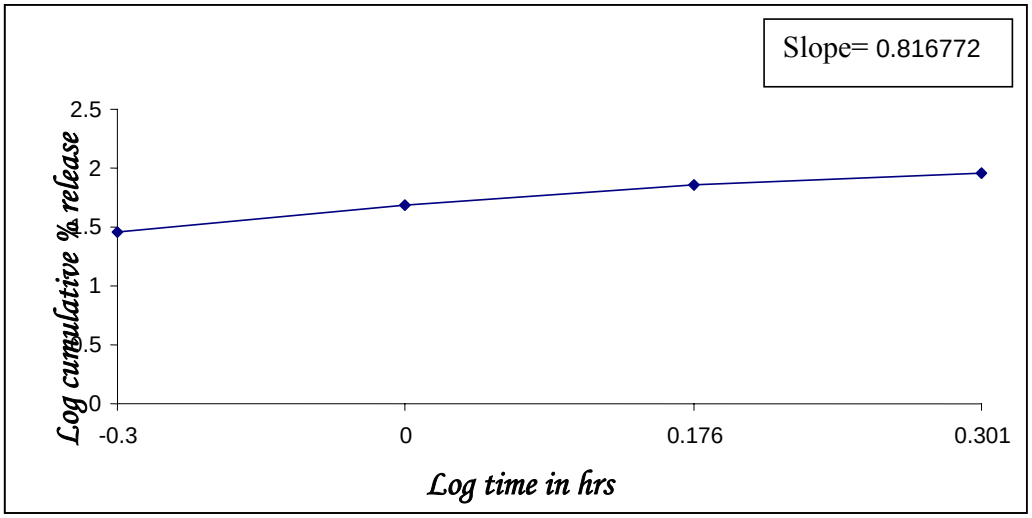


Fig 18: KORSMEYER PEPPAS PLOT of 0.2% MC



6.2.8.2. PLGA IN 3% SODIUM ALGINATE GELS

Fig 19: ZERO ORDER RELEASE of 0.2% PLGA

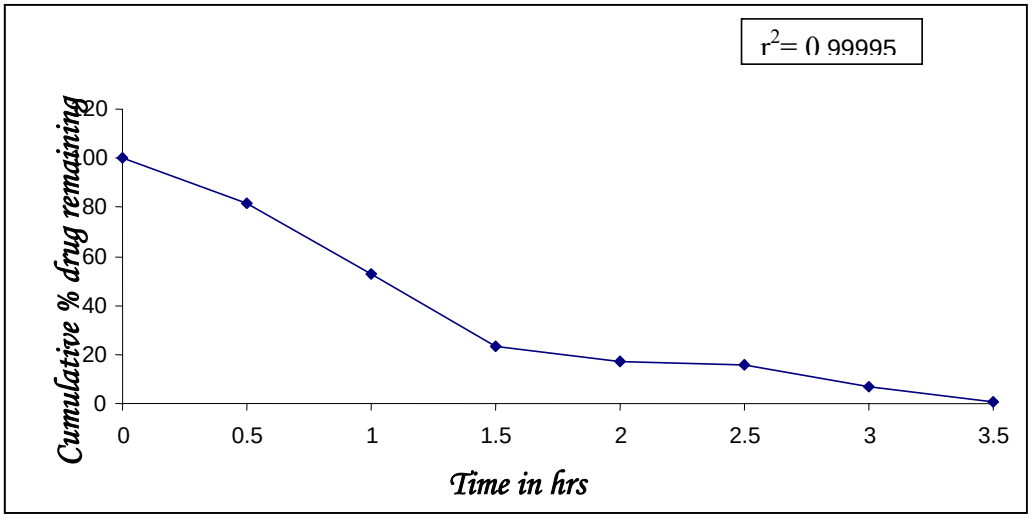


Fig 20: FIRST ORDER RELEASE of 0.2% PLGA

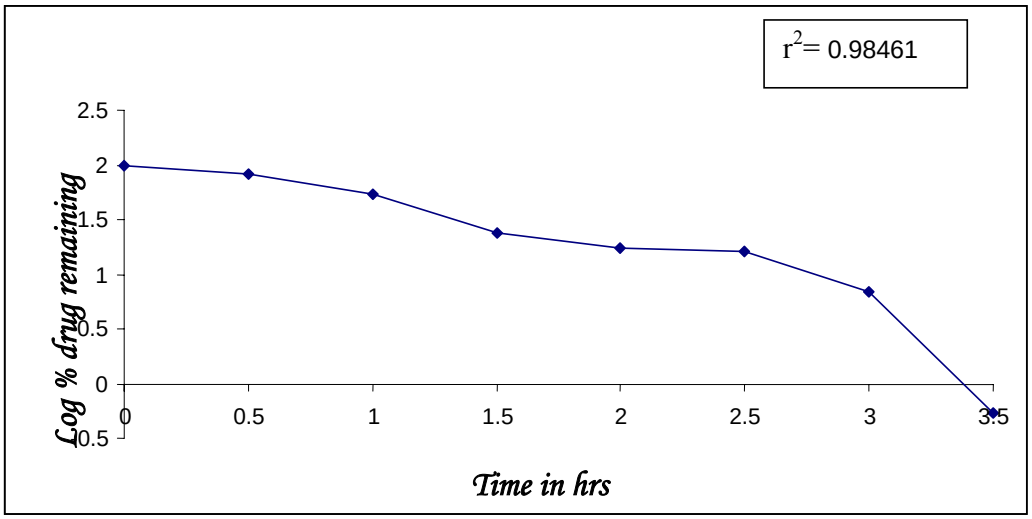


Fig 21: HIGUCHI PLOT of 0.2% PLGA

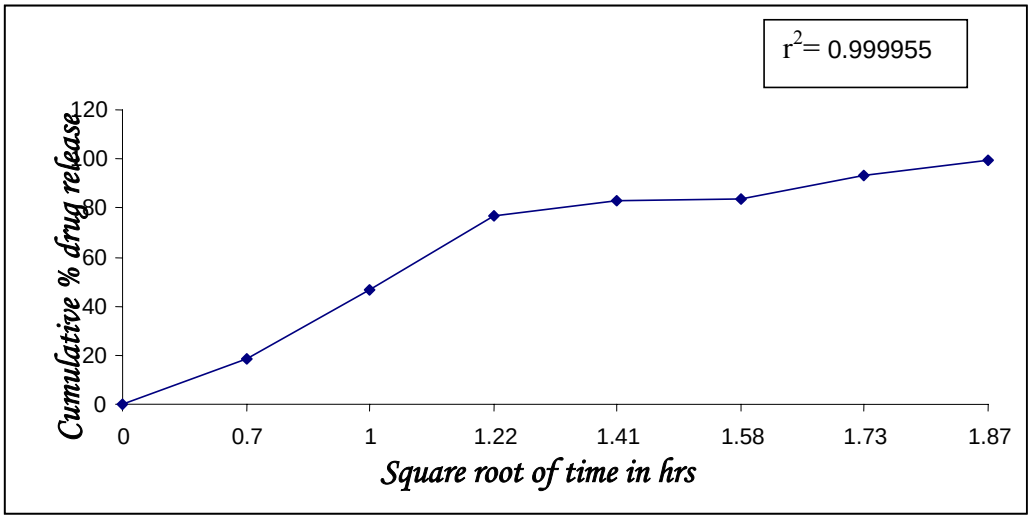
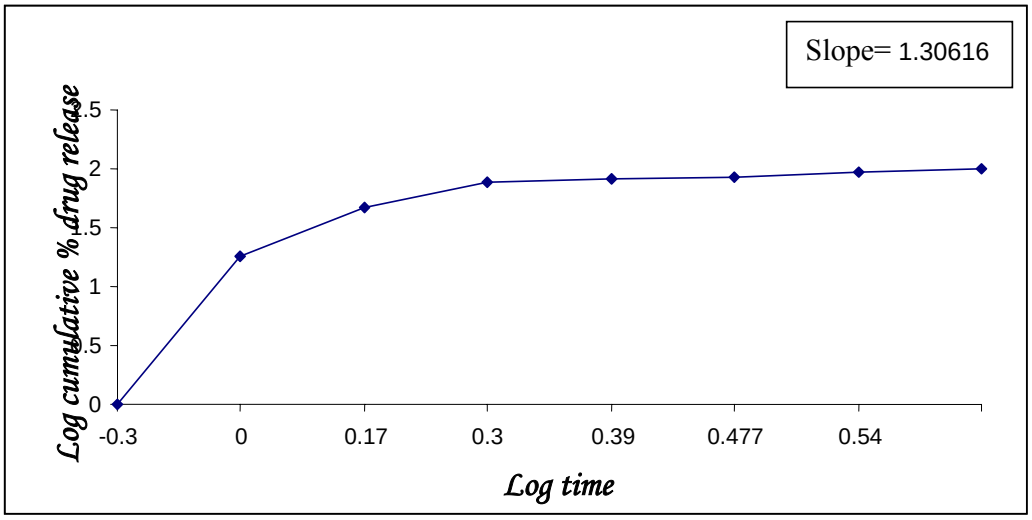


Fig 22: KORSMEYER PEPPAS PLOT of 0.2% PLGA



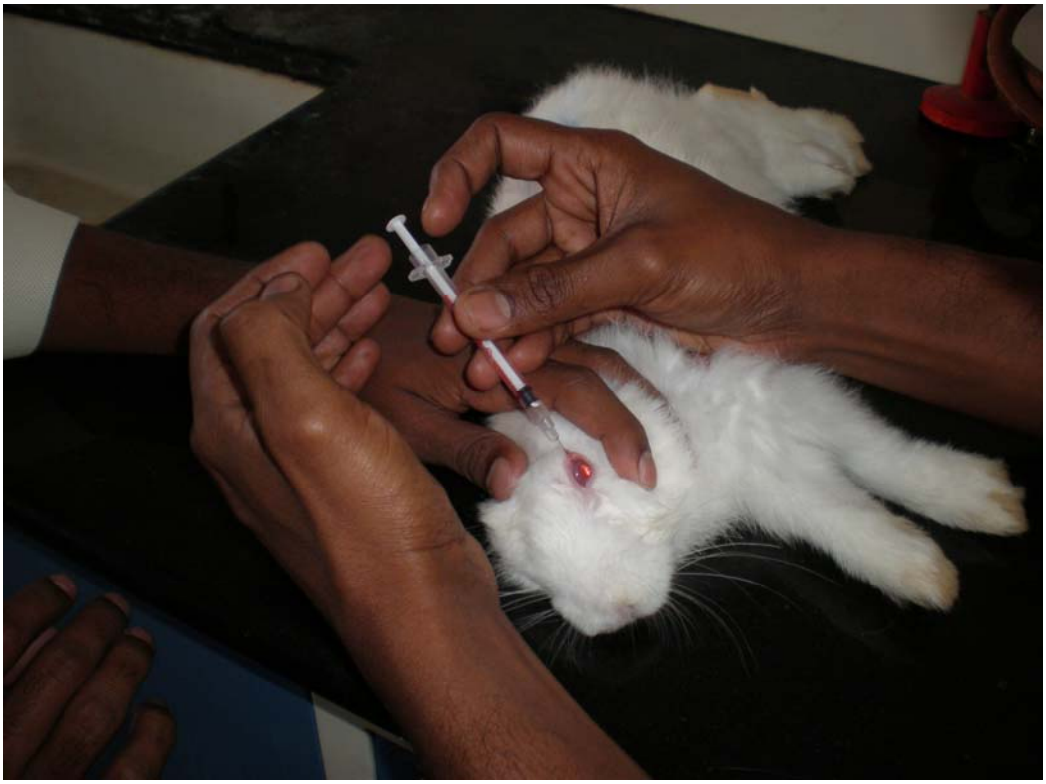
6.2.9. STERILIZATION.

Sterilization of the ophthalmic gels was carried by moist heat sterilization. The ophthalmic gels were autoclave at 121^{o}C for 20 mins.

6.2.10. *IN VIVO* STUDIES.

The animal ethical committee (PSG Institute of Medical Sciences and Research, Reg no: 158/ 1999/ CPCSEA, dated on 5th Jan 2009) had given permission for the *in vivo* study. The healthy albino rabbits were taken (weighing 1.5 to 2 kg). The animals with any ocular abnormalities were excluded after thorough examination. The animals were housed in individual cages. The drug content present in aqueous humor, of the ophthalmic gel formulation and Drug in solution was determined using three groups of healthy rabbits (A, B & C) four each. The rabbits were anesthetized with intramuscular injection of Ketamine (40 mg/kg).

Fig 23: Collection of Aqueous Humor



Aqueous humor (50 µl) was collected (Fig 23) by using a syringe connected to a 30- gauge needle in the sclero-corneal limbus. The aqueous humor was collected at every 15 mins interval for 1 hour. The absorbance of the withdrawn sample was measured after suitable dilution with phosphate buffer and assayed for drug content by UV spectrophotometer method at 242 nm. The *in vivo* release data was showed in Table 16- 18. The comparative *in vivo* release profile showed in Fig 24 & Table 19.

Table 16: *IN VIVO* RELEASE DATA OF 0.2% MC IN 1% SODIUM ALGINATE GEL

n = 4

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
15	0.21924	8.139892	0.00814	0.244197	24.41968 ± 0.48
30	0.34521	12.98117	0.012981	0.389435	38.9435 ± 1.40
45	0.62781	23.84204	0.023842	0.715261	71.52613 ± 1.28
60	0.6626	25.17909	0.025179	0.755373	75.53728 ± 0.69

Table 17: *IN VIVO* RELEASE DATA OF PLGA IN 3% SODIUM ALGINATE GEL

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
15	0.11865	4.27402	0.004274	0.128221	12.82206 ± 0.93
30	0.18274	6.737125	0.006737	0.202114	20.21138 ± 0.36
45	0.71533	27.20561	0.027206	0.816168	81.61683 ± 0.93
60	0.72241	27.47771	0.027478	0.824331	82.43313 ± 0.56

Table 18: *IN VIVO* RELEASE DATA OF DRUG IN SOLUTION

n = 4

Time in Mins	Absorbance in nm	Concentration in mcg/ml	Concentration in mg	Concentration in 10 ml	Cumulative % Release
15	0.35571	13.3847	0.013385	0.401541	40.15411 ± 0.59
30	0.74915	28.50538	0.028505	0.855161	85.51614 ± 1.2

Fig 24: COMPARATIVE *IN VIVO* RELEASE PROFILE OF OPHTHALMIC GELS:

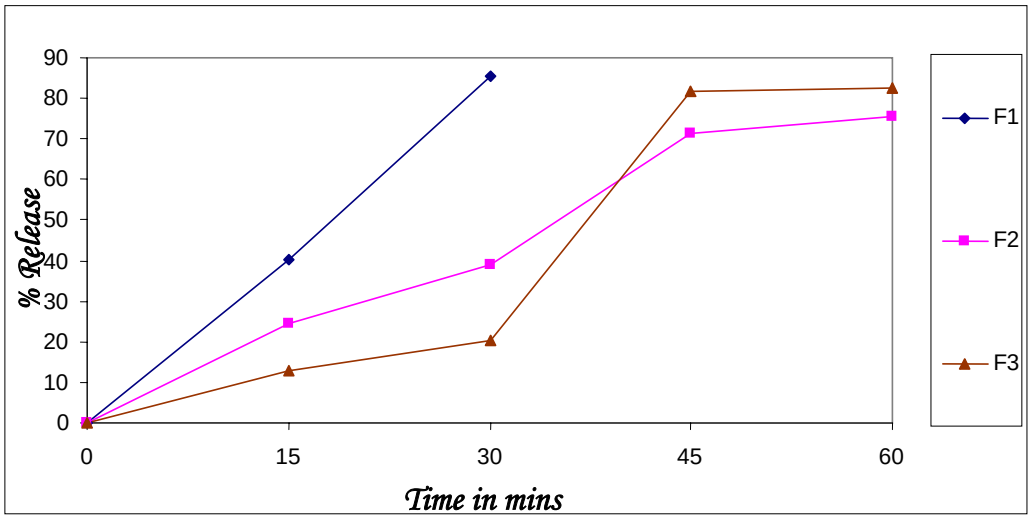


Table 19: COMPARATIVE *IN VIVO* RELEASE PROFILE OF OPHTHALMIC GELS

TIME IN MINS	PERCENTAGE RELEASE		
	F1	F2	F3
30	40.15411 ± 0.59	24.41968 ± 0.48	12.82206 ± 0.93
60	85.51614 ± 1.2	38.9435 ± 1.40	20.21138 ± 0.36
90	-	71.52613 ± 1.28	81.61683 ± 0.93
120	-	75.53728 ± 0.69	82.43313 ± 0.56

6.2.11. STATISTICAL ANALYSIS:

Comparison between the *in vivo* release in 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gel was performed by Analysis of Variance (one way ANOVA with Turkeys multiple comparison post test) with graph pad prism (version 3.0) software.

RESULTS AND DISCUSSION

7.1. Construction of standard graph:

The pure drug Dexamethasone sodium phosphate solution was analyzed spectrophotometrically at 242 nm. Linearity was found to be in the concentration (1-10 µg/ml) and the r^2 value was found to be 0.99780. (Fig-1, 2 & Table-1)

7.2. Formulation of ophthalmic particulate solution:

The ophthalmic particulate formulation was formulated using solvent evaporation method. The particles in the particulate solutions were found to be good and visible in the formulation prepared with 0.2% MC, 0.2% CMC, 0.4% CMC and PLGA. Hence these formulations were selected for the further investigations. (Table 2, 3)

7.3. Particle Size

The particle size in the prepared formulation was found to be less in PLGA (2.22 µm) and bigger in 0.4% MC (5.26 µm), (Table 5). Formulation with 0.2% MC and 0.2 CMC were also found to be smaller. Microparticles [49] (mean diameter 1-3 µm) may be better suited for controlled release, but the presence of coarse particle above 25 µm makes them less tolerable and cause irritation to the eye. This confirms formulated particulate ophthalmic solutions are within the limit.

7.4. Drug Content uniformity and pH

The drug content uniformity in 1% & 3% sodium alginate gel with PLGA and 0.2% MC was seemed to be high as 87.57%, 77.36% and 75.27%, 79.17%.

Even though drug content uniformity in PLGA and 0.2% MC were seemed to be higher in Carbopol gel, the drug content uniformity is less when compared with sodium alginate formulations (1% & 3%). (Table 6)

The pH of all ophthalmic formulated gels (1% & 3% Sodium alginate gel and 0.1% Carbopol gel) was found to be in limit (pH 7-7.5). (Table 6)

7.5. Viscosity of Gels.

When the Nanoparticulate solutions dispersed in lower polymer concentrations, (i.e.) 1% sodium alginate gel the viscosity was found to be low. When the polymeric concentration (3 % sodium alginate gel) increased the viscosity seems to be increased.

Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye. The viscosity range of 3% sodium alginate gels are 3- 4 times higher than that of 1% sodium alginate gel. Viscosity values are promising; hence it may increase the contact time in conjunctival sac during application. (Table 7)

7.6. *IN VITRO* RELEASE STUDIES.

The release data of ophthalmic gels are showed in Table 8-15 & Fig 11-14.

Drug release from 1% sodium alginate gel, with the formulation Methyl cellulose (0.2%) shows 90.08% at 120 mins, where as Carboxy methyl cellulose (0.2%) was 26.12% in 120 mins, the release after 150 mins from CMC was 33.17%.

The higher polymeric concentration of Carboxy methyl cellulose (0.4%) shows 98.53% at 120 mins.

The release from 0.2% MC & 0.4% CMC shows the maximum percentage release of about 90% at 120 mins.

The release profile from PLGA formulation was found to be 33.65% at 300 mins. (Fig 11)

Drug release from 3% sodium alginate gel shows 69.52% of release in lowest polymeric concentration of Methyl cellulose (0.2%) at 120 mins; where as the drug release was not found from the Carboxy methyl cellulose (0.2%) in the initial hours.

Formulation containing carboxy methyl cellulose (0.4%) concentration dispersed in 3% sodium alginate gel shows 76.47% of drug in 60 mins.

PLGA shows the release of 82.80% at 120 mins, and it extends upto 210 mins where release of drug was 99.46%.

From the release study of 3% sodium alginate gel 0.2% MC and PLGA shows the maximum percentage release. (Fig 12)

Formulation was dispersed in 1% and 0.5% Carbopol gel and the drug was not released from this formulation even after 2 hour.

From the 0.2% MC formulation dispersed in 0.1% Carbopol the release was found to be 78.48% in 270 mins.

Formulations 0.2% CMC, 0.4% CMC and PLGA were dispersed in 0.1% Carbopol gel found that the release was less than 40% of drug in 270 mins. Hence these formulations were not taken for further studies. (Fig 13)

The release data of drug in solution shows 73.85% of drug released in 45mins (Fig 14)

The drug released from 0.2% MC in 1% sodium alginate gel & 3% sodium alginate was 90.08% & 69.52% at 120 mins, where as PLGA in 1% sodium alginate gel & 3% sodium alginate was 33.65% & 99.46% in 210 mins. The formulations, 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gels were selected for further studies.

The *in vitro* drug release from 0.2% MC in 1% sodium alginate gel & PLGA in 3% sodium alginate gel was about 20% in the initial 30 mins.

The difference in drug release from the formulation may be due to the

- Polymeric concentration in the particulate formulation
- Permeation of gel through the dialysis membrane and
- Diffusion of drug particulates from the formulation into the gel

From the kinetics data, the regression value for Higuchi plot shows the release from both formulations 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gel follow diffusion.

From the slope value of korsmeyer Peppas plot 0.2% MC in 1% sodium alginate gel follows non fickian diffusion type of release and PLGA in 3% sodium alginate gel follows analomous type diffusion (diffusion and erosion).(Fig 15-22)

7.7. *IN VIVO* STUDIES.

Percentage of drug release in the aqueous humor from drug in solution (F1) was 85.51% at 60 mins (Table 16), where as 0.2% MC in 1% sodium alginate gel (F2) & PLGA in 3% sodium alginate gel (F3) it was 75.53% & 82.42% at 120 mins (Table 17, 18).

Both the formulations F2 & F3 were found to be effective in extending the drug release. Formulation (0.2% MC in 1% sodium alginate gel) F2 seemed to be the best formulation than PLGA in 3% sodium alginate gel (F3). (Fig 24, Table 19).

The drug concentration in aqueous humor was found to increase significantly ($P< 0.001$) in formulation F1 when compared with formulation F2 & F3.

But when the formulations F2 & F3 were compared F2 was seemed to be significant ($P<0.001$) at 60 mins.

CONCLUSION

From above results we conclude that gel formulation prepared with 0.2% MC in 1% sodium alginate and PLGA in 3% sodium alginate gives promising results in viscosity, drug content uniformity, and *in vitro* studies.

Nanoparticulate formulation 0.2% MC dispersed in 1% sodium alginate gel (F2) found to have significant ($P<0.001$) drug concentration in the rabbit aqueous humour than the formulation PLGA in 3% sodium alginate gel (F3).

From the in vivo drug release, the formulation F2 (0.2% MC in 1% sodium alginate gel) confirms that the drug release can be extended upto 2 to 2.5 hours through the nanoparticulate formulation for the post cataract treatment.

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